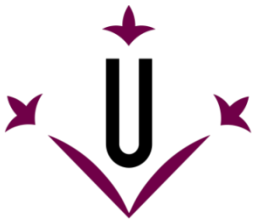

Colostrum and milk pasteurization improve health status and decrease mortality in neonatal calves receiving appropriate colostrum ingestion.

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Interpretative summary

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Colostrum and milk pasteurization improve health status and decrease mortality in neonatal calves receiving appropriate colostrum ingestion.

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Running head: Colostrum and milk pasteurization is an efficient measure to control diseases

ABSTRACT

The objective of the study was to evaluate if on-farm heat treatment of colostrum and bulk tank milk can improve calf health status and morbidity and mortality rates during the first 21 days of life in neonatal Holstein calves receiving appropriate colostrum ingestion. A total of 587 calves were randomly assigned into 2 groups of males and females over 18 months. Non-pasteurized group (NP) (n=287- 143 males and 144 females) was fed with frozen (-20ºC) colostrum (6-8 L during the first 12 hours of life) that was previously reheated up to 40ºC. They were also fed with refrigerated (4ºC) raw milk from the bulk tank that was also reheated up to 40ºC (1.8 L every 12 hours). Pasteurized group (P) (n=300- 150 males and 150 females) was also fed with colostrum and milk but both were pasteurized prior to freezing. Blood samples were drawn from all calves to obtain serum at 2-5 days of life. Serum total protein (STP, g/dL) was determined using a commercially available refractometer. Colostrum and milk underwent routine bacteriological analysis to determine total plate counts (TPC; cfu/mL) and total coliform counts (TCC; cfu/mL). All the calves underwent clinical examination every 24 hours during the first 21 days of life. Every day, calves were clinically diagnosed either as being healthy or suffering from respiratory disease, neonatal calf diarrhea or suffering other diseases. On-farm heat treatment for colostrum and milk reduces between 1 and 2 $\log_{10}$ of TPC and TCC. Pasteurization of colostrum and milk significantly decreased the morbidity and mortality (5.2 and 2.8%) in comparison with calves receiving non-pasteurized colostrum and milk (15.0 and 6.5%), respectively during the first 21 days of life even in animals receiving appropriate colostrum ingestion.

Key words: On-farm heat treatment, colostrum, milk, health status.

INTRODUCTION

Passive immunity is the only source of early immunity for calves due to the inability of bovine placenta to transmit maternal immunoglobulins to the foetus (Richter and Götze, 1993; Baintner, 2007). Thus, it is compulsory to acquire these natural defenses by colostrum ingestion. In this sense, the immune status of calves during pre-weaning period depends directly on the quality and quantity of colostrum ingested during the first hours of life (Heinrichs and Elizondo-Salazar, 2008).The gold standard method accepted to evaluate passive transfer is a direct measurement of IgG concentration through radial immunodiffusion (RID). Failure of passive transfer (FPT) appears if the calf serum IgG concentration is less than 10 mg/mL, when sampled between 24 and 48 hours of age (Weaver et al., 2000; Godden, 2008), because a value below 10 mg/mL is a risk factor for developing diseases during the neonatal period (Godden, 2008). Unfortunately, RID is not useful as an on-farm method. Evaluation of serum total proteins (STP) by refractometry is widely used by veterinarians and producers to evaluate adequate passive transfer of immunoglobulin in calves (Tyler et al., 1996) because the correlation between STP and IgG in blood is very good the first days of life, considering that IgG is the most abundant protein ingested through colostrum (Calloway et al., 2002)). Thus, a value of STP between 5.0 and 6.0 g/dL is considered to prevent FTP after colostrum intake (Donovan et al.,1998; Windeyer et al., 2014). Moreover, a cut-off for STP of 5.2 g/dL is accepted to guarantee the equivalent threshold value of 10 mg/mL of IgG in calf serum (Tyler et al., 1996; Calloway et al., 2002; Windeyer et al., 2014). An appropriate colostrum ingestion program shall begin feeding the calf within 4 to 6 hours after birth and has to ensure at least 4 to 5 L of colostrum intake during the first 8 hours of life. This protocol allows achieving high blood levels of circulating maternal immunoglobulins in 48-hour-old calves until their immune system becomes

