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The effect of various heat-treatment methods on colostrum quality, health and performance of dairy calves

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ABSTRACT. To investigate the effect of feeding heat-treated colostrum at different duration on the health and performance, 48 Holstein calves were enrolled randomly into four treatment groups before first feeding and consumed untreated colostrum (H0, n = 12), heat-treated colostrum at 60ºC for 30 min. (H30, n = 12), heat-treated colostrum at 60ºC for 60 min. (H60, n = 12) and heat-treated colostrum at 60ºC for 90 min. (H90, n = 12). Blood samples were collected for analyses of IgG and protein profile at 0, 6, and 24h of age. The colostrum sample from treated and untreated batches and feces sample from each calf also were taken. The results showed heat-treatment of colostrum at 60ºC for 60 (p = 0.03) and 90 min. (p = 0.01) reduced total bacterial count, while colostral IgG concentration maintained up to 60 min. Serum total protein (p = 0.02), IgG concentrations (p = 0.03), and apparent efficiency of IgG absorption (p = 0.02) were significantly greater at 6 and 24h in calves that were fed heat-treated colostrum (H90) compared to calves fed unheated colostrum (H0). General health status of calves that were received heat-treated colostrum was better and the prevalence of diarrhea-induced pathogens was lower than calves were fed unheated colostrum. In conclusion, the consumption of heat-treated colostrum had a positive effect on health, growth characteristics, and performance of calves during the suckling period.

Keywords: IgG; growth; health; Holstein calf; heat-treating; suckling period.

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Introduction

The newborn calf is born into a world laden with challenges it must overcome in order to grow and develop into a healthy, productive adult. The syndesmochorial placenta of the bovine forms a syncytium between the maternal endometrium and the fetal trophectoderm, separating the maternal and fetal blood supplies and preventing transmission of Ig in utero (Elizondo-Salazar & Heinriches, 2009; Constable, Hincheliff, Done, & Gruenberg, 2016; Godden, Lombard, & Woolums, 2019). Thereupon calf borne agammaglobulinemic and in the early days of life are highly dependent on colostrum consumption till growth, health, and future economic performance be guaranteed (Arguello, Castro, & Capote, 2005; Wheeler, Hodgkinson, Prosser, & Davis, 2007; Zakian et al., 2018). Immunoglobulins, which are highest in concentration at first milking, are critical to protect the newborn from the transmission of pathogenic infections in early life (Godden, 2008). Colostrum also contains a high concentration of nutrients including protein (much of which consists of the immunoglobulins), lipids, carbohydrates, minerals, and vitamins. Nearly all components of colostrum are present at higher concentrations compared to those measured in mature milk, with the main exception being lactose (Blum & Hammond, 2000). Inadequate or improper colostrum feeding and management cause a significant portion of the calf morbidity and mortality on dairy farms. The importance of consumption of high-quality colostrum with low microbial pollution in the critical first 24h of life is well documented (Staley & Bush, 1980; Godden 2008; Zakian et al., 2018). In addition to reduced risk for preweaning morbidity and mortality, additional long-term benefits associated with successful passive transfer include reduced mortality in the postweaning period, improved rate of weight gain, reduced age at first calving, improved first and second lactation milk production, and reduced tendency for culling during the first lactation (Wells, Dargatz, & Ott, 1996; Faber, Faber, McCauley, & Ax, 2005).

Although it is an important source of nutrients and immune factors, colostrum can also represent one of the earliest potential exposures of dairy calves to infectious agents, such as *Mycobacterium avium subsp paratuberculosis* (Streeter, Hoffsis, Bech-Nielson, Shulaw, & Rings, 1995; Stabel & Goff, 2004), *Salmonella* spp. (Houser, Donaldson, Kehoe, Heinrichs, & Jayarao, 2008), *Escherichia coli* (Farber, Sanders, & Malcolm, 1988; Steele et al., 1997) *Listeria monocytogenes* (Farber et al., 1988; Doyle et al., 1987) and *Campylobacter* spp. (Lovett, Francis, & Hunt, 1983) that can cause infections that may directly via colostrum or during the time of collection and storage colostrum to be disposed and transferred to neonates. Microbial contamination and lower colostrum immunoglobulin G [IgG ⩽ 50 g L^{-1}] are two important factors which affecting the quality of bovine colostrum; and if contaminated colostrum consumed for calf feeding, colostrum bacteria bonded with free Ig in the small intestine and IgG cannot absorb as well as reducing the apparent efficiency of absorption (AEA) (James, Polan, & Cummins, 1981; Morrill et al., 2012).

A dairy's colostrum management program is one of the very few processes in the animal health world that can be easily evaluated and should be routinely reviewed by veterinarians. One of the best methods for reduction or elimination of infectious pathogens is heat-treatment (McMartin et al., 2006; Godden 2008). A disadvantage of this method is the potential heat denaturation of colostral immunoglobulins and other essential constituents which could increase calf morbidity and mortality rates. Previous studies showed colostrum heating at 60°C as safe and without affecting at immunoglobulin G concentration of colostrum and colostrum viscosity (Godden et al., 2006; McMartin et al., 2006), but still, about the most effective duration of this treatment process (30, 60, 75, 90 or 120 min.) there are many discussion and disagreement (Godden et al., 2006; McMartin et al., 2006; Elizondo-Salazar, Jones, & Heinrichs, 2010).

