NUEVOS CONOCIMIENTOS RESPECTO A LA ETIOLOGIA Y LA PREVENCION DE LAS ENFERMEDADES DE LOS TERNEROS

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Introduction

Efficient growth of pre-weaned dairy calves together with low incidence of disease, especially diarrhea and pneumonia, are prerequisites for their optimal performance after weaning and contribute in the profitability of a dairy enterprise; both are largely influenced by colostrum and pre-weaning liquid feed management.
Colostrum importance for the neonatal calf cannot be overemphasized; it provides immunoglobulins, nonspecific immune factors and nutrients (Weaver et al., 2000). On the other hand it may expose calves to many different pathogens (Johnson et al., 2007). Colostrum pasteurization will reduce pathogen numbers but it may also change its composition, particularly the concentration of IgG (Godden et al., 2006; Indyk et al., 2008). It has been reported that commercial on-farm, high temperature-short time pasteurization (HTST) (72°C for 15 seconds) of colostrum can cause a 30% reduction of IgG concentration and lead to undesirable viscosity (Stabel et al., 2004). Godden et al. (2006) showed a 10% reduction in colostrum IgG concentration using high quality colostrum (>75mg/ml) processed in a 30-L commercial batch pasteurization device at 60°C for 120 minutes (low temperature-long time (LTLT)). Batch pasteurization of colostrum at 60°C for 60 minutes was shown to be efficient in reducing *Salmonella* spp., *Escherichia coli*, *Mycoplasma* spp., *Listeria* and *Campylobacter* spp., while an acceptable viscosity was maintained (Gao et al., 2002; Jones et al., 2004; Elizondo-Salazar et al., 2010). It was also recently demonstrated that calves fed pasteurized colostrum (63ºC for 30 minutes) had a greater serum total protein than calves fed unheated colostrum (Godden et al., 2003). Johnson et al. (2007) reported that batch heat-treatment of colostrum at 60°C for 60 min resulted in reduced bacteria concentrations in colostrum and preserved the colostrum IgG concentration, while efficiency of absorption of IgG and calf serum IgG and total protein concentrations at 24 h of age were significantly greater for calves fed heat treated vs. raw colostrum.
Feeding calves with milk replacer can be costly and therefore the use of pasteurized, non-saleable milk (hospital milk) is considered to be an attractive alternative. Hospital milk includes milk from mastitic cows which may dramatically increase bacterial contamination. However, pasteurization of the hospital milk results in a lower bacteria count (Moore et al., 2009). Stabel et al. (2004) demonstrated that HTST pasteurization is effective in the destruction of *Mycobacterium paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in hospital milk. Additionally, Godden (2005) demonstrated that calves that received pasteurized hospital milk during the pre-weaning period out-performed (showing higher growth rates and lower morbidity and mortality rates) calves fed conventional milk replacer; an economic advantage of $0.69/calf per day for the calves fed pasteurized hospital milk was also shown. On the other hand, the pasteurization process might affect the nutritional value of the milk by heat-induce protein (e.g. lactoferrin) denaturation. Joslin et al. (2002) demonstrated that calves supplemented with lactoferrin had a decreased weaning age and improved average daily gain.
Ultraviolet (UV) light disinfection systems have been used for water and wastewater treatment in US and Europe (Lindenauer and Darby, 1994; Guo et al., 2009). UV light was shown to be effective against several pathogenic microorganisms that can be found in drinking water (Hijnen et al., 2006). UV light effectively inactivated *Staphylococcus aureus* in milk (Krishnamurthy et al., 2007) and *Listeria monocytogenes* in raw goat's milk (Matak et al., 2005). Pulsed UV light adequately controlled bacteria growth in bulk tanks with cold milk and cold spiked milk (Smith et al., 2002). A potential advantage of UV light treatment (UVLT) of hospital milk and colostrum is that it requires much less energy than the traditional heat treatment pasteurization protocols, since milk has to be heated only to feeding temperature (around 40ºC) and not up to 71ºC (HTST). Therefore, UVLT could be an alternative to traditional hospital milk or colostrum pasteurization protocols. To our knowledge, the use of UVLT for colostrum and hospital milk fed to dairy calves has not yet been evaluated.
Therefore, the objective of this study was to evaluate the effect of different colostrum (raw, LTLT pasteurization, and UVLT) and hospital milk (HTST pasteurization and UVLT) treatments and related risk factors on calf health, growth and survivability. The effect of treatment on colostrum and hospital milk bacteriology, and IgG and lactoferrin concentration was also assessed.

Study design and data collection

A randomized field trial study design was used. Newborn Holstein heifer calves were firstly randomly allocated into one of three colostrum treatments (raw, UVLT, and LTLT pasteurization) and subsequently block randomized (by colostrum treatment) into one of two hospital milk treatments HTST or UVLT. Colostrum was harvested twice daily from all multiparous fresh cows; primiparous cows' colostrum was not used during the trial period. The harvested colostrum was pooled and subsequently divided into three distinct portions. One third of the colostrum did not receive any treatment and was bottled in 4 litter jars and refrigerated (1.7 - 3.3ºC) until used. One third of the pooled colostrum was heat pasteurized using a LTLT batch pasteurizer (DT-10G Platinum, Dairy Tech Inc., Severance, Colorado); this colostrum was heated to 63ºC

for 60 minutes, rapidly cooled, bottled in 4 litter jars, and refrigerated (1.7 - 3.3°C) until used. The remaining colostrum was treated with UV light using the UV Pure system (GEA Farm Technologies, Naperville, Illinois) according to the manufacturer's recommendations. Different treatment colostrum bottles were number identified and kept in a refrigerator near the maternity pen. Maternity pen employees were trained by the research team and were monitored on a daily basis; they were advised to feed calves the colostrum treatment according to a previously created random table containing the randomly assigned treatments. Colostrum pooling, treatment, aliquoting, and storage were always done by a veterinarian who was a member of the research team.

Newborn calves were transported twice daily from the maternity pen to the calf barn where they would spend the next 60 days. Each newborn calf was randomly assigned into one of two hospital milk treatments within colostrum treatment group; HTST pasteurization or UVLT. Calves assigned to the HTST hospital milk treatment group received 6 liters twice daily of milk pasteurized immediately before each feeding by a GoodNature calf milk pasteurizer (Goodnature Products Inc., Orchard Park, New York). Calves allocated into the UVLT also received 6 liters of hospital milk twice daily treated by the UV Pure system (GEA Farm Technologies, Naperville, Illinois) according to the manufacturer's recommendations.

