



Effect of feeding heat-treated and unheated colostrum on immunoglobulin G absorption, health and performance of neonatal Holstein dairy calves

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ABSTRACT. First colostrum is an important source of nutrients and immune factors which are necessary for calves in the first weeks of life. Despite these benefits, colostrum can also represent one of the earliest potential exposures of dairy calves to infectious agents which these patogens can act directly on growth and cause diseases such as scours or septicemia. With recent increased interest in pasteurized milk feeding systems, producers have been curious to learn if there may also be benefits from feeding pasteurized colostrum. This study was realized to determine the effects of feeding heat-treated colostrum or unheated colostrum on passive transfer of immunity, immunoglobulin G (IgG) concentration, total plate count, health and performance of neonatal dairy calves. First-milking colostrum was collected from Holstein cows and frozen at -20°C to accumulate a large batch. Pooled batches of colostrum were mixed and divided equally: One half was fed unheated colostrum; whereas the other half was fed after heat treatment at 60°C for 30 min. Forty newborn male Holstein dairy calves were fed either unheated (n = 20) and heat-treated colostrum (n = 20), 10% of their birth weight. Calves received 4 L within 1 to 2h after birth and residuals was fed 6h after birth. Serum samples collected from calves and were assayed for serum total protein (STP) and IgG. Feed intake recorded weekly and body weight and skeletal growth measures recorded at d 3 and d 63 (weaning). Every day, calves clinically diagnosed either as being healthy or suffering from respiratory disease and neonatal calf diarrhea. Heat-treated colostrum resulted in lower colostrum bacterial concentration (2.01 vs. 3.96 cfu mL⁻¹). Calves fed heat treated colostrum had greater STP in 24, 72h and 23d, IgG concentrations at 24 and 72h plus unheated colostrum. Also weaning weight and average daily gain were greater in calves feed heated colostrum. There were no differences in starter intake and feed efficiency between two groups. Calves fed heat-treated colostrum had lower fecal scores, diarrhea and pneumonia incidence. There were not differences in skeletal growth measurements except body barrel. These results shows that feeding heated colostrum can provide better growth and health in neonatal calves.

Keywords: immunoglobulin G; colostrum; health; serum total protein; weaning.

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Introduction

Calves are agammaglobulinemic at birth because syndesmochorial structure of placenta so depend on ingestion and absorption of colostral immunoglobulins, especially immunoglobulin G (IgG) across the intestinal epithelium during the first 24h of life to establish a protective serum IgG concentration (Elizondo-Salazar & Heinrichs, 2008). Successful passive transfer of IgG in a calf is defined as having a serum IgG concentration ≥10 mg ML⁻¹ at 24 to 48h of age and is associated with such benefits as decreased risk for morbidity and mortality in the preweaning period and improved improved milk production later in life (DeNise, Robison, Stott, & Armstrong, 1989; Donovan, Dohoo, Montgomery, & Bennett, 1998). Despite aforementioned benefits, colostrum can be a potential early source of exposure to microbial pathogens. Contamination of colostrum with pathogen may result to the multiple sources, including secretion from mammary gland, during milking, or proliferation during storage feeding or storage (Stewart et al., 2005). In any case, failure of passive transfer can occur and the incidence of respiratory or digestive disease may increase in these animals (Godden et al., 2006; Pardon et al., 2015).

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Recent researches on heat treatment of colostrum have shown great advantage in reducing number of bacteria (Donahue et al., 2012; Godden et al., 2006; Johnson, Godden, Molitor, Ames, & Hagman, 2007) and it has been known that feeding heat-treated colostrum may increase IgG absorption and as result increase serum IgG concentration (Elizondo-Salazar & Heinrichs, 2009a; Johnson et al., 2007). This mechanism is unknown, but Johnson et al. (2007) hypothesized that because antibodies in colostrum may bind pathogens present in gut before absorption, by reducing the contamination in heat-treated colostrum, and consequently the number of pathogen in the gut, more antibodies are potentially free for absorption. Corley, Staley, Bush, and Jones (1977) reported microbes in colostrum might also act competitively by occupying binding sites on the apical plasma membrane of the epithelial cell. They observed microbial attachment, exfoliation of microvilli and intracellular penetration of ileal epithelial cells when E. coli O55 was administered to colostrum-deprived calves. Furthermore, some studies have reported that high concentrations of bacteria in colostrum may be associated with decreasing IgG absorption, thereby potentially contributing to failure of passive transfer (Elizondo-Salazar & Heinrichs, 2009a, 2009b). National US statistics estimate that the proportions of preweaned calves with failure of passive transfer, treated for scours or other digestive problems, and treated for respiratory disease are 19.2, 17.9, and 11.4%, respectively (USDA-APHIS, 2007). 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 d of life (Meganck, Hoflack, & Opsomer, 2014; Windeyer et al., 2014). So, a good colostrum feeding protocol should also avoid bacterial contamination (Meganck et al., 2014).