fully functional, at 3-6 weeks of age (Heinrichs and Elizondo-Salazar, 2008). In any case, FPT can occur and the incidence of respiratory or digestive disease may increase in these animals (Virtala et al., 1999; Godden et al., 2012; Pardon et al., 2015). As a consequence, cost for treatment of bovine respiratory disease (BRD) and neonatal calf diarrhea (NCD) and death rates due to both diseases may increase during the first 21 days of life (Meganck et al., 2014; Windeyer et al., 2014). Additionally, management of milk, buckets and sucklers, timing at feeding and quantity of milk are also important factors to assure a good health status of calves and good production parameters in the first 21 days of life (Wells et al., 1996). A good colostrum feeding protocol should also avoid its bacterial contamination (Meganck et al., 2014) through a strict hygiene management of buckets and sucklers because bacteria in colostrum may interfere with passive absorption of colostral antibodies into the calf's circulation (James et al., 1981). Moreover, it has been described that colostrum and milk can contain pathogens such as *Mycobacterium avium ssp. Paratuberculosis* (Streeter et al., 1995; Sweeney, 1996), *Mycoplasma spp.* (Butler et al., 2000; Stabel et al., 2004), *Escherichia coli*, *and Salmonella spp*. (Smith et al., 1989; Spier et al., 1991; Stabel et al., 2004).Fortunately, pasteurization is a good way to decrease the bacterial load in colostrum but heat treatment must avoid colostrum denaturalization and an increase in its viscosity. Previous studies observed that the conventional pasteurizing protocol for milk may alter colostrum characteristics (Meylan et al., 1996; Godden et al., 2003). It has been established that heating colostrum at 60ºC for 30 to 60 minutes can be a good treatment to maintain colostrum quality (McMartin et al., 2006;Heinrichs and Elizondo-Salazar, 2008) while significantly reducing important pathogens that can contaminate it such as *Listeria monocytogenes*, *E. coli* O157:H7, *Salmonella enteritidis*, and *M. avium ssp. paratuberculosis* (Stabel et al., 2004; Godden et al., 2006).

Animals fed with on-farm pasteurized colostrum and waste milk showed higher average daily gain, a lower prevalence of pneumonia and diarrhea and a lower mortality rate when compared to calves fed with non-pasteurized colostrum and waste milk (Jamaluddin et al., 1996; Godden et al,. 2005) .However, the experimental design used in these studies did not allow determining the effect of colostrum and milk pasteurization on health status and mortality of calves when high levels of colostrum ingestion are guaranteed.

The objective of this study was to evaluate if on-farm pasteurization of raw colostrum and bulk tank milk could improve the health status, reduce neonatal illness and decrease death rates during the first 21 days of life in calves receiving appropiate colostrum ingestion monitored through a refractometer.

MATERIAL AND METHODS

Animals and Farm

The study was carried out in a dairy housing with an average of 330 lactating Holstein cows and an average production of 11,100 kg of milk (3.6% Fat and 3.3% Protein) by cow. This farm was located in Lleida (northeast Spain). Cows were milked three times daily (at 4, 12 and 20 h). Milk from each cow was sampled and analyzed for milk quality (fat, protein, and lactose concentration) and somatic cell count by technicians of the Central Laboratory for Milk Recording (ALLIC, Catalonia) once a month. This farm is positive for Bovine herpesvirustype I (BoHV-1) and Bovine viral diarrhea virus (BVDV), and these viruses can play an important role as respiratory pathogens in calves. Cows and heifers were vaccinated against (BoHV-1), BVDV, Bovine respiratory

syncytial virus (BRSV) and influenza virus (Hiprabovis IBR Marker live and Hiprabovis BVD balance, Hipra, Spain) every 6 months. Vaccination was carried out by veterinarians and a single use needle was used per each cow. Additionally, the farm was under an official eradication program of BoHV-1 using gE deleted marked vaccine. Moreover, individual serology was carried out once a year to detect infected (gE-positive) animals. Control of BVDV is also very strict with vaccination and identification of persistently infected animals through PCR. Finally, bulk tank milk sampling was carried out every 4 months by PCR for BVDV and ELISA gE for BoHV-1 detection. Cows were moved three weeks before calving to a facility where parturitionin group occurred (5-10 cows). Newborn calves were removed from the pen immediately after calving to avoid suckling. First milking of colostrum was obtained between 30 to 90 minutes after calving. Calves were not fed with colostrum coming from their dam but they were fed frozen colostrum, whether heat treated or not, from a single cow, because mixing colostrum from different cows was not allowed. Colostrum bags were stored frozen an average of 6 days before calves received it. Only colostrum with a specific gravity ≥1065 was considered suitable for feeding calves (Fleenor and Stott, 1980).

A total of 587 female and male calves were randomly assigned into 2 groups considering ear tag number as allocating criteria (even and odd numbers) during 18 months. As inclusion criteria, animals had to be singletons, born from a normal parturition and with STP values ≥ 5.8 g/dL. This value was chosen taking into account previous studies. Although a cut-off for STP of 5.2 g/dL is accepted by some authors as good enough to guarantee the equivalent threshold value of 10 mg/mL of IgG in calf serum (Tyler et al., 1996; Calloway et al., 2002; Windeyer et al., 2014), others observed

slight improvements in mortality and BRD morbidity rates during the calf life with STP values of 6.0 g/dL (Donovan et al., 1997) and 5.7 g/dL, respectively (Windeyer et al., 2014). Thus, it was considered that an STP value of 5.8 g/dL can undoubtedly be associated with appropriate colostrum ingestion. Blood samples were drawn from all calves at 2-5 days of life and serum was obtained. Serum total protein (STP, g/dL) was determined using a commercially available refractometer (AtagoMaster-sur/NE, Atago U.S.A., Inc, Washington, U.S.A.) at room temperature (20ºC). If the value obtained was higher than 7.5 g/dL, haematocrit was checked to determine if dehydration could cause a misreading. Dehydrated animals were excluded from the study