A blood sample was collected from all study calves on day 3 of life; blood was collected by jugular venipuncture using "red top" vacutainer (Becton Dickinson and Company, Franklin Lakes, NJ). Blood samples were refrigerated and transported to our laboratory in Ithaca, New York where serum was harvested after centrifugation at 20,000 × *g* for 20 minutes and stored in a -80 freezer. Once weekly, calf health was assessed visually by using objective criteria of appetite, fecal consistency, hydration status, respiratory effort, and attitude (Berge et al., 2009). The body weight of the calves was measured at birth and then weekly until weaning; a Waypig 15, 62" digital scale (Vittetoe inc., Keota, Iowa) was used.

Results

Descriptive statistics

A total of 893 calves were enrolled in this trial of which 290 calves received UVLT colostrum, 318 calves received LTLT pasteurized colostrum, and 285 received raw colostrum. Additionally, of the 893 calves enrolled on the study 458 calves were fed hospital milk pasteurized by HTST method whereas 435 calves were fed UVLT hospital milk.

A total of 418 calves were born from primiparous cows and 475 calves were born from multiparous cows, 8% of the calves were born with some assistance by the maternity pen workers, and the observed mortality for the study period was 4.5 %. Additionally, 89 % of the calves were born in the maternity pen and 11 % of the calves were born in the "close-up" free-stall barn.

Effect of colostrum treatment (UVLT, Raw, LTLT) on colostrum IgG and lactoferrin

A total of 281 polled colostrum samples were used in this analysis; 94 for LTLT, 91 for Raw, and 96 for UVLT colostrum. Both heat pasteurization and UVLT of colostrum significantly decreased the IgG concentration in the colostrum; 39.5, 52.4, and 69.1 for UVLT, LTLT, and raw colostrum, respectively ($P < 0.001$). Ultraviolet light treated colostrum IgG concentration was also significantly lower than heat pasteurized colostrum IgG concentration (Figure 1). Both UVLT and heat pasteurized colostrum had lower concentration of lactoferrin than raw colostrum ($P < 0.001$) (Figure 2).

Effect of hospital milk treatment (UVLT or HTST) on lactoferrin

Lactoferrin concentration was significantly decreased in HTST pasteurized hospital milk, while no significant difference was observed between UVLT hospital milk and hospital milk before any treatment (P < 0.01) (Figure 2).

Effect of colostrum treatment (UVLT, LTLT) on log-reduction of colony forming units

A total of 281 polled colostrum samples were used in this analysis; 94 for LTLT, 91 for Raw, and 96 for UVLT. Low temperature-long time pasteurization was more effective in decreasing CFU counts of SPC, *Escherichia coli*, and *Streptococcus* spp. than UVLT ($P < 0.001$) (Table 1). However, there was no difference between the CFU log-reduction for *Staphylococcus aureus* between LTLT and UVLT milk.

Effect of hospital milk treatment (UVLT or HTST) on log-reduction of colony forming units

A total of 270 samples of hospital milk were evaluated before and after treatments using standard microbiological techniques to estimate CFU counts of SPC, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus* spp. *Staphylococcus aureus* was relatively rare and was only detected in hospital milk during the months of March and April, and *Staphylococcus aureus* CFU log-reduction following UVLT or HTST treatment did not differ ($P = 0.88$). Additionally, the CFU log-reduction of *Escherichia coli* and *Streptococcus* spp. did not differ between UVLT and HTST treatment. High temperature short time pasteurization was more effective in decreasing CFU counts of SPC when compared to UVLT ($P < 0.01$) (Table 2).

Effect of colostrum treatment on calf serum IgG and total protein at 3 days of life

The variables retained in the general linear model were colostrum treatment, pre-treatment colostrum *Escherichia coli* CFU quartile, pre-treatment colostrum *Streptococcus* spp. CFU quartile, parity, and birth weight quartile. The serum IgG concentration at 3 days of life had a statistical tendency (P = 0.08) to be higher for calves that were fed LTLT-pasteurized colostrum compared to serum IgG concentration of calves that were fed UVLT and raw colostrum (Table 3). Calves that were born from primiparous cows also tended to have higher serum IgG concentration compared to calves born from multiparous cows ($P = 0.05$). Birth weight, *Escherichia coli* CFU, and *Streptococccus spp.* CFU quartiles were significantly

associated with serum IgG concentration (Table 3). Colostrum treatment had no effect on serum total protein concentration. The mean total protein concentration was 5.4, 5.7 and 5.8 (P = 0.13) for UVLT, LTLT treated and raw colostrum respectively.

Effect of colostrum and hospital milk treatments on calf survivability

The variables colostrum and hospital milk treatment were not significantly associated with survivability and forced into the Cox's Proportional Hazards model. The independent variables parity of the dam, dam diagnosed with metritis, calf serum IgG status, pre-treatment colostrum *Escherichia coli* CFU quartile, and birth weight quartile were significantly associated with survivability. Calves that were born from cows that subsequently developed metritis were at a 3.3 times higher hazard of death compared to calves born from cows that did not develop metritis (P = 0.003). Calves that were born from multiparous cows were at a 2.3 times higher hazard of death compared to calves born from primiparous cows (P = 0.05). Calves with birth weight from 22 to 37 kg were at a 5.4 times higher hazard of death compared to calves with birth weight from 38 to 41 kg (P = 0.02). Calves with serum IgG status <1,250 (mg/dl) were at a 2.7 times higher hazard of death compared to calves with serum IgG status >1,250 (mg/dl) (P = 0.01) (Table 4).

Effect of colostrum and hospital milk treatments on the odds of diarrhea incidence

The variables colostrum and hospital milk treatments were not significantly associated with the odds of diarrhea and were forced into the logistic regression model. Parity of the dam and birth weight quartile were significantly associated with the odds diarrhea; the largest quartile of birth weight calves were at the smallest risk of diarrhea with an adjusted diarrhea incidence of 35.7% compared to an adjusted incidence of 46% encountered for the smallest weight quartile calves (P = 0.01). Calves born from multiparous cows were at a 1.6 higher hazard of having diarrhea compared to calves born from primiparous cows (P = 0.001) (Table 5).

Effect of colostrum and hospital milk treatments on the odds of pneumonia incidence

The variables colostrum and hospital milk treatment were not significantly associated with the odds of pneumonia and were forced into the logistic regression model. Calves born from cows that subsequently developed metritis were at a 2.05 higher hazard of having pneumonia compared to calves born from cows that did not develop metritis (P = 0.02). Calves born from cows that subsequently developed retained placenta were at a 3.80 higher hazard of having pneumonia compared to calves born from cows that did not develop retained placenta (P = 0.001). Calves born from multiparous cows were at a 1.65 higher hazard of having pneumonia compared to calves born from primiparous cows (P = 0.001) (Table 6).