Heat treatment may be one approach to reduce microbial contamination in colostrum. The history of developing a technique to heat-treat colostrum has been reviewed elsewhere (Donahue et al., 2012). Ragsdale and Brody (1923) reported that colostrum can be safely heated at 60°C for up to 3h without denaturing Ig or creating an undesirable, heat-coagulated product while inactivating tuberculosis organisms. The recent introduction of commercial on-farm pasteurizers has since created interest in feeding heat-treated colostrum to dairy calves. Experiments to pasteurize colostrum using traditional Pasteurized Milk Ordinance pasteurization temperatures, ranging from 62.5 to 73°C, yielded unacceptable IgG denaturation and changes in viscosity (Godden et al., 2003; Meylan et al., 1996; Tyler et al., 2000). In most situations, heating colostrum at 60°C for 30 or 60 min should be sufficient to maintain IgG concentrations and fluid characteristics while eliminating or significantly reducing important pathogens including Listeria monocytogenes, *E. coli* O157:H7, Salmonella enteritidis, and M. avium ssp. paratuberculosis (Godden et al., 2006; McMartin et al., 2006). There is lack of researches to indicate effects of heat-treated colostrum on health and growth parameters.

The first objective of this study was to compare the effects of heat-treated colostrum on total plate count and IgG absorption. The second objective of this study was to compare effects of heat-treated colostrum on health and diarrhea and pneumonia incidence.

Material and methods

Colostrum collection and heat treatment

First milking colostrum was collected from Holstein cows into 1.5 L plastic bottles and frozen immediately at -20°C to inhibit bacterial growth. At the start of the experiment, colostrum samples were thawed at 4°C and then thoroughly mixed for 10 min. Thawed colostrum divided into equal aliquots. One aliquot was left unheated whereas the other was heat-treated at 60°C for 30 min by on farm colostrum pasteurization system (V4, SHIRMAK GROUP). Fresh and heat treated colostrum were transferred to sanitized 1.5 L bottles and refrigerated at -20°C for later feeding to calves enrolled in the study.

Immediately before administration, an additional 10 mL aliquot of colostrum from heated and unheated colostrum were collected and frozen at -20°C for later bacterial culture to assess how much bacterial growth had occurred and colostrum IgG measurements. Colostrum samples were thawed to 4°C, mixed by vortex, and serially diluted 1:10 for 5 dilutions. Each dilution was plated on plate count agar for total plate count and plates were incubated for 48h at 37°C and the number of colonies recorded log10 (cfu mL⁻¹).

Calf enrollment

The protocol for animal experiments was approved by University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Calves were removed from the dam within 20 to 30 min of birth before suckling

could occur. To be eligible for enrollment, calves had to be singletons, weight from 40 to 45 kg, similar median calving ease score 3 (1 to 5 scale), and have similar median parity of dam. Forty male newborn Holstein calves were fed either unheated (n = 20) and heat-treated colostrum (n = 20), 10% of their birth weight. During first 24h, calves received colostrum 4 L, within 1 to 2h after birth and residual was fed 6h after birth. Systematic, rather than random, assignment to treatment groups was used to ensure that the age (duration of storage) of heat-treated and unheated colostrum was approximately equal at the time of feeding, because the storage period will allow for bacterial growth in colostrum. After assignment to a colostrum feeding group, the appropriate bottle of colostrum (heat-treated or unheated) was removed from refrigerator and warmed approximately to 39°C. During the days 2 and 3, calves were fed pasteurized milk 2 L at 9 AM and 2 L at 6 PM. Milk feeding program during days 4 to 63 (9 weeks) was 4, 4, 5, 7, 7, 7, 5, 2 and 1 litter per each day which except week 9, calves were fed just 1 L in the morning and half of milk was received in the morning and residual was fed in afternoon. The composition of the diets is presented in Table 1. Solid feed (starter) was offered ad libitum to all calves after d 3. During the research all the calves receive same amount of milk in the milk feeding program.

Table 1. Ingredient composition (% of DM) of calf starter diets and chemical compositions of starters (% of DM)

Ingredients	Diet
Ground corn	45
Ground barley	11
Soybean meal	34
Calcium carbonate	1.4
Salt	0.2
Sodium bicarbonate	1.5
Dicalcium phosphate	0.2
Milk replacement	5
Vitamins ¹	1
Minerals ²	0.7
Chemical composition	
DM (%)	90.07
ME (Mcal kg ⁻¹)	2.6
NEm (Mcal kg ⁻¹)	2.06
NEg (Mcal kg ⁻¹)	1.57
CP (%)	20.2
Ether extract (%)	3.00

 1 Minerals contained (mg kg-1): Ca (800), P (80), Mg (100), Cu (10), Mn (18), Zn (35.2), Co (0.12), I (0.28), and Se (0.17) on a DM basis. 2 Vitamins contained: vitamin A (1280000 IU kg-1) , vitamin D3 (72000 IU kg-1), vitamin E (4000 IU kg-1) and 1.25 g of butylated hydroxytoluene kg-1 as a synthetic antioxidant. on a DM basis.