Calves were housed individually, straw bedded and following calving time order. If an animal died, straw of the house was removed; walls and floor were cleaned with high pressure water and soap, disinfected with Kenocox® (CID Lines, Ark Animal Care Ltd, Newbridge, Ireland) and not used to allocate another newborn calf at least during a 15 days wash-out period.. Feeding of the animals as well as colostrum and milk management was always carried out by the same employee except for one day a week (Saturday) and two 15-day-periods of vacation, when these duties were carried out by the farmer. Colostrum and milk were always provided with nipple buckets, cleaned after every use and disinfected with chlorhexidine between feedings. The feeding protocol guaranteed an ingestion of 6-8 L of colostrum by the calf during the first 12 hours of life (Figure 1). Afterwards, calves were fed an average of 1.8 L (1.3-2.3 L) of milk from the bulk tank (pasteurized or not) every 12 hours (8:00 and 20:00).Calves assigned to the non-pasteurized group (NP) (even numbers with n=287- 143 males and 144 females) were fed with frozen (-20ºC) colostrum that was previously reheated up to 40ºC. They were then fed with refrigerated (4ºC) raw milk from the bulk tank that was also reheated up to 40ºC. Calves assigned to the pasteurized group (P) (odd numbers withn=300- 150

198 males and 150 females) were under a similar feeding protocol, but both colostrum and
199 milk had been pasteurized before freezing (Figure 2). All calves were allowed fresh
200 water and dry food *ad libitum* from day 2 onwards.
201
202 ***Colostrum and milk management***
203 Colostrum immunoglobulin concentration was measured on-farm at 21ºC with a
204 colostrometer (Biogenics, Oregon, USA) through specific gravity. Only colostrum with
205 density ≥ 1065 was used. Colostrum was stored in 4 L aluminium bags identified with
206 the cow number (Perfect Udder 4, DairyTech Inc., CO, USA). Colostrum of P group
207 was heat-treated at 60°C for 60 min using a commercial on-farm batch pasteurization
208 system (DT Silver, DairyTech Inc., CO, USA). The temperature was subsequently
209 lowered to 37ºC and bags were frozen at -20ºC. Afterwards, colostrum was heated to
210 40ºC before feeding the calves (Figure 2). Bulk tank milk for the P group was heat-
211 treated at 63ºC for 30 minutes using a commercial on-farm batch pasteurization system
212 (Urban MilkShuttle Pasteur, Urban Gmbh & Co, Wüsting, Germany) and then cooled
213 down to 40ºC before feeding the calves (Figure 2). Timing and temperatures used for
214 pasteurization were strictly followed according manufacturer's instructions and have
215 been previously used in other studies (Johnson et al.,2007). Paired colostrum and milk
216 samples, assigned to NP and P groups, were collected just before feeding the calves at
217 40ºC. They underwent routine bacteriological analysis to determine total plate counts
218 (TPC; cfu/mL) and total coliform counts (TCC; cfu/mL) as previously described by
219 Jayarao et al 2004.
220
221 ***Health monitoring of calves***
222 All calves underwent a clinical examination every 24 hours during the first 21 days of

223 life. For each animal, rectal temperature, breathing rate, consistency of the faeces,
224 attitude and navel were checked. Calves that showed clinical signs of disease were
225 allocated to 3 different categories. The first group included calves that were clinically
226 diagnosed with BRD. Calves were included in this category when at least two of the
227 following clinical signs were observed: inducible cough on tracheal massage, abnormal
228 sounds on respiratory tract auscultation, high rectal temperature (>39.5ºC) with
229 increased respiratory rate, serous nasal discharge, coughing and hyporexia or anorexia
230 (Virtala et al., 1999). The second group included calves with NCD, which was clinically
231 diagnosed using a score from 1 to 4 based on consistency of the faeces up to day 2 of
232 life (1, solid; 2, semi-solid; 3, liquid faeces; 4, watery). Animals with a score of 3 or 4
233 were considered to have NCD (Larson et al., 1977). The third group included calves
234 suffering from non-respiratory, non-digestive diseases, and they were assigned to this
235 group when any of the following clinical signs were observed: trauma, congenital
236 disease, hypothermia (<37.5ºC)(Berchtold, 2009), neurological signs, weakness,
237 reluctance to stand, difficulty in suckling or absence of suckling reflex, swollen joints,
238 cloudy eyes or omphalitis and fever (>39.5ºC), in the absence of respiratory or digestive
239 signs.
240
241 Clinical examination and establishment of curative treatment for sick animals was
242 carried out by the same veterinarians (the two authors of this research work) for both
243 experimental groups in order to avoid any bias. A necropsy was performed on dead
244 animals and causes of death were divided into three groups based on the main system
245 affected. Thus, a respiratory cause (BRD) was established if the lung was consolidated,
246 fibrin was noted on pleural surfaces or emphysema, atelectasis, or tracheitis was
247 observed. A digestive cause (NCD) was established if lesions (enteritis and fluid

content) were noted in the digestive tract. Cause of death was established as "other" if lesions were observed in systems other than respiratory or digestive.