Effect of colostrum and hospital milk treatments on weekly body weight (Kg)

The variables colostrum and hospital milk treatment were not significantly associated with body weight and were forced into the mixed general linear model (Table 7). The variable birth weight was used as a covariate to adjust for potential imbalance within the different levels of the categorical independent variables retained in the model. The independent variables pre-treatment colostrum *Escherichia coli* and SPC CFU quartiles, pneumonia, calving location, calf serum IgG status, and parity were significantly associated with body weight and were kept in the model. Calves that were affected with pneumonia had an average body weight gain for the study period of 54.96 Kg compared to 58. 06 Kg for calves not affected with pneumonia (P < 0.001) (Table 7). The interactions of calf pneumonia, calf serum IgG status, *Escherichia coli* and SPC quartiles with weekly body weight measurement were significant and used in the model (Figures 3-6).

Discussion

Both UVLT and LTLT pasteurization reduced the number of CFU in colostrum. However, LTLT pasteurization was more efficient in reducing SPC, *Escherichia coli* and *Streptococcus* spp. CFU but not in reducing *Staphylococcus aureus* CFU. Pulsed UV light was already known to effectively inactivate *Staphylococcus aureus* in milk (Krishnamurthy et al., 2007). A significant difference in the log-reduction of total bacteria was also noticed between UVLT and HTST pasteurization of the hospital milk, with the latter being again more efficient. Colostrum has a thicker consistency when compared to hospital milk (Foley and Otterby, 1978) and liquid composition and consistency can change UV light penetration and efficiency (Lindenauer and Darby, 1994). This could be an explanation for the reduced efficiency of colostrum UVLT. Photoreactivation, i.e. the use of near-UV light and the enzyme photolyase to repair DNA lesions, is a potential bacterial defense against UV damage especially when the amount of UV light does not reach the disinfection dose (Tosa and Hirata, 1999; Hijnen et al., 2006).

Both UVLT and LTLT pasteurization caused a significant reduction of colostrum IgG concentration. As far as LTLT pasteurization is concerned this is consistent with Godden et al. (2003) study which showed that batch pasteurization at 63°C for 30 minutes significantly decreased IgG concentration. However, Elizondo-Salazar and Heinrichs (2009) using different holding time and colostrum volume and colostrum of known, high, quality, reported that batch pasteurization did not significantly decrease colostrum IgG concentration. The fact that Elizondo-Salazar and Heinrichs (2009), used only high quality colostrum (immunoglobulin concentration >50 g/L), which was not the case in the present study, could partially explain this discrepancy between the results.

Serum IgG concentration was higher (a difference that tended to be statistically significant, P = 0.08) for calves fed LTLT treated colostrum than calves fed raw or UVLT colostrum. The pool of colostrum can be highly contaminated with bacteria which may bind to enterocytes receptors that are also responsible for IgG absorption. Consequently, colostrum bacterial contamination can lead in decreased IgG absorption (James and Polan, 1978;

Staley and Bush, 1985). Elizondo-Salazar and Heinrichs (2009) recently reported that calves fed heat-treated colostrum (60°C for 30 min) had a higher IgG serum level, probably due to higher IgG absorption, than calves fed unheated colostrum. Ultraviolet light treatment was less efficient in reducing SPC, while it also had a greater negative effect on colostrum IgG concentration; this might explain why calves enrolled in that group tended to have lower IgG serum concentration.

Calves that were born from primiparous cows tended to have higher serum IgG concentration compared to calves born from multiparous cows ($P = 0.05$). This was not the case in a study conducted by Perino (1995) where age of the dam was not related with calves' plasma protein and IgG concentrations 24 hours after their birth. Additionally, birth weight in this study was found to be significantly related with serum IgG concentration. Calves in the third birth weight quartile (42 to 44 kg) had the higher serum IgG concentrations, while calves in the first birth weight quartile (22 to 37 kg) had the lowest serum IgG concentration. Calves in the first birth weight quartile were most likely immature at birth and this could be related with a decreased intestinal ability to absorb IgG. Birth weight was not related with serum IgG concentration in other studies though (Perino, 1995; Jones et al., 2004).

A negative effect of HTST pasteurization on hospital milk lactoferrin concentration is shown here. Pasteurization temperatures have already been reported to cause denaturation and conformational changes on bovine lactoferrin (Kulmyrzaev et al., 2005; Schwarcz et al., 2008). Lakritz et al., (2000) showed that calves receiving pasteurized colostrum had lower serum concentrations of lactoferrin compared to calves that received raw colostrum. On the other hand, UVLT did not seem to have any effect on hospital milk lactoferrin concentration.

In the present study, there was no effect of colostrum and hospital milk treatments on body weight gain, pneumonia, diarrhea or calves' survivability until weaning. Pneumonia was found to have the most significant impact on body weight gain. Calves that were affected with pneumonia were on average 3.1kg lighter at weaning than calves not affected with pneumonia. This finding is in agreement with Virtala et al. (1996) study which reported that verified and treated pneumonia was related with a decrease in average daily gain of 66 g. Virtala et al. (1996) also reported that failure of passive transfer of immunity led in a reduction of average daily gain by 48 g during the 1st month of the calves' life. We also report here that failure of passive transfer of immunity resulted in significantly lower body weight at weaning.

Colostrum and hospital milk treatments did not affect pneumonia incidence. However, some interesting observations were made regarding possible risk factors for calves' pneumonia. Specifically, calves born from multiparous cows, or from cows that subsequently developed metritis or retained placenta, were more likely to have pneumonia before weaning. Perez et al. (1990) also reported that calves born to primiparous cows were less prone to pneumonia, while Lundborg et al. (2003) reported that dam's morbidity during late pregnancy and retained placenta were associated with the relative risk of pneumonia in the calf; calves born to cows that had a disease 280–50 days before calving or cows with retained placenta at parturition had a higher relative risk of developing pneumonia than did calves born to healthy cows.