Blood sample analyses for Ig and serum total protein measures in calves

Precolostrum (0h) and postcolostrum (24, 72h and 23, 43 and 63d) blood samples collected from jugular vein from all calves into serum vacutainer tubes, centrifuged and the serum separated from the clot within 1 h of collection. STP concentrations were determined using spectrophotometer (Pars Azmoon kit, Iran). Sera were then stored at -20°C until it could be analyzed for serum IgG concentration (mg mL $^{-1}$). Serum IgG concentration were determined by immunoprecipitation using single radial immunodiffusion (SRID; Sera vet, Spain). Serum samples (3 μ L) were applied to serial RID plates containing agarose gel with anti-bovine IgG. Plates were left undisturbed for 24h at room temperature after adding samples. Resulting ring diameters were measured with a monocular comparator, and IgG content of samples was calculated by regression analysis. A standard curve was generated with reference sera supplied by the manufacturer.

Growth factor and health analyses

Intake of starter was recorded weekly throughout the experiment. Overall average daily gains and feed efficiency (feed efficiency = kg of body weight gain kg $^{-1}$ of total DMI) were determined during 63 d of life. Body length (distance between the points of the shoulder and rump), heart girth (circumference of the chest), withers height (distance from base of the front feet to the withers), hip height (distance from the base of the rear feet to the hip bone), body barrel (circumference of the belly before feeding), and hip width (distance between the left and right trochanter major) measurements of calves were recorded at d 3 and d 63 (weaning). Fecal scoring was performed daily, as follows: 1 = normal; 2 = soft to loose; 3 = loose to watery; 4 = watery, mucous, and slightly bloody; and 5 = watery, mucous, and bloody.

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Statistical analysis

Repeated measure design using PROC MIXED of Statistical Analysis System software (SAS, 2004) was used for body weight, average daily gain, skeletal growth, feed intake, feed efficiency, STP and IgG data analysis. The statistical model used for analyses was yijklm = μ + ti + dij + pk + (t × p)ik + β (body weight) + eijklm, where yijklm = observations or dependent variables, μ = overall mean, ti = effect of treatment i, dij = animal random effect with mean 0 and variance that is equal to the covariance between repeated measurements within animals, pk = the effect of period k, (t × p)ik = the effect of interaction between treatment i and period k, β = regression coefficient of observations on birth weight as a covariate considered only for growth traits (starter intake, average daily gain, feed efficiency, body weight, and other body measurements) and eijklm = random residual effect. Health and fecal scores were tested by performing χ 2 tests using the frequency procedure of SAS. All the statistical tests were carried out at 5 percent probability level (p < 0.05).

Results and discussion

Effect of heat treatment on colostrum bacteria counts and immunoglobulin G concentration.

There was no difference in the mean colostral IgG concentration for heat-treated vs. unheated colostrum $(57.6 \text{ mg mL}^{-1} \text{ vs. } 60.6 \text{ mg mL})$ (p = 0.22) (Table 2). Early studies with colostrum pasteurization using the same times and temperatures recommended for milk found that heating denatured 12 to 30% of colostral IgG and increased viscosity (Meylan et al., 1996). Elizondo-Salazar, Jayarao, and Heinrichs (2010) reported when colostrum was heated at 60°C there was a reduction in IgG, especially in IgG1, even when colostrum was heated for just 30 min. The greatest reduction in IgG concentration was observed when colostrum was heated at 63°C. Previous laboratory studies have also reported success in reducing or eliminating pathogens when colostrum has been heat-treated (Godden et al., 2006; Johnson et al., 2007). In our study, heating temperature was monitored automatically and temperature fluctuations during heating were +0.1 to -0.1° C, so heating denatured 5% of the colostral IgG. Mean total plate count was lower (p < 0.0002) in heat-treated colostrum (2.01 cfu mL⁻¹) vs. unheated colostrum (3.96 cfu mL⁻¹). Calf birth weight, median parity of the dam and median calving ease score did not vary among treatments (Table 2). Researches have shown that colostral quality continues to increase with parity after the second calving, and older cows generally have the best quality colostrum, while dystocia would reduce colostrum IgG absorption (Donovan, Badinga, Collier, Wilcox, & Braun, 1986; Morrill et al., 2012). Therefore, to reduce the test error in this study, parity of the dam and calving ease score were considered among the two groups.