Statistical Analysis

All statistical analyses were carried out using the SAS system V.9.1.3 (SAS institute Inc, Cary, NC, USA). For all analyses, the individual calf was used as the experimental unit. The significance level (P) was set at 0.05 with statistical tendencies reported when $P<0.10$. The treatment variable was the pasteurization process (NP and P) and the outcome variables were its effect on TPC and TPP in colostrum and on the health of the calves (disease and/or death).The variables included in the statistical analyses were classified as categorical (pasteurization status (NP/P), illness(Yes/No), death (Yes/No) and cause of death (BRD, NCD and other), or continuous (STP, TPC and TPP). Shapiro Wilk´s and Levene tests were used to evaluate the normality of the distribution of the continuous variables and the homogeneity of variances, respectively. To study the association between pasteurization status with the continuous non-normally distributed variables (TPC and TPP), the Wilcoxon test (with the U Mann-Whitney test to compare each pair of values) was used whereas an ANOVA test (with Student's T-test to compare each pair of values) was used to analyze the association between continuous normally distributed variables (STP). Contingency tables (Chi-square or Fischer exact tests) were used to assess the association between categorical variables. Finally, conditional logistic regression was used to estimate the univariate odds ratios (ORs) and 95 % confidence intervals (CIs) for death and illness with pasteurization. A multivariate logistic regression analysis was also carried out to decipher the effect of STP and pasteurization and its interaction on death and illness.

RESULTS

Descriptive statistics of the results

Average serum total protein was 7.27 and 7.34 g/dL for the NP and P group, respectively. In both cases, the lowest value observed was 5.8 g/dL and the maximum value observed was 9.2 and 9 g/dL for the NP and P group, respectively. For the NP and P group, the coefficient of variation was 7.8% (Table 1).Regarding bacterial load, TPC and TCC were always higher in colostrum than in milk (Table 2).

Morbidity was 15 and 5.2% for NP and P group, respectively and the prevalence of BRD, NCD and other causes was 40.9, 39.1 and 20% for the NP group and 43.3, 35.7 and 21% for the P group, respectively (Table 1).

Mortality was 6.5 and 2.8% for the NP and P group, respectively and the cause of death associated to BRD, NCD and other causes was 36.6, 43.9 and 19.5% for the NP group and 38.9, 33.3 and 27.8% for the P group, respectively (Table 1).

Effect of heat treatment on serum total protein and bacterial counts

Mean TPC and TCC were significantly lower in pasteurized colostrum and milk ($P<0.001$ in all cases) than in non-pasteurized ones (Table 2).No significant difference ($P=0.12$) was observed for STP between NP (7.27±0.57 g/dL) and P (7.34 ±0.54g/dL) groups (Table 1).

Effect of heat treatment of colostrum and milk on the health status of animals

Calves from NP group were at greater risk of illness (odds ratio (OR) = 3.8; 2.5-5.8) and death (OR=2.5; 1.39-4.3) than calves from P group during the first 21 days of life. Thus, pasteurization of colostrum with a density ≥1065 and milk significantly decreased the

morbidity (P<0.0001) and mortality (P<0.001) in comparison with calves receiving non-pasteurized colostrum and milk (Table 2). However, the distribution of the cause of illness or death was not significantly different (P>0.05) between NP and P groups during the first 21 days of life (Table 1). The proportion of diseased or dead calves were not significantly affected by STP neither in the NP (P>0.05) nor in the P group (P>0.05) in the range of values studied. Pasteurization was the only significant variable in a multivariate logistic regression analysis on death (P=0.002) and illness in calves (P<0.0001) without observing a significant interaction (P>0.05) with STP.

DISCUSSION

Colostrum with density ≥1065 and milk pasteurization improve health status and decrease mortality during the first 21 days of life in neonatal calves receiving appropriate colostrum ingestion (STP ≥ 5,8 g/dL).