It is already well accepted that the transition period poses serious challenges for the cows' immune system; metritis and retained placenta are usually related with compromised immune functions (Kimura et al., 2002; Hammon et al., 2006). Severe negative energy balance –characterized by elevated blood concentration of non-esterified fatty acids and â-hydroxybutyrate, increased oxidative stress (due to increased production of oxygen ions, free radicals and lipid hydroperoxides) and depletion of important micronutrients may all be related with the transition cows' immunosuppression (Sordillo et al., 2009). Factors related with this immunosuppression could, through blood circulation also affect the fetus and subsequently the calf's immune functions and thus the greater the metabolic stress a cow undergoes during late gestation, the greater the susceptibility of her offspring to diseases like pneumonia.

Colostrum and hospital milk treatments did not affect diarrhea incidence either. However, dam's parity, assisted parturition, and calf's birth weight were identified as risk factors for calves' diarrhea during the pre-weaning period. Calves born to primiparous cows in the present study were found to have higher serum IgG concentration and this could partially explain the fact that they also had lower diarrhea incidence. Calves born to cows that required assistant during parturition were more susceptible to diarrhea during the pre-weaning period. Similarly, Lombard et al. (2007), reported that, the odds of heifer calves having a digestive event were increased for those calves born to dams that had a mild or severe dystocia. Low birth weight was associated with higher diarrhea incidence which is in agreement with results presented by Berge et al (2009). According to Berge et al. (2009) increased diarrhea incidence could be a result of low-weight calves being fed more milk in relation to body weight, resulting in greater fecal output than the heavier calves (Berge et al., 2009).

Survivability of the calves was also not affected by colostrum or hospital milk treatment. On the other hand, we show here that calf's serum IgG status, calf's birth weight and dam's metritis were affecting calf's survivability until weaning. Failure of passive transfer of immunity has already been reported to be an important risk factor for pre-weaned calves' mortality (Donovan et al., 1998; Berge et al., 2009). Calves born from cows that developed metritis were reported here to be more susceptible to pneumonia and this could be the reason why dam's metritis was also associated with calves' survivability.

Conclusion

Raw colostrum IgG concentration was significantly higher than LTLT and UVLT colostrum and IgG concentration was lowest for UVLT colostrum. Colostrum and hospital milk lactoferrin was also affected by treatment; however, UVLT of hospital milk did not significantly affect lactoferrin

concentration. Pasteurization of colostrum and hospital milk was more effective than UVLT in reducing standard aerobic bacteria counts. However, colostrum and hospital milk treatments were not associated with calves' survivability, diarrhea, pneumonia or body weight gain.

References

●Berge, A. C. B., T. E. Besser, D. A. Moore and W. M. Sischo. 2009. Evaluation of the effects of oral colostrum supplementation during the first fourteen days on the health and performance of preweaned calves. J. Dairy Sci. 92:286-295.

●Donovan, G. A., I. R. Dohoo, D. M. Montgomery and F. L. Bennett. 1998. Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. Prev. Vet. Med. 34:31-46.

●Elizondo-Salazar, J. A. and A. J. Heinrichs. 2009. Feeding heat-treated colostrum or unheated colostrum with two different bacterial concentrations to neonatal dairy calves. J. Dairy Sci. 92:4565-4571.

●Elizondo-Salazar, J. A., B. M. Jayarao and A. J. Heinrichs. 2010. Effect of heat treatment of bovine colostrum on bacterial counts, viscosity, and immunoglobulin G concentration. J. Dairy Sci. 93:961-967.

●Foley, J. A. and D. E. Otterby. 1978. Availability, storage, treatment, composition, and feeding value of surplus colostrum: A review. J. Dairy Sci. 61:1033-1060.

●Gao, A., L. Mutharia, S. Chen, K. Rahn and J. Odumeru. 2002. Effect of pasteurization on survival of mycobacterium paratuberculosis in milk. J. Dairy Sci. 85:3198-3205.

●Godden, S. M., S. Smith, J. M. Feirtag, L. R. Green, S. J. Wells and J. P. Fetrow. 2003. Effect of on-farm commercial batch pasteurization of colostrum on colostrum and serum immunoglobulin concentrations in dairy calves. J. Dairy Sci. 86:1503-1512.

●Godden, S. 2005. Economic analysis of feeding pasteurized nonsaleable milk versus conventional milk replacer to dairy calves. Journal of the American Veterinary Medical Association. - 1547.

●Godden, S., S. McMartin, J. Feirtag, J. Stabel, R. Bey, S. Goyal, L. Metzger, J. Fetrow, S. Wells and H. Chester-Jones. 2006. Heat-treatment of bovine colostrum. II: Effects of heating duration on pathogen viability and immunoglobulin G. J. Dairy Sci. 89:3476-3483.

●Guo, M., H. Hu, J. R. Bolton and M. G. El-Din. 2009. Comparison of low- and medium-pressure ultraviolet lamps: Photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants. Water Res. 43:815-821.

●Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet. Immunol. Immunopathol. 113:21-29.

●Hijnen, W. A. M., E. F. Beerendonk and G. J. Medema. 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan oocysts in water: A review. Water Res. 40:3-22.

●Indyk, H. E., J. W. Williams and H. A. Patel. 2008. Analysis of denaturation of bovine IgG by heat and high pressure using an optical biosensor. Int. Dairy J. 18:359-366.

●James, R. E. and C. E. Polan. 1978. Effect of orally administered duodenal fluid on serum proteins in neonatal calves. J. Dairy Sci. 61:1444-1449.

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

●Jones, C. M., R. E. James, J. D. Quigley III and M. L. McGilliard. 2004. Influence of pooled colostrum or colostrum replacement on IgG and evaluation of animal plasma in milk replacer. J. Dairy Sci. 87:1806-1814.

●Joslin, R. S., P. S. Erickson, H. M. Santoro, N. L. Whitehouse, C. G. Schwab and J. J. Rejman. 2002. Lactoferrin supplementation to dairy calves. J. Dairy Sci. 85:1237-1242.

●Kimura, K., J. P. Goff, M. E. Kehrli Jr. and T. A. Reinhardt. 2002. Decreased neutrophil function as a cause of retained placenta in dairy cattle. J. Dairy Sci. 85:544-550.
Krishnamurthy, K., A. Demirci and J. M. Irudayaraj. 2007. Inactivation of *Staphylococcus aureus* in milk using flow-through pulsed UV-light treatment system. J. Food Sci. 72:M233-M239.

●Kulmyrzaev, A.A., D. Levieux, and E. Dufour. 2005. Front-face fluorescence spectroscopy allows the characterization of mild heat treatments applied to milk. Relations with the denaturation of milk proteins. J. Agric. Food Chem. 53:502-507.