In our study heating colostrum at 60°C for 30 min was tend to reduction of colostrum pathogens. Despite the fact that Johnson et al. (2007) reported heat treated colostrum at 60°C for 60 min was tend to significant reduction in standard plate count and coliform count and it appears that 60°C for 30 min is adequate for achieving a goal of bacterial reduction in heat-treated colostrum. An additional 30 min at 60°C would probably eliminate more pathogenic bacteria; however, a different response in calf IgG concentration or apparent efficiency of absoption would not be expected (Elizondo-Salazar & Heinrichs, 2009b). Donahue et al. (2012) in multi-herd study confirms the results of these previous smaller, single-herd trials. Heat treatment of colostrum at 60°C for 60 min, as performed by farm staff on 6 commercial dairies, decreased total coliform count by 2.25 log10 and decreased total plate count by 2.49 log10, without decreasing overall colostral IgG centration.

Parameter	Treatment group		CEM	D cooler
	Raw ¹	Heat-Treated ²	- SEM	P < value
Colostrum characteristics				
IgG concentration (mg mL ⁻¹)	60.59	57.56	0.42	0.22
Total plate count (log10 cfu mL ⁻¹)	3.96 a	2.01^{b}	0.38	0.0002
Calf characteristics				
Mean birth weight (kg)	43 - (40 to 45)	43.15 - (40 to 45)	0.27	0.78
Median parity of dam	2 - (1 to 5)	2 – (1 to 5)	0.22	0.71
Median calving ease score	2 - (1 to 3)	1 – (1 to 3)	0.93	0.14

Table 2. Description of calf and colostrum parameter.

¹Raw = Unheated colostrum. ²Heat-treated = Heated colostrum at 60°C for 30 min. a,b Least squares means within a row with different superscript letters differ at p < 0.05 for treatment effect.

In theory, decreasing bacterial exposure to calves through colostrum while maintaining colostrum quality (IgG concentrations) should result in a healthier calf. Also, Donahue et al. (2012) reported there was a positive relationship between colostrum quality and magnitude of IgG loss when heat treating colostrum. Specifically, when expressed either as an absolute change in pre-versus post-heat-treated IgG concentrations, batches of high quality colostrum experienced a significantly greater magnitude of change in IgG, as compared with the referent category (50 – 59.9 mg of IgG mL⁻¹).

Effect of feeding heat-treated colostrum on serum total protein, serum immunoglobulin G concentrations and apparent efficiency of absorption.

According the results in Table 3, there was no difference in STP at 0h (precolostrum feeding) between two groups. However, STP at 24 and 72h and 23d were greater for calves fed heat-treated colostrum (6.80, 6.61 and 6.01) vs. calves fed unheated colostrum (6.31, 6.21 and 5.72). Also there was no difference in STP at 43 and 63 d between two groups. Serum IgG concentrations at birth (0h) were below detectable concentrations of the assay and did not produce ring. IgG concentrations at 24 and 72h of life were greater for calves fed heat-treated colostrum (15.37 and 12.95) vs. calves fed unheated colostrum (12.53, 11.37) (Table 3). A value of 50 g L-1 for STP at 24h of age is establishing the cutoff point for successful passive transfer (Donovan et al., 1998). Tyler et al. (1996) compared the performance of commonly used tests for passive transfer, demonstrated STP concentration of 52 g L-1 was equivalent to an IgG concentration of 10 g L⁻¹. Elizondo-Salazar and Heinrichs (2009a) reported calves received heat-treated colostrum showed greater STP concentrations at 8, 12, 16, and 20h than calves fed unheated colostrum and it was due to absorption of colostral IgG. The concentrations of STP and IgG in serum at 24h have been shown to be positively correlated (Elizondo-Salazar & Heinrichs, 2009a). In Our research STP and serum IgG concentration were significantly greater in calves fed heated colostrum and results are consistent with aforementioned researches. In a research on 1,071 new born calf demonstrated colostral total plate count and total coliform count had negative association with serum IgG (Godden et al., 2012). Microbes might also act competitively by occupying binding sites on the apical plasma membrane of the epithelial cell. Evidence of interaction of intestinal microflora with the absorptive surface of intestinal epithelial cells was demonstrated by Corley et al. (1977). Johnson et al. (2007) in a research hypothesized that because antibodies in colostrum can bind pathogens present in the gut before absorption can occur, by reducing the number of pathogens in heat-treated colostrum, and consequently the number of pathogens in the gut, more antibodies are potentially free for absorption.

Apparent efficiency of absoption (AEA, %) of IgG at 24 and 72h of life was also greater for calves fed heat-treated colostrum (23.94 and 20.19%) vs. calves fed unheated colostrum (19.29 and 17.50%) (Table 3). AEA (%) of IgG, a calculated measure that estimates what proportion of the total IgG mass fed is actually absorbed into the calf's circulation, was calculated using the equation described by Quigley III and Drewry (1998), assuming a plasma volume of 8.5% of birth weight. By increasing serum IgG concentration in calves fed heat treated colostrum and reduction in total plate count during this process, AEA in calves fed heated colostrum had been increased and it was due to rising binding sites for absorbing IgG in gut and it was approved by authors hypothesis.