Effect of heat treatment on serum total protein and bacterial counts

Measurement of STP by refractometer is a good descriptive marker to estimate the amount of immunoglobulins in neonatal calf serum with the goal of assessing the calf passive immunity transfer after colostrum ingestion (Tyler et al., 1996; Donovan et al.,1998). It has been described that STP values above 5.2g/dL are linked with an appropiate passive immunity transfer (Tyler et al., 1996; Calloway et al., 2002). Previous studies have concluded that animals fed with heat treated colostrum at 60ºC during 30-60 min had significantly greater STP when compared with calves fed with raw colostrum (Johnson et al., 2007; Gelsinger et al., 2014). Thus, colostrum pasteurization seems to be a good tool to increase the STP in calves. However, we did not detect significant differences in STP between NP and P calves in this study. In our

case, it must be taken into account that we are only including in the study a calf population fed with high quality colostrum (density≥1065) and showing high STP values (cut-off ≥5.8 g/dL). Thus, it seems that the pasteurization process is unable to increase the STP value in calves receiving appropriate colostrum ingestion as suggested by other authors in previous studies (Gelsinger et al., 2014). What this study did find, as expected, is that heat treatment of raw colostrum and milk can reduce the number of pathogens as previously described by other authors (Godden et al., 2006; McMartin et al., 2006, Johnson et al., 2007). The exposure of calves to pathogenic bacteria via colostrum and milk could cause diseases such as diarrhea or septicemia and facilitate the transmission of microorganisms such as *Mycoplasma spp*, *Mycobacterium avium subsp paratuberculosis*, fecal coliforms, and *Salmonella spp* (Jayarao et al., 2004; Godden 2008). Fortunately, feeding pasteurized colostrum and milk can be helpful to reduce fecal-oral transmission of pathogens (Godden et al., 2003;Jayarao et al., 2004; McGuirk and Collins, 2004).

Effect of heat treatment on the health status of animals

Although neonatal calf diseases are multifactorial (Lorenz et al, 2011; Al Mawly et al, 2015), a proper quantity of good quality colostrum and milk intake is essential to control them (Meganck et al, 2014). Other factors that could alter the prevalence of neonatal calf diseases are individual farm management practices and the preventive medicine programs applied to each farm. The current study, designed to test the potential benefit of pasteurization when calves are fed with high quality colostrum, was carried out within the same farm to reduce inter-farm variability (Mac Farlane et al, 2015). With this design, the internal validity of the current is sound because potential confounding factors have been greatly minimized. The study has been carried out in a

conventional Spanish dairy farm using Holstein cows and standard operation procedures. Even with these factors in mind, the external validity of this study could be limited mainly due to the fact that it has been carried out in a single farm. For this reason, it would be advisable to have similar studies carried out in other farms where there are differences in terms of management and facilities.

In our study, we have observed a similar reduction in terms of morbidity (9.8%) and mortality (3.7%) in calves fed with pasteurized colostrum and milk in comparison with animals receiving non-pasteurized colostrum and milk during the first 21 days of life.) As reported by Godden et al 2005, dairy calves fed pasteurized non-saleable milk have lower morbidity and mortality rates (11.6 and 2.2%, respectively) than do calves fed with milk replacer (32.1 and 12.1%, respectively). In the same study, calves fed with milk replacer had a higher risk for treatment during summer and winter months (OR=3.99) as well as death during winter (OR=29.81) than calves fed pasteurized non-saleable milk. Godden et al 2012 also reported a significant increase in risk for treatment of NCD in calves fed fresh colostrum (OR = 1.32) compared with calves fed heat treated colostrum at 60°C during 60 min (16.5%) over the pre-weaning period.

Finally, the cause of illness or death was not significantly different between NP and P groups during the first 21 days of life in our study but the difference in mortality between NP and P group was 8.3 and 10.6% for NCD and other causes, respectively (Table 1). Obviously, this difference is biologically relevant although a significant difference was not observed. A plausible explanation for this apparent contradiction could be that our study has a low statistical potency to detect differences in causes of illness or death and consequently increase the probability to have a type 2 error. Additional studies are necessary to tackle this question.

373 Curiously, the clinical improvement observed in our study cannot be explained by a
374 significant increase of STP in calves receiving pasteurized colostrum and milk versus
375 calves receiving raw feed. Our study paves the way to carry out additional studies to
376 decipher the mechanisms involved in the better health status in calves that received
377 pasteurized colostrum and milk. Several research lines can be explored such as whether
378 pasteurization can have a positive effect on the calf gut microbiota and/or on the
379 absorption of cellular immunity. It must be taken into account that the positive effect of
380 colostrum is relevant not only during the neonatal phase of life but also during the
381 whole productive life of animals. (Robison et al., 1988; DeNise et al., 1989; Wells et al.,
382 1996; Donovan et al., 1998; Weaver et al., 2000; Faber et al., 2005).
383

384 **CONCLUSIONS**

385 Pasteurization of colostrum (density ≥1065) and milk significantly improves calf health
386 status and reduces morbidity and mortality during the first three weeks of life even in
387 calves with STP values ≥5.8 g/dL.
388