●Lakritz, J., J. W. Tyler, D. E. Hostetler, A. E. Marsh, D. M. Weaver, J. M. Holle, B. J. Steevens, and J. L. Denbigh. 2000. Effects of pasteurization of colostrum on subsequent serum lactoferrin concentration and neutrophil superoxide production in calves. Am. J. Vet. Res. 61: 1021-1025.

●Lindenauer, K. G. and J. L. Darby. 1994. Ultraviolet disinfection of wastewater: Effect of dose on subsequent photoreactivation. Water Res. 28:805-817.
Lombard, J. E., F. B. Garry, S. M. Tomlinson, and L. P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. J. Dairy Sci. 90:1751–1760.

●Lundborg, G. K., P. A. Oltenacu, D. O. Maizon, E. C. Svensson and P. G. A. Liberg. 2003. Dam-related effects on heart girth at birth, morbidity and growth rate from birth to 90 days of age in Swedish dairy calves. Prev. Vet. Med. 60:175-190.

●Matak, K. E., J. J. Churey, R. W. Worobo, S. S. Sumner, E. Hovingh, C. R. Hackney and M. D. Pierson. 2005. Efficacy of UV light for the reduction of *Listeria monocytogenes* in goat's milk. J. Food Prot. 68:2212-2216.

●Moore, D. A., J. Taylor, M. L. Hartman and W. M. Sischo. 2009. Quality assessments of waste milk at a calf ranch. J. Dairy Sci. 92:3503-3509.

●Perez, E., J. P. T. M. Noordhuizen, L. A. van Wuijkhuise and E. N. Stassen. 1990. Management factors related to calf morbidity and mortality rates. Livest. Prod. Sci. 25:79-93.

●Perino, L. J. 1995. Effects of various risk factors on plasma protein and serum immunoglobulin concentrations of calves at postpartum hours 10 and 24. Am. J. Vet. Res. 56:1144.

●Schwarcz W. S., L. Carnelocce1, J. L. Silva, A. C. Oliveira and R. B. Goncalves. 2008. Conformational changes in bovine lactoferrin induced by slow or fast temperature increases. Biol. Chem. 389:1137–1142.

●Smith W.L., M.C. Lagunas Solar and J.S. Cullori. 2002. Use of pulsed ultraviolet laser light for the cold pasteurization of bovine milk. J. Food Prot. 65:1480-1482. An. Health Res. Rev. 10: 53–63.

●Sordillo L. M., G. A. Contreras and S. L. Aitken. 2009. Metabolic factors affecting the inflammatory response of periparturient dairy cows.

●Stabel, J. R., S. Hurd, L. Calvente and R. F. Rosenbusch. 2004. Destruction of *Mycobacterium paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk by a commercial on-farm high-temperature, short-time pasteurizer. J. Dairy Sci. 87:2177-2183.

●Staley, T. E. and L. J. Bush. 1985. Receptor mechanisms of the neonatal intestine and their relationship to

immunoglobulin absorption and disease. J. Dairy Sci. 68:184-205.
●Tosa, K. and T. Hirata. 1999. Photoreactivation of enterohemorrhagic *Escherichia coli* following UV disinfection. Water Res. 33:361-366.
●Virtala, A. -. K., G. D. Mechor, Y. T. Gröhn and H. N. Erb. 1996. The effect of calfhood diseases on growth of female dairy calves during the first 3 months of life in New York State. J. Dairy Sci. 79:1040-1049.
●Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. Journal of Veterinary Internal Medicine. 14:569-577.

Table 1: Log-reduction of colony forming units following ultraviolet light treatment (UVLT) or low-temperature, long-time (LTLT) pasteurization of colostrum. Average log-reduction by study month and respective standard deviation are presented as well as the average log-reduction for the entire study period and respective standard deviation

	SPC[2]		*E. coli*[3]		*S. aureus*[4]		*Streptococcus* spp.[5]	
Month	UVLT[6]	LTLT[7]	UVLT	LTLT	UVLT	LTLT	UVLT	LTLT
February	2.7(0.1)	2.7 (0.1)						
March	2.2(2.5)	3.4 (2.2)	1.5 (2.0)	2.7 (1.3)	0.3 (1.1)	0.3 (1.1)	0.2 (1.4)	1.2 (2.9)
April	1.8(0.5)	3.5 (1.9)	1.3 (1.9)	2.9 (3.1)	0.3 (1.1)	0.3 (1.1)	2.9 (2.5)	3.0 (2.5)
May	1.3 (0.5)	3.1 (1.1)	1.7 (0.7)	5.7 (1.7)	0.2 (0.9)	0.2 (0.9)	2.1 (2.2)	2.3 (2.4)
June	1.6 (0.4)	3.3 (0.9)	2.3 (1.5)	5.3 (2.5)	0.2 (0.8)	0.2 (0.8)	2.7 (1.6)	3.6 (2.3)
July	1.7 (0.4)	3.2 (0.7)	2.7 (2.1)	4.6 (3.0)	0.5 (1.2)	0.5 (1.2)	3.3 (1.3)	3.5 (1.5)
August	1.7 (0.4)	3.7 (1.3)	2.7 (2.2)	4.4 (3.0)	0.5 (1.3)	0.5 (1.3)	3.4 (1.3)	3.9 (1.6)
September	1.5 (0.3)	4.7 (2.1)	3.0 (2.1)	5.3 (2.3)	1.3 (1.8)	1.3 (1.8)	2.9 (1.6)	4.5 (1.9)
Mean (S.D.)[1]	1.7[a] (1.0)	3.5[b] (1.5)	2.2[a] (1.9)	4.5[b] (2.7)	0.4[a] (1.2)	0.4[a] (1.2)	2.5[a] (1.9)	3.1[b] (2.3)

[a, b] = when *P* value is less than 0.05 the superscript is represented by different letters within the same column.
[1]Mean (S.D.) = mean post-pasteurization colony forming unit log-reduction and respective standard deviation.
[2]SPC = standard plate count agar technique for enumeration of aerobic bacteria. EMD, Chemicals Inc.
[3]*E. coli* = detection and enumeration of *Escherichia coli* using chromogenic medium – CHROMagar™ *E. coli*.
[4]*S. aureus* = detection and enumeration of *Staphylococcus aureus* using chromogenic medium – CHROMagar™ *Staph aureus*.
[5]*Streptococcus* spp. = detection and enumeration of group B *Streptococcus* spp. using chromogenic medium – CHROMagar™ *StrepB*.
[6]UVLT = ultraviolet light treatment.
[7]LTLT = low-temperature, long-time pasteurization.