Table 3. Serum total protein (g dL⁻¹), immunoglobulin G concentration (mg mL⁻¹) and apparent efficiency of absorption (%) in calves receiving unheated or heat-treated colostrum.

Parameter	Treatm	Treatment group		D 1
	Raw ¹	Heat-Treated ²	SEM	P- values
STP ³ (g dL ⁻¹)				
0h	4.16	4.19	0.93	0.83
24h	6.31 ^b	6.80^{a}	0.06	0.017
72h	6.21 ^b	6.61 ^a	0.06	0.03
23d	$5.72^{\rm b}$	6.01 ^a	0.07	0.04
43d	5.90	6.03	0.11	0.06
63d	6.57	6.68	0.16	0.80
IgG ⁴ (mg mL ⁻¹)				
0h	0	0	0	0
24h	12.53 ^b	15.37 ^a	0.93	0.01
72h	11.37 ^b	12.95ª	0.93	0.03
AEA ⁵ for IgG (%)				
24h	19.29 ^b	23.94^{a}	0.93	0.003
72h	$17.50^{\rm b}$	20.19^{a}	0.93	0.003

¹Raw = Unheated colostrum. ²Heat-treated = Heated colostrum at 60°C for 30 min. ³STP = Serum total protein. ⁴IgG = immunoglobulin G. ⁵AEA = Apparent efficiency of absorption. a,b Least squares means within a row with different superscript letters differ at p < 0.05 for treatment effect.

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Effect of feeding heat-treated colostrum on fecal score, diarrhea and pneumonia incidence

Calves fed unheated colostrums had greater fecal score vs. calves fed heat treated colostrum (1.61 vs. 1.22; p < 0.0001) (Table 4). The percent of diarrhea incidence during 63 d was greater significantly for calves received unheated colostrum vs. calves fed heated colostrum (12.16 vs. 3.8 %; p = 0.002). Rate of incidence to pneumonia during 63 day of lives between treatments shows that calves fed unheated colostrum had greater rate (18 incidences) than calves fed unheated colostrum (5 incidences) (χ 2 = 0.03) (Figure 1).

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Table 4 Fecal score and	i diarrhea incidence hetwe	en calves ted heat-trea	ted and unheated colostrum.

Parameter	Treatment group		CEM	D
	Raw ¹	Heat-Treated ²	– SEM	P- values
Fecal score	1.66ª	1.22 ^b	0.01	<0.0001
Diarrhea incidence (%)	12.16 ^a	3.8^{b}	1.4	0.002

¹Raw = Unheated colostrum. ² Heat-treated = Heated colostrum at 60°C for 30 min. a,b Least squares means within a row with different superscript letters differ at p < 0.05 for treatment effect.

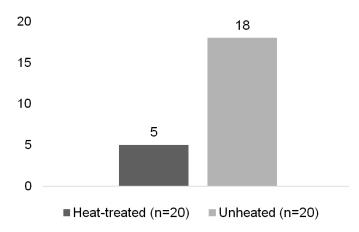


Figure 1. Rate of incidence to Pneumonia between Holstein dairy calves fed heat-treated (n=20) and unheated (n = 20) colostrum (χ^2 = 0.03).

Neonatal calf diseases are multifactorial (Al Mawly et al., 2015; Lorenz et al., 2011), 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. Godden et al. (2012) in a research on 1,071 calves confirmed unheated colostrum had a positive relationship with total plate count, total coliform count, and risk for any illness and scours. Several clinical studies had been demonstrated that colostrum or blood plasma immunoglobulins can provide local immunity against several pathogens associated with calf diarrheal disease such as *Escherichia coli* and corona virus (Acres, 1985; Crouch et al., 2000). Gelsinger, Jones, and Heinrichs (2015) reported calves that received heated and low-bacteria colostrum nearly tended to experience a fecal score ≤2 more often than calves that received unheated or low-bacteria colostrum. In the present study calves with higher serum IgG concentration had less diarrhea incidence and fecal score (Table 4).

Armengol and Fraile (2016) in a research on 287 calf stated a reduction in terms of morbidity (9.8%) and mortality (3.7%) in calves fed heated (60° C for 60 min) colostrum and milk in comparison with animals receiving unheated colostrum and milk during the first 21 d of life.

Effect of feeding heat-treated colostrum on performance of neonatal calves.