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392

393 **REFERENCES**

394 Al Mawly, J., A. Grinberg, D. Prattley, J. Moffat, J. Marshall, and N. French.2015. Risk
395 factors for neonatal calf diarrhoea and enteropathogen shedding in New Zealand dairy
396 farms. Vet. J. 203: 155-160.
397 Bach, A. 2012.Nourishing and managing the dam and postnatal calf for optimal

398 lactation, reproduction and immunity. J. Anim. Sci. 2012.90:1835–1845.
399 Baintner, K. 2007. Transmission of antibodies from mother to young: Evolutionary
400 strategies in a proteolytic environenment. Vet. Immunol. Immunopathol. 117:153–161.
401 Berchtold, J. 2009. Bovine Neonatology. Treatment of Calf Diarrhea: Intravenous Fluid
402 Therapy.Vet Clin North Am Food Anim Pract. 25: 73-99
403 Butler, J. A., S. A. Sickles, C. J. Johanns, and R. F. Rosenbusch. 2000. Pasteurization of
404 discard *mycoplasma* mastitic milk used to feed calves: Thermal effects on various
405 *mycoplasma*. J. Dairy Sci. 83: 2285–2288.
406 Calloway, C. D., J. W. Tyler, R. K. Tessman, D. Hostetler, and J. Holle. 2002.
407 Comparison of refractometers and test endpoints inthe measurement of serum protein
408 concentration to assess passivetransfer status in calves. J. Am. Vet. Med. Assoc.
409 221:1605–1608.
410 DeNise SK, Robison JD, Stott GH, et al. Effects of passive immunity on subsequent
411 production in dairy heifers. J Dairy Sci 1989;72:552–4.
412 Donovan, G. A., I. R. Dohoo, D. M. Montgomery, and F. L. Bennett. 1998. Associations
413 between passive immunity and morbidity and mortality in dairy heifers in Florida, USA.
414 Prev. Vet. Med. 34:31–46.
415 Faber SN, Faber NE, McCauley TC, et al. Effects of colostrum ingestion on lactational
416 performance. The Professional Animal Scientist 2005;21:420–5.
417 Fleenor, W.A., and G. H. Stott. 1980. Hydrometer Test for Estimation of
418 Immunoglobulin Concentration in Bovine Colostrum. J. Dairy Sci. 63: 973–977.
419 Gelsinger, S. L., S. M. Gray, C. M. Jones, and A. J. Heinrichs. 2014. Heat treatment of
420 colostrum increases immunoglobulin G absorption efficiency in high-, medium-, and
421 low-quality colostrum. J. Dairy Sci. 97: 2355–2360.
422 Godden, S. M., S. Smith, J. M. Feirtag, L. R. Green, S. J. Wells, and J. P. Fetrow. 2003.

423 Effect of on-farm commercial batch pasteurization of colostrum on colostrum and
424 serum immunoglobulin concentrations in dairy calves. J. Dairy Sci. 86: 1503–1512.
425 Godden S. M., J. P. Fetrow, J. M. Feirtag, L. R. Green, and S. J. Wells. 2005. Economic
426 analysis of feeding pasteurized nonsaleable milk versus conventional milk replacer to
427 dairy calves. J. Am. Vet. Med. Assoc. 226(9):1547-54.
428 Godden, S. M., S. McMartin, J. Feirtag, R. Stabel, R. Bey, S. Goyal, L. Metzger, J.
429 Fetrow, S. J. Wells, and H. Chester-Jones. 2006. Heat treatment of bovine colostrum II:
430 Effects of heating duration on pathogen viability and immunoglobulin G. J. Dairy Sci.
431 89: 3476–3483.
432 Godden, S. 2008. Colostrum management for dairy calves. Vet. Clin. Food. Anim.
433 24:19–39.
434 Godden, S. M., D. J. Smolenski, M. Donahue, J. M. Oakes, R. Bey, S. Wells, S.
435 Sreevatsan, J. Stabel, and J. Fetrow. 2012. Heat-treated colostrum and reduced
436 morbidity in preweaned dairy calves: Results of a randomized trial and examination of
437 mechanisms of effectiveness. J. Dairy Sci. 95:4029–4040.
438 Heinrichs, J. A., and A. J. Elizondo-Salazar. 2008. Reducing failure of passive
439 immunoglobulin transfer in dairy calves. Rev Méd Vét 160: 8-9, 436-440.
440 Jamaluddin, A. A., D. W. Hird, M. C. Thurmond, and T. E. Carpenter. 1996. Effect of
441 preweaning feeding of pasteurized and nonpasteurized milk on postweaning weight gain
442 of heifer calves on a Californian dairy. Prevent. Vet. Med. 28: 91-99.
443 James, R. E., C. E. Polan, and K. A. Cummins.1981. Influence of administered
444 indigenous microorganisms on uptake of [iodine- 125] gamma-globulin in vivo by
445 intestinal segments of neonatal calves. J. Dairy Sci. 64: 52–61.
446 Jayarao, B. M., S. R. Pillai, A. A. Sawant, D. R. Wolfgang, and N. V. Hegde. 2004.
447 Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J. Dairy Sci.