Table 2: Log-reduction of colony forming units following ultraviolet light treatment (UVLT) or high temperature-short time (HTST) pasteurization of hospital milk. Average log-reduction by study month and respective standard deviations are presented as well as the average log-reduction for the entire study period and respective standard deviation

	SPC[2]		*E. coli*[3]		*S. aureus*[4]		*Streptococcus* spp.[5]	
Month	UVLT[6]	[7]HTST	UVLT	HTST	UVLT	HTST	UVLT	HTST
February	5.3 (0.5)	4.9 (0.6)	2.5 (1.6)	2.4 (1.3)				
March	5.4 (1.7)	5.8 (0.8)	2.5 (1.8)	2.6 (1.9)	0.2 (0.8)	0.2 (0.9)	2.3 (2.6)	2.3 (2.4)
April	3.8 (1.7)	4.8 (1.3)	2.4 (1.9)	2.3 (2.3)		0.2 (0.8)	3.0 (2.0)	3.4 (2.0)
May	3.4 (1.7)	5.0 (0.9)	2.1 (1.8)	1.4 (1.9)			2.5 (2.2)	2.9 (2.3)
June	3.0 (1.6)	4.8 (1.5)	1.2 (1.7)	0.9 (1.6)			1.7 (2.2)	2.3 (2.3)
July	2.9 (1.4)	5.2 (0.3)	0.9 (1.6)	0.0 (0.0)			1.5 (2.1)	1.5 (2.0)
August	3.0 (1.7)	5.2 (0.4)	1.3 (1.7)	0.0 (0.0)			1.3 (2.0)	1.9 (2.0)
September	2.0 (1.2)	5.3 (1.7)	1.3 (1.2)	0.6 (1.4)			1.5 (2.2)	2.5 (2.5)
October	2.2 (1.6)	5.6 (0.5)	1.9 (1.6)	1.4 (1.9)			2.1 (2.4)	1.6 (2.3)
November	1.8 (0.5)	5.6 (0.4)	1.7 (1.8)	1.5 (2.1)			2.6 (2.4)	2.9 (2.7)
Mean (S.D.)[1]	3.3[a] (1.8)	5.2[b] (1.1)	1.7[a] (1.7)	1.2[a] (1.8)	0.2[a] (0.8)	0.2[a] (0.8)	2.0[a] (2.2)	2.4[a] (2.3)

[a b] = when *P* value is less than 0.01 the superscript is represented by different letters within the same column
[1]Mean (S.D.) = mean post-pasteurization colony forming unit log-reduction and respective standard deviation
[2]SPC = standard plate count agar technique for enumeration of aerobic bacteria. EMD, Chemicals Inc.
[3]*E. coli* = detection and enumeration of *Escherichia coli* using chromogenic medium – CHROMagar™ *E. coli*
[4]*S. aureus* = detection and enumeration of *Staphylococcus aureus* using chromogenic medium – CHROMagar™ *Staph aureus*
[5]*Streptococcus* spp. = detection and enumeration of group B *Streptococcus* spp. using chromogenic medium – CHROMagar™ *StrepB*
[6]UVLT = ultraviolet light treatment
[7]HTST = high temperature short time pasteurization

Table 3: Least Square Means (LSM) and respective 95% confidence intervals of calf serum IgG (units) by the different categorical variables included in the analysis of variance. Blood was collected by jugular venipuncture in the third day of life and serum was harvested by centrifugation within 6h of collection

		LSM (95% C.I.) of serum IgG[1]	*P-value*
Colostrum treatment	Raw[2]	2,249.7 (2,060.3 – 2,438.8)	*0.08*
	LTLT[3]	2,310.2 (2,132.1 – 2,488.3)	
	UVLT[4]	2,039.3 (1,853.3 – 2,225.2)	
E. coli C.F.U/ml[5]	< 3,000	1,819.4 (1,602.6 – 2,036.3)	*0.001*
	3,000 – 60,000	2,343.6 (2,143.5 – 2,543.8)	
	60,001 – 10,000,000	2,265.9 (1,943.6 – 2,588.3)	
	> 10,000,000	2,369.7 (2,180.6 – 2,558.8)	
Streptococcus spp. C.F.U/ml[6]	< 3,000	2,246.4 (2,031.2 – 2,461.5)	*0.02*
	3,000 – 120,000	2,469.8 (2,247.1 – 2,692.5)	
	120,001 – 1,800,000	2,027.5 (1,821.0 – 2,234.1)	
	>1,800,000	2,055.0 (1,807.3 – 2,302.8)	
Parity of the Dam	primiparous	2,306.3 (2,146.6 – 2,466.0)	*0.05*
	multiparous	2,093.0 (1,941.1 – 2,245.0)	
Birth weight (Kg) quartiles	22 – 37	1,932.0 (1,719.3 – 2,145.8)	*0.001*
	38 – 41	2,331.8 (2,117.2 – 2,546.5)	
	42 – 44	2,389.9 (2,170.6 – 2,609.2)	
	45 - 59	2,145.0 (1,937.3 – 2,352.7)	

[1]LSM (95% C.I.) of serum IgG = Least Square Means (LSM) of calf serum IgG collected on the third day of life
[2]Raw = Raw colostrum
[3]LTLT = low-temperature, long-time pasteurization
[4]UVLT = ultraviolet light treatment
[5]*E. coli* C.F.U/ml = colony-forming units quartiles for *Escherichia coli* cultured before colostrum treatment/pasteurization
[6] *Streptococcus* spp. C.F.U/ml = colony-forming units quartiles for *Streptococcus sp.* cultured before colostrum treatment/pasteurization

Table 4: Cox's proportional hazards survival analysis evaluating the effect of several risk factors on calf survivability during the first 60 days of life (from birth until weaning)

		Mortality %	Hazard ratio	*P-value*
Colostrum treatment	LTLT[1]	3.8	1.2	*0.85*
	Raw[2]	3.2	1.3	
	UVLT[3]	2.8	*Reff.*	
Hospital milk treatment	UVLT	4.2	1.9	*0.10*
	HTST[4]	2.4	*Reff.*	
Parity of the dam	primiparous	1.9	*Reff.*	*0.05*
	multiparous	4.4	2.3	
Dam diagnosed with metritis	Yes	9.5	3.3	*0.003*
	No	2.4	*Reff.*	
Calf serum IgG status (mg/dl)[5]	< 1,250	5.4	2.7	*0.01*
	⩾ 1,250	2.0	*Reff.*	
E. coli C.F.U/ml[6]	< 3,000	2.3	1.1	*0.05*
	3,000 – 60,000	6.2	3.5	
	60,001 – 10,000,000	2.9	2.0	
	> 10,000,000	1.8	*Reff.*	
Birth weight (Kg) quartiles	22 – 37	6.5	5.4	*0.02*
	38 – 41	1.4	*Reff*	
	42 – 44	2.6	2.7	
	45 - 59	2.3	1.5	