According to the results (Table 5), weaning weight (63d) was greater for calves were fed heated colostrum vs. unheated colostrum (90.94 vs. 87.10 kg; p = 0.01). Average daily gain was significantly greater for calves fed heat-treated colostrum vs. unheated colostrum (0.77 vs. 0.71 kg d⁻¹; p = 0.01) (Table 5). Whereas there were no differences in starter intake and feed efficiency between two groups (Table 5). Colditz (2004) reported calves with failure of passive transfer also had lesser weight gains likely because of decreased nutrient utilization and reduced feed intake associated with more disease in these calves compared with calves with adequate passive transfer. Berge, Besser, Moore, and Sischo (2009) demonstrated calves with greater serum IgG concentration had a significantly greater average daily gain to 28d of age. In an other

research, Yang, Zou, Wu, Li, and Cao (2015) shows calves that receive colostrum and According to aforementioned researches and ours research results, calves with heated colostrum by having more serum IgG concentration have more average daily gain and body weight versus calves fed unheated colostrum. In contrast, Elizondo-Salazar and Heinrichs (2009a) reported feeding heated colostrum in 60°C for 30 min had no differences among treatments in body weight and feed intake from birth until 8 weeks. In the present study calves birth weight, median ease score and median parity of dam were same between heated and unheated groups (Table 2). In addition, the average daily gain and weaning weight in the heat—treated clostrum were greater, while feed intake was not significantly different between the two groups. It seems that increasing the performance in heat-treated calves is due to an increase in the immune system and a reduction in the mortality (Tables 3, 4).

Table 5. weaning weight, total intake of starter, average daily gain, and feed efficiency Holstein calves fed heat-treated and unheated colostrum.

Parameter	Treatment group		CEM	D
	Raw ¹	Heat-Treated ²	– SEM	P- values
Weaning weight (kg)	87.10 ^b	90.94ª	1.01	0.01
Starter intake (kg)	40.56	45.70	0.27	0. 1
Average daily gain (kg d ⁻¹)	0.71 ^b	0.77^{a}	0.01	0.01
Feed efficiency	0.63	0.64	0.93	0.62

¹Raw = Unheated colostrum. ²Heat-treated = Heated colostrum at 60°C for 30 min. a,b Least squares means within a row with different superscript letters differ at p < 0.05 for treatment effect.

Effect of feeding heat-treated colostrum on body skeletal growth measurements of neonatal calves

Skeletal growth measurement results have shown in Table 6 and there was no significantly different in skeletal body measurements between two treatments except body barrel that was greater for calves fed heat-treated colostrums.

Overall all the calves fed heating colostrum had greater skeletal growth than other group. Elizondo-Salazar and Heinrichs (2009a) reported there was no different in colostrum minerals composition between heated and unheated colostrums. Also in this research there was no significant difference between treatments in starter intake (Table 3).

In our research as it was expected, there was no difference in skeletal growth except body barrel between calves received heated and unheated colostrum during 63 days (Table 6). This different seems to be because of bloat in calves that increased body barrel diameter in changing diets from milk to starter.

 $\textbf{Table 6}. \ \textbf{Skeletal growth measurements between calves fed heat-treated and unheated colostrum}$

Parameter	Treatment group		CEM	D
	Raw ¹	Heat-Treated ²	– SEM	P- values
Body length, cm	74.43	74.92	0.73	0.67
Hip height, cm	85.71	87.08	0.65	0.48
Hip width, cm	16.08	16.48	0.24	0.25
Heart girth, cm	96.53	96.80	0.99	0.85
Body barrel, cm	93.44 ^b	96.11 ^a	0.91	0.04
Withers height, cm	85.71	87.08	0.59	0.11

¹Raw = Unheated colostrum. ²Heat-treated = Heated colostrum at 60°C for 30 min. a,b Least squares means within a row with different superscript letters differ at p < 0.05 for treatment effect.

Conclusion

Feeding heat-treated colostrum at 60°C for 30 min reduced bacterial concentration while preserving the colostral IgG concentration. AEA of IgG and calf serum IgG was greater at 24 and 48h of age for calves fed heat-treated versus unheated. STP concentration at 24, 72h and 23d were greater for calves fed heated colostrum. The average daily gain and weaning weight in the heat-treated clostrum were greater, while feed intake was not significantly different between the two groups. It seems that increasing the performance in heat-treated calves is due to an increase in the immune system and a reduction in the mortality. Moreover, fecal score, percent of diarrhea incidence and Pneumonia incidence during 63 were greater for calves fed unheated colostrum. Body skeletal growth measurements of neonatal calves between treatments were similar. Future studies will also need to investigate the effects of heating colostrum in colostral GH and IGF1 hormones and intestinal development.