448 87:3561–3573.

449 Johnson, J. L., S. M. Godden, T. Molitor, T. Ames, and D. Hagman. 2007. Effects of
450 feeding heat-treated colostrum on passive transfer of immune and nutritional parameters
451 in neonatal dairy calves. J. Dairy Sci. 90:5189–5198.

452 Larson L.L., F. G. Owen, J. L. Albright , R. D. Appleman, R. C. Lamb, and L. D.
453 Muller.1977.Guidelines Toward More Uniformity in Measuring and Reporting Calf
454 Experimental Data. J. Dairy Sci. 60- 6:989-991

455 Lorenz, I., B. Earley, J. Gilmore, I. Hogan, E. Kennedy, and S. J. More1. 2011. Calf
456 health from birth to weaning. III. Housing and management of calf pneumonia. I. Vet. J.
457 64:14.

458 MacFarlane, J. A., D. H. Grove-White, M. D. Royal, and R. F. Smith. 2015.
459 Identification and quantification of factors affecting neonatal immunological transfer in
460 dairy calves in the UK. Vet. Rec. 176: 625.

461 McGuirk SM, Collins M. 2004. Managing the production, storage and delivery of
462 colostrum. Vet Clin North Am Food Anim Pract. 20:593–603.

463 McMartin, S., S. Godden, L. Metzger, J. Feirtag, R. Bey, J. R. Stabel, S. Goyal, J.
464 Fetrow, S. J. Wells, and H. Chester-Jones. 2006. Heat-treatment of bovine colostrum I:
465 Effects of temperature on viscosity and immunoglobulin G. J. Dairy Sci. 89: 2110–
466 2118.

467 Meganck, V., G. Geert Hoflack, and G. Opsomer. 2014. Advances in prevention and
468 therapy of neonatal dairy calf diarrhoea: a systematical review with emphasis on
469 colostrum management and fluid therapy. Acta Vet. Scan. 56:75.

470 Meylan, M., D. M. Rings, W. P. Shulaw, J. J. Kowalski, S. Bech- Nielsen, and G. F.
471 Hoffsis. 1996. Survival of *Mycobacterium paratuberculosis* and preservation of
472 immunoglobulin G in bovine colostrum under experimental conditions simulating

473 pasteurization. Am. J. Vet. Res. 57:1580–1585.
474 Pardon B., J. Alliët, R. Booneb, S. Roelandt, B. Valgaerena, and P. Depreza. 2015.
475 Prediction of respiratory disease and diarrhea in veal calves based on immunoglobulin
476 levels and the serostatus for respiratory pathogens measured at arrival. Prev. Vet. Med.
477 120:169–176
478 Richter, J., and R. Götze. 1993. Tiergeburtshilfe. Pages 31-35.4th ed. Verlag Paul Parey,
479 Berlin und Hamburg, DE.
480 Robison JD, Stott GH, DeNise SK. Effects of passive immunity on growth and survival
481 in the dairy heifer. J Dairy Sci 1988;71:1283–7.
482 Smith, B. P., D. G. Oliver, P. Singh, G. Dilling, P. A. Martin, B. P. Ram, L. S. Jang, N.
483 Sharkov, J. S. Orsborn, and K. Jacket. 1989. Detection of *Salmonella dublin* mammary
484 gland infection in carrier cows, using an enzyme-linked immunosorbent assay for
485 antibody in milk or serum. Am. J. Vet. Res. 50: 1352–1360.
486 Spier, S. J., B. P. Smith, J. S. Cullor, H. J. Olander, L. D. Roden, and G. W. Dilling.
487 1991. Persistent experimental *Salmonella Dublin* intramammary infection in dairy
488 cows. J.Vet. Int. Med. 5: 341–350.
489 Stabel, J. R., S. Hurd, L. Calvente, R. F. Rosenbusch. 2004. Destruction of
490 *Mycobacterium paratuberculosis*, *Salmonella spp*., and *Mycoplasma spp*. in raw milk
491 by a commercial on-farm hightemperature, short-time pasteurizer. J. Dairy Sci.
492 87:2177–2183.
493 Streeter, R. N., G. F. Hoffsis, S. Bech-Nielsen, W. P. Shulaw, and D. M. Rings. 1995.
494 Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically
495 infected cows. Am. J. Vet. Res. 56: 1322–1324.
496 Sweeney, R. W. 1996. Transmission of paratuberculosis. Vet Clin North Am.F.An.Pr. 12:
497 305–312.