[1]LTLT = low-temperature, long-time pasteurization of colostrum
[2]Raw = raw colostrum
[3]UVLT = ultraviolet light treatment
[4]HTST = high-temperature, short-time pasteurization of hospital milk
[5]Calf serum IgG status (mg/dl) = IgG < 1,250 mg/dl (failure of passive transfer of immunity) or IgG e" 1,250 mg/dl
[6]*E. coli* C.F.U/ml = colony-forming units quartiles for *Escherichia coli* cultured before colostrum treatment/pasteurization

Table 5: Effect of colostrum and hospital milk treatments on the odds of diarrhea analyzed by multivariable logistic regression

		Adjusted probability %	Adjusted odds ratio	*P-value*
Colostrum treatment	Raw[1]	42.8	1.4	*0.17*
	UVLT[2]	40.2	1.1	
	LTLT[3]	36.7	*Reff.*	
Hospital milk treatment	HTST[4]	38.6	1.0	*0.82*
	UVLT	37.1	*Reff.*	
Parturition assistance	Yes	46.3	1.6	*0.05*
	No	37.0	*Reff.*	
Parity of the Dam	primiparous	32.1	*Reff.*	*0.001*
	multiparous	43.2	1.6	
Birth weight (Kg) quartiles	22 – 37	46.0	1.8	*0.01*
	38 – 41	44.5	1.6	
	42 – 44	40.1	1.3	
	45 - 59	35.7	*Reff*	

[1]Raw = raw colostrum
[2]UVLT = ultraviolet light treatment
[3]LTLT = low-temperature, long-time pasteurization
[4]HTST = high-temperature, short-time pasteurization

Table 6: Effect of colostrum and hospital milk treatments on the odds of pneumonia analyzed by multivariable logistic regression

		Adjusted probability %	Adjusted odds ratio
Colostrum treatment	Raw[1]	12.3	1.55
	UVLT[2]	9.0	1.16
	LTLT[3]	8.6	*Reff.*
Hospital milk treatment	HTST[4]	9.2	*Reff.*
	UVLT	10.7	1.20
Dam diagnosed with metritis	Yes	20.9	2.05
	No	8.4	*Reff.*
Dam diagnosed with retained placenta	Yes	36.4	*3.80*
	No	8.5	*Reff.*
Parity of the Dam	primiparous	7.2	*Reff.*
	multiparous	12.3	1.65
E. coli C.F.U/ml[5]	< 3,000	13.1	2.30
	3,000 – 60,000	11.5	1.97
	60,001 – 10,000,000	11.8	1.98
	> 10,000,000	6.1	*Reff.*

[1]Raw = raw colostrum
[2]UVLT = ultraviolet light treatment.
[3]LTLT = low-temperature, long-time pasteurization
[4]HTST = high-temperature, short-time pasteurization
[5]*E. coli* C.F.U/ml = colony-forming units quartiles for *Escherichia coli* cultured before colostrum treatment/pasteurization.

Table 7: Least Square Means (LSM) with 95% confidence intervals of calf weight by the different categorical variables included in the analysis of variance. Weight was measured weekly from birth until weaning in a total of eight weeks

		LSM (95% C.I.) of calf weight	*P-value*
Colostrum treatment	Raw[1]	56.56 (55.88 – 57.24)	*0.86*
	LTLT[2]	56.41 (55.74 - 57.08)	
	UVLT[3]	56.56 (55.87 - 57.24)	
Hospital milk treatment	UVLT	56.58 (55.96 – 57.20)	*0.5*
	HTST[4]	56.43 (55.79 – 57.07)	
E. coli C.F.U/ml[5]	< 3,000	57.19 (56.39 – 58.00)	*0.003*
	3,000 – 60,000	57.10 (56.41 - 57.78)	
	60,001 – 10,000,000	56.31 (55.55 - 57.06)	
	> 10,000,000	55.44 (54.64 - 56.24)	
SPC C.F.U/ml[6]	< 2,800,000	57.19 (56.39 – 58.00)	*0.0002*
	2,800,001 – 14,000,000	57.10 (56.41 – 57.78)	
	14,000,001 – 22,000,000	56.31 (55.56 – 57.06)	
	>22,000,000	55.44 (54.64 – 56.24)	
Pneumonia	Yes	54.96 (54.00 – 55.92)	*<.0001*
	No	58.06 (57.63 – 58.49)	
Calving location	Maternity pen	57.05 (56.56 – 57.54)	*0.007*
	Close-up free-stall	55.97 (55.10 – 56.83)	
Calf serum IgG status (mg/dl)[7]	< 1,250	56.09 (55.45 – 56.72)	*0.05*
	⩾ 1,250	56.93 (56.30 – 57.56)	
Parity of the Dam	primiparous	56.18 (55.51 – 56.84)	*0.01*
	multiparous	56.84 (56.24 – 57.45)	
Birth weight			*<.0001*
Pneumonia*week		*Refer to figure 3*	*<.0001*
Calf serum IgG status*week		*Refer to figure 4*	*<.0001*
SPC*week		*Refer to figure 5*	*<.0001*
*Escherichia coli**week		*Refer to figure 6*	*<.0001*

[1]Raw = raw colostrum
[2]LTLT = low-temperature, long-time pasteurization
[3]UVLT = ultraviolet light treatment
[4]HTST = high-temperature, short-time pasteurization
[5]*E. coli* C.F.U/ml = colony-forming units quartiles for *Escherichia coli* cultured before any colostrum treatment process.
[6]SPC = standard plate count agar technique for enumeration of aerobic bacteria. EMD, Chemicals Inc.
[7]Calf serum IgG status (mg/dl) = IgG < 1,250 mg/dl (failure of passive transfer of immunity) or IgG e" 1,250 mg/dl

Figure 1: Average colostrum IgG and respective 95% confidence intervals by the different colostrum treatment groups; low temperature long time pasteurization (LTLT), raw colostrum (Raw), and ultraviolet light treatment (UVLT). The average colostrum IgG was 52.4 mg/ml (35 – 44), 69.1 mg/ml (64 – 74), and 39.5 mg/ml (35 – 44) for LTLT, Raw, and UVLT colostrum treatments, respectively (*P* – value < 0.001).