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References

- Acres, S. D. (1985). Enterotoxigenic *Escherichia coli* infections in newborn calves: a review. *Journal of Dairy Science*, *68*(1), 229-256. doi: 10.3168/jds.S0022-0302(85)80814-6
- Al Mawly, J., Grinberg, A., Prattley, D., Moffat, J., Marshall, J., & French, N. (2015). Risk factors for neonatal calf diarrhoea and enteropathogen shedding in New Zealand dairy farms. *The Veterinary Journal*, *203*(2), 155-160. doi: 10.1016/j.tvjl.2015.01.010
- Armengol, R., & Fraile, L. (2016). Colostrum and milk pasteurization improve health status and decrease mortality in neonatal calves receiving appropriate colostrum ingestion. *Journal of Dairy Science*, 99(6), 4718-4725. doi: 10.3168/jds.2015-10728
- Berge, A. C. B., Besser, T. E., Moore, D. A., & Sischo, W. M. (2009). Evaluation of the effects of oral colostrum supplementation during the first fourteen days on the health and performance of preweaned calves. *Journal of Dairy Science*, *92*(1), 286-295. doi: 10.3168/jds.2008-1433
- Colditz, I. G. (2004). Some mechanisms regulating nutrient utilisation in livestock during immune activation: an overview. *Australian Journal of Experimental Agriculture, 44*(5), 453-457. doi: 10.1071/EA02066
- Corley, L. D., Staley, T. E., Bush, L. J., & Jones, E. W. (1977). Influence of colostrum on transepithelial movement of *Escherichia coli* 055. *Journal of Dairy Science*, 60(9), 1416-1421. doi: 10.3168/jds.S0022-0302(77)84046-0
- Crouch, C. F., Oliver, S., Hearle, D. C., Buckley, A., Chapman, A. J., & Francis, M. J. (2000). Lactogenic immunity following vaccination of cattle with bovine coronavirus. *Vaccine*, *19*(2-3), 189-196. doi: 10.1016/S0264-410X(00)00177-8
- DeNise, S. K., Robison, J. D., Stott, G. H., & Armstrong, D. V. (1989). Effects of passive immunity on subsequent production in dairy heifers. *Journal of Dairy Science*, 72(2), 552-554. doi: 10.3168/jds.S0022-0302(89)79140-2
- Donahue, M., Godden, S. M., Bey, R., Wells, S., Oakes, J. M., Sreevatsan, S., ... Fetrow, J. (2012). Heat treatment of colostrum on commercial dairy farms decreases colostrum microbial counts while maintaining colostrum immunoglobulin G concentrations. *Journal of Dairy Science*, *95*(5), 2697-2702. doi: 10.3168/jds.2011-5220
- Donovan, G. A., Badinga, L., Collier, R. J., Wilcox, C. J., & Braun, R. K. (1986). Factors influencing passive transfer in dairy calves. *Journal of Dairy Science*, 69(3), 754-759. doi: 10.3168/jds.S0022-0302(86)80464-7.
- Donovan, G. A., Dohoo, I. R., Montgomery, D. M., & Bennett, F. L. (1998). Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Preventive Veterinary Medicine*, *34*(1), 31-46. doi: 10.1016/S0167-5877(97)00060-3
- Elizondo-Salazar, J. A., & Heinrichs, A. J. (2008). Heat treating bovine colostrum. *The Professional Animal Scientist*, *24*(6), 530-538. doi: 10.15232/S1080-7446(15)30902-5
- Elizondo-Salazar, J. A., & Heinrichs, A. J. (2009a). Feeding heat-treated colostrum or unheated colostrum with two different bacterial concentrations to neonatal dairy calves. *Journal of Dairy Science*, *92*(9), 4565-4571. doi: 10.3168/jds.2009-2188
- Elizondo-Salazar, J. A., & Heinrichs, A. J. (2009b). Feeding heat-treated colostrum to neonatal dairy heifers: Effects on growth characteristics and blood parameters. *Journal of Dairy Science*, *92*(7), 3265-3273. doi: /10.3168/jds.2008-1667
- Elizondo-Salazar, J. A., Jayarao, B. M., & Heinrichs, A. J. (2010). Effect of heat treatment of bovine colostrum on bacterial counts, viscosity, and immunoglobulin G concentration. *Journal of Dairy Science*, 93(3), 961-967. doi: 10.3168/jds.2009-2388