498 Tyler, J. W., D. D. Hancock, S. M. Parish, D. E. Rea, T. E. Besser, S. G. Sanders, and L.
499 K. Wilson. 1996. Evaluation of 3 assays for failure of passive transfer in calves. J. Vet.
500 Intern. Med. 10:304–307.
501 Virtala, M. K., Y. T. Gröhn, D. D. Mechor, and H. N. Erb. 1999. The effect of
502 maternally derived immunoglobulin G on the risk of respiratory disease in heifers
503 during the first 3 months of life. Prev. Vet. Med. 39: 25-37.
504 Weaver DM, Tyler JW, VanMetre DC, et al. Passive transfer of colostral
505 immunoglobulins in calves. J Vet Intern Med 2000;14:569–77.
506 Wells, S. J., D. A. Dargatz, and S. L. Ott. 1996. Factors associated with mortality to 21
507 days of life in dairy heifers in the United States. Prev. Vet. Med. 29: 9-19.
508 Windeyer MC, K.E. Leslie, S.M. Godden, D.C. Hodgins, K.D. Lissemore, and S.J.
509 LeBlanc. 2014. Factors associated with morbidity, mortality, and growth of dairy heifer
510 calves up to 3 months of age. Prev. Vet. Med. 113:231–240.
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Table 1. Effect of heat treatment of colostrum and milk on STP levels,mortality/morbidity rates and cause of illness and death in 21 day-old Holstein calves fed with raw (NP Group) and pasteurized (P Group) colostrum and milk.

	Treatment Group		
Parameter[1]	NP Group (n=287)	P Group (n=300)	P value
STP[1] (g/dL)	7.27 (5.8-9.2)[a]	7.34 (5.8-9.0)[a]	P=0.12
Mortality (%)	6.5[a]	2.8[b]	P<0.001
Distribution of mortality			
BRD[2] (%)	36.6 [a]	38.9 [a]	P=0.80
NCD[3] (%)	43.9 [a]	33.3 [a]	P=0.50
Other (%)	19.5 [a]	27.8[a]	P=0.55
Morbidity (%)	15.0[a]	5.2 [b]	P<0.0001
Distribution of morbidity			
BRD[2] (%)	40.9 [a]	43.3 [a]	P=0.82
NCD[3] (%)	39.1 [a]	35.7 [a]	P=0.70
Other (%)	20.0 [a]	21.0 [a]	P=0.90

[a b]Values within a row with different superscript letters are significantly different.
[1]STP= Serum Total Protein. Reported values reflect mean and range in parentheses.
[2] BRD= Bovine Respiratory Disease.
[3]NCD= Neonatal Calf Diarrhea.

Table 2. Colostrum and milk bacteriology parameters before and after heat treatment[1,2].

	Treatment Group		
Parameter[3]	Raw	Heat-treated	P value
Colostrum			
TPC (log_{10} CFU/mL)	7.43 ±0.42[a]	6.55 ± 0.41[b]	P<0.001
TCC (log_{10} CFU/mL)	6.15 ±0.34[a]	5.90 ±0.28[b]	P<0.001
Milk			
TPC (log_{10} CFU/mL)	6.60 ± 0.64[a]	4.80 ± 0.39[b]	P<0.001
TCC (log_{10} CFU/mL)	4.88 ± 1.10[a]	2.53 ± 0.33[b]	P<0.001

[a b]Values within a row with different superscript letters are significantly different.
[1]Reported values reflect mean±SE.
[2]Heat treatment for colostrum: 60ºC for 60 min; Heat treatment for milk: 63ºC for 30 min.
[3]TPC = total plate count; TCC = total coliform count.

551 **Figure Caption list**

552 Figure 1. Colostrum and milkfeeding protocol of the study farm.

553 Figure 2. Processing of colostrum and bulk tank milk for P (calves fed pasteurized

554 colostrum and milk) and NP (calves fed non pasteurized colostrum and milk) groups.

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Figure 1. Armengol

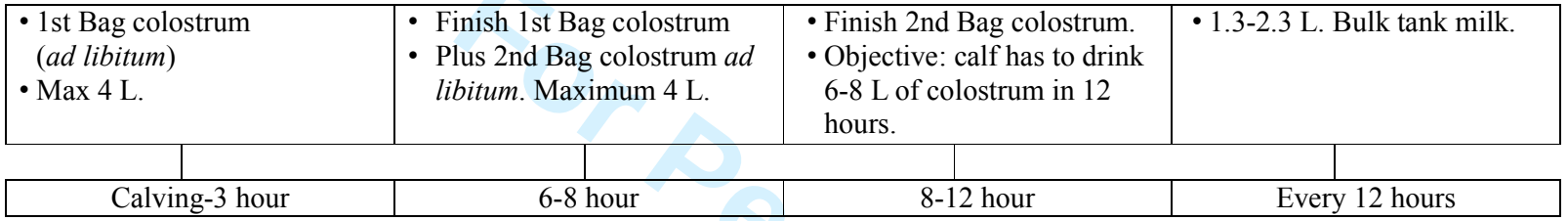

Figure 2. Armengol