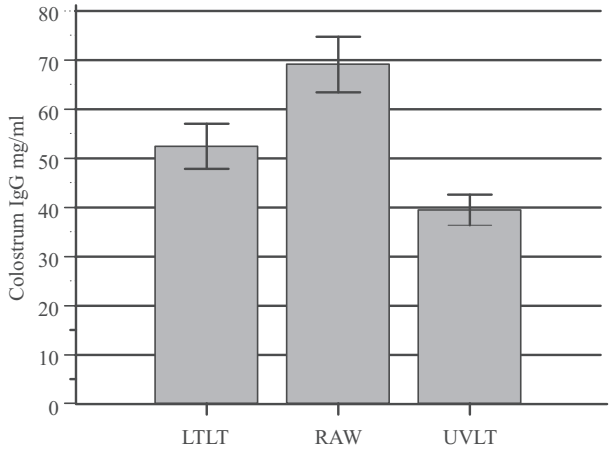

Figure 2: Effect of hospital milk and colostrum treatment on lactoferrin concentration (µg/ml). Results are presented as average lactoferrin and respective 95% confidence intervals.

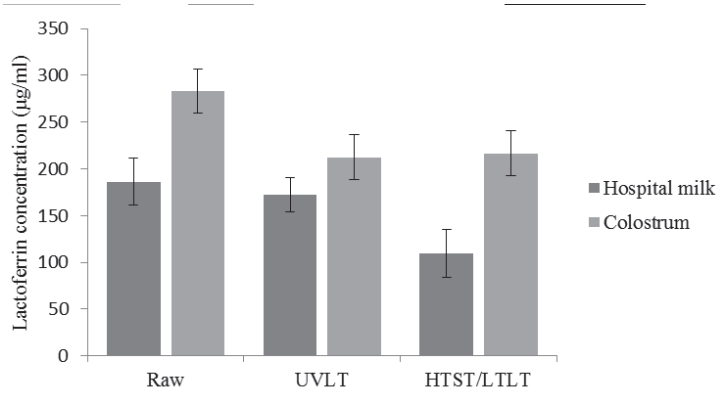

Figure 3. Weekly least square means of body weight (Kg) from the day of birth until weaning. The dark gray line represents the calves diagnosed with pneumonia and the light gray line represents the healthy calves. Standard errors of the means are depicted by the error bars.

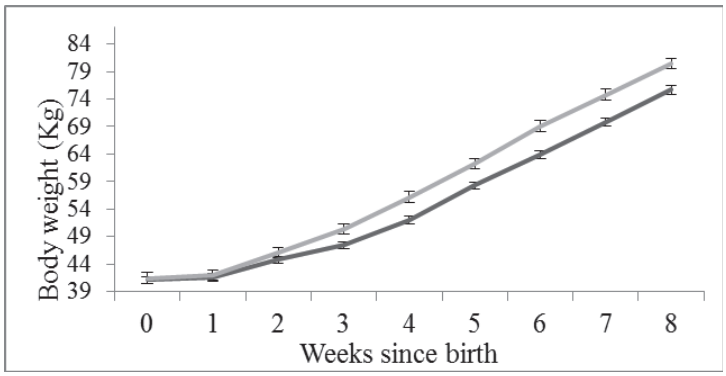

Figure 4. Weekly least square means of body weight (Kg) from the day of birth until weaning. The light grey line represents the calves with failure of passive transfer of immunity (serum IgG < 1,250 mg/dl) the dark gray line represents the calves without failure passive transfer. Standard errors of the means are depicted by the error bars.

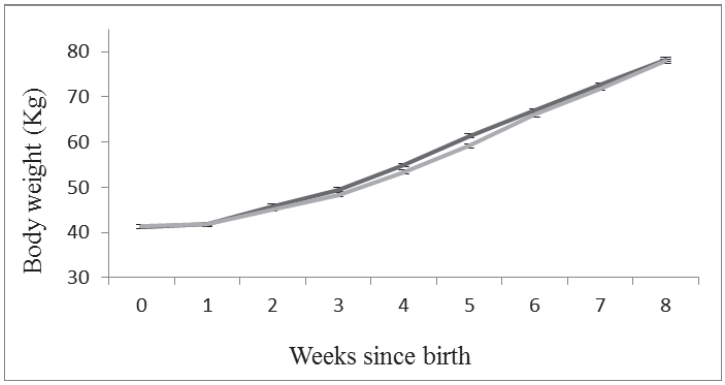

Figure 5. Weekly least square means of body weight (Kg) from the day of birth until weaning. The lines represent the quartiles of the total bacteria (CFU/ml) count of the hospital milk before pasteurization or ultraviolet light treatment (UVLT). The black full line represents the first quartile of the total bacteria count (below 2,800,000 CFU/ml), the dotted light gray line represents the second quartile of the total bacteria count (between 2,801,000 to 14,000,000 CFU/ml), the dashed dark gray line represents the third quartile of the total bacteria count (between 14,001,000 to 22,000,000 CFU/ml) and the full light gray line represents the fourth quartile of the total bacteria count (over 22,001,000 CFU/ml). Standard errors of the means are depicted by the error bars.

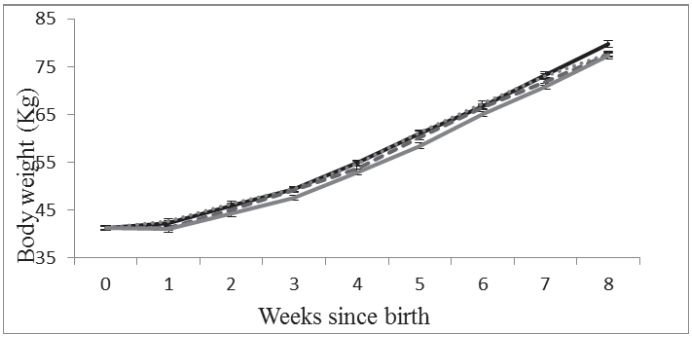

Figure 6. Weekly least square means of body weight (Kg) from the day of birth until weaning. The lines represent the quartiles of the *Escherichia coli* count (CFU/ml) count of the hospital milk before any treatment. The black full line represents the first quartile of the bacteria count (below 3,000 CFU/ml), the dotted light gray line represents the second quartile of the bacteria count (between 3,000 to 60,000 CFU/ml), the dashed gray line represents the third quartile of the bacteria count (between 60,001 to 10,000,000 CFU/ml) and the full light gray line represents the fourth quartile of the bacteria count (over 10,000,000 CFU/ml). Standard errors of the means are depicted by the error bars.