- Gelsinger, S. L., Jones, C. M., & Heinrichs, A. J. (2015). Effect of colostrum heat treatment and bacterial population on immunoglobulin G absorption and health of neonatal calves. *Journal of Dairy Science*, 98(7), 4640-4645. doi: 10.3168/jds.2014-8790
- Godden, S., McMartin, S., Feirtag, J., Stabel, J., Bey, R., Goyal, S., ... Chester-Jones, H. (2006). Heat-treatment of bovine colostrum. II: effects of heating duration on pathogen viability and immunoglobulin G. *Journal of Dairy Science*, 89(9), 3476-3483. doi: 10.3168/jds.S0022-0302(06)72386-4
- Godden, S. M., Smith, S., Feirtag, J. M., Green, L. R., Wells, S. J., & Fetrow, J. P. (2003). Effect of on-farm commercial batch pasteurization of colostrum on colostrum and serum immunoglobulin concentrations in dairy calves. *Journal of Dairy Science*, 86(4), 1503-1512. doi: 10.3168/jds.S0022-0302(03)73736-9
- Godden, S. M., Smolenski, D. J., Donahue, M., Oakes, J. M., Bey, R., Wells, S., ... Fetrow, J. (2012). Heattreated colostrum and reduced morbidity in preweaned dairy calves: Results of a randomized trial and examination of mechanisms of effectiveness. *Journal of Dairy Science*, *95*(7), 4029-4040. doi: 10.3168/jds.2011-5275
- Johnson, J. L., Godden, S. M., Molitor, T., Ames, T., & Hagman, D. (2007). Effects of feeding heat-treated colostrum on passive transfer of immune and nutritional parameters in neonatal dairy calves. *Journal of Dairy Science*, *90*(11), 5189-5198. doi: 10.3168/jds.2007-0219
- Lorenz, I., Earley, B., Gilmore, J., Hogan, I., Kennedy, E., & More, S. J. (2011). Calf health from birth to weaning. III. housing and management of calf pneumonia. *Irish Veterinary Journal*, *64*(14), 1-9. doi: /10.1186/2046-0481-64-14
- McMartin, S., Godden, S., Metzger, L., Feirtag, J., Bey, R., Stabel, J., ... Chester-Jones, H. (2006). Heat treatment of bovine colostrum. I: Effects of temperature on viscosity and immunoglobulin G level. *Journal of Dairy Science*, 89(6), 2110-2118. doi: 10.3168/jds.S0022-0302(06)72281-0
- Meganck, V., Hoflack, G., & Opsomer, G. (2014). Advances in prevention and therapy of neonatal dairy calf diarrhoea: a systematical review with emphasis on colostrum management and fluid therapy. *Acta Veterinaria Scandinavica*, *56*(75), 1-8. doi: 10.1186/s13028-014-0075-x
- Meylan, M., Rings, D. M., Shulaw, W. P., Kowalski, J. J., Bech-Nielsen, S., & Hoffsis, G. F. (1996). Survival of *Mycobacterium paratuberculosis* and preservation of immunoglobulin G in bovine colostrum under experimental conditions simulating pasteurization. *America Journal of Veterinary Research*, *57*(11), 1580-1585.
- Morrill, K. M., Conrad, E., Lago, A., Campbell, J., Quigley, J., & Tyler, H. (2012). Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. *Journal of Dairy Science*, *95*(7), 3997-4005. doi: 10.3168/jds.2011-5174
- Pardon, B., Alliët, J., Boone, R., Roelandt, S., Valgaeren, B., & Deprez, P. (2015). Prediction of respiratory disease and diarrhea in veal calves based on immunoglobulin levels and the serostatus for respiratory pathogens measured at arrival. *Preventive Veterinary Medicine, 120*(2), 169-176. doi: 10.1016/j.prevetmed.2015.04.009
- Quigley III, J. D., & Drewry, J. J. (1998). Nutrient and immunity transfer from cow to calf pre-and postcalving. *Journal of Dairy Science*, 81(10), 2779-2790. doi: 10.3168/jds.S0022-0302(98)75836-9.
- Ragsdale, A. C., & Brody, S. (1923). The colostrum problem and it solution. *Journal of Dairy Science*, *6*(2), 137-144. doi: 10.3168/jds.S0022-0302(23)94072-5
- Statistical Analysis System [SAS]. (2004). SAS/STAT User guide, Version 9.0. Cary, NC: SAS Institute Inc.
- Stewart, S., Godden, S., Bey, R., Rapnicki, P., Fetrow, J., Farnsworth, R., ... Mueller, K. (2005). Preventing bacterial contamination and proliferation during the harvest, storage, and feeding of fresh bovine colostrum. *Journal of Dairy Science*, *88*(7), 2571-2578. doi: 10.3168/jds.S0022-0302(05)72933-7
- Tyler, J. W., Hancock, D. D., Parish, S. M., Rea, D. E., Besser, T. E., Sanders, S. G., & Wilson, L. K. (1996). Evaluation of 3 assays for failure of passive transfer in calves. *Journal of Veterinary Internal Medicine*, 10(5), 304-307. doi: 10.1111/j.1939-1676.1996.tb02067.x
- Tyler, J. W., Lakritz, J., Hostetler, D. E., Douglas, V., Weaver, D. M., Steevens, B. J., ... Denbigh, J. (2000). Effect of pasteurization at 76 and 63 C on the absorption of colostral IgG in calves. *Journal of Dairy Research*, *67*(4), 619-623. doi: 10.1017/S0022029900004441

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Windeyer, M. C., Leslie, K. E., Godden, S. M., Hodgins, D. C., Lissemore, K. D., & LeBlanc, S. J. (2014). Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Preventive Veterinary Medicine*, *113*(2), 231-240. doi: 10.1016/j.prevetmed.2013.10.019

Yang, M., Zou, Y., Wu, Z. H., Li, S. L., & Cao, Z. J. (2015). Colostrum quality affects immune system establishment and intestinal development of neonatal calves. *Journal of Dairy Science*, *98*(10), 7153-7163. doi: 10.3168/jds.2014-9238