Recently, there is a growing body of literature that recognises the importance of failure of passive transfer of immunity (FPTI) in the management of the dairy cow industry. The current hypothesis suggests that the presence of pathogen in gastrointestinal tract at the time of colostrum consumption could interfere with the absorption of Ig biomolecule (Staley & Bush, 1985). To our knowledge, there is a lack of published data on the difference between feeding heat-treated colostrum and unheated to calves and the effect of heat-treated colostrum consumption on growth, health and performance of dairy calves in pre-weaned period (Elizondo-Salazar & Heinriches, 2009; Rafiei, Ghoorchi, Toghdory, Moazeni, & Khalili, 2019). Hence, the specific objective of this study was to determine the effects of feeding heat-treated colostrum at different time duration (30, 60, and 90 min.) on apparent efficiency of Ig absorption, growth, health, and skeletal development of Holstein calves. In addition, the second purpose of this investigation is to explore the effects of heat-treatment at different time duration on colostrum composition, bacterial contamination, and pathogen viability at the on-farm situation.

Material and methods

Farm selection and herd management

The present research was conducted in summer season at Fazeel, a large dairy farm in the Isfahan province, Iran where 3,321 Holstein cows were milked two times a day with a herd average of 12107 Liters milk (305-day mature equivalent (305ME) fat, and protein yields: 3.3 ± 0.33% and 3.2 ± 0.14%, respectively). Geographical conditions of the study period were included ambient air temperature 29.23 ± 5.68°C, and relative humidity 26.33 ± 3.72. The study was confirmed according to the guidelines of the Iranian Council on Animal Care [ICAC] (1995). Cows were kept in free-stall barns and were fed a total mixed ration with free access to fresh water. When parturition was imminent, cows were moved from free stalls into shared maternity pens, which were bedded with dry straw and supervised 24hours per day.

Colostrum harvesting, batch preparation, and heat-treatment

First milking colostrum of 50 multiparous Holstein (Dry period: 80 ± 13.46 day) during the month before starting study were collected and 240 liters colostrum were stored in 60 sterile bags with 4 liters volume frozen at -20°C. Colostrum was thawed at 15-20°C and thoroughly hemogenized together into a plastic tub with 300-liter capacity and gently shaken for 2 min via the sterile plastic applicator. Then, hemogenized colostrum was divided into 4 batches of 60 L; unheated batch (3 × 20 L; H0), and three batches of colostrum

heat-treated at 60°C for 30 min. (3 × 20 L; H30), heat-treated at 60°C for 60 min. (H60; 3 × 20 L) and heat-treated at 60°C for 90 min. (H90; 3 × 20 L) by an on-farm colostrum batch pasturizator (20 liters capacity; Shir-mak Company, Isfahan, Iran). It should be noted pasteurizers were programmed to heat colostrum to a target temperature of 60°C for 30, 60 and 90 min., with a maximum allowable fluctuation of 0.71 ± 0.02ºC during the 30 min., 0.84 ± 0.02ºC during 60 min. and 0.96 ± 0.03ºC during 90 min. holding phase. The colostrum was then automatically cooled to 37ºC (approximately 20 minutes for each heating cycle) ready for feeding to calves. Times and temperatures were reported by the digital display on the equipment during the heat-treatment process and were recorded each pasteurization cycle and time-dependent temperature change of pasturizator at 30, 60, and 90 min. observed completely. Colostrum was agitated during the entire heat-treatment process.

Calf enrollment, experimental design, feeding and weaning protocol

Forty-eight female calves were immediately removed from the maternity pen after birth and transferred into individual calf units from birth to weaning (bedded with fresh wood-shavings), 2 m apart from each other, and then dipped navels with 7% (vol:vol) iodine. Calves eligible for inclusion in the study were born from multiparous cows with similar body condition score (3.5 on the scale of 1-5; according to the method of Edmonson, Lean, Weaver, Farver and Webster, 1989) at calving time, without dystocia and obvious congenital abnormalities based on clinical examination by farm veterinarian. Male calves and twins were excluded from enrollment. The animals were assigned randomly into 4 experimental groups with 12 animals each. For the first and second feeding, 3 and 2 L of colostrum prepared as described above (H0, H30, H60, and H90) was fed by 2 and 12h of age, respectively. From the second day of experiment to weaning, the milk was supplied as follows: calves were fed 3.0 L of milk twice daily from day 2 to 10±2, then received 4.0 L of milk twice daily from day 10 ± 2 to 40 ± 2, then received 5.0 L of milk twice daily from day 40 ± 2 to 65 ± 2, then received 2.0 L of milk in the morning from day 65 ± 2 to 70 ± 2 and were weaned from milk at day 70 ± 2. All milk was from the farm's hospital herd and pasteurized before being fed to the calves at 63°C for 30 min. Calves were offered starter grain (Table 1) and water (20-25°C) *ad libitum* from day 2 to 70 ± 2.

Table 1. Ingredients and chemical composition of the starter ration of all calves consumed in this study during suckling period.

Ingredients	(% DM)
Corn Grain, ground, dry	39
Barley Grain, ground	15
Wheat grain, ground	5
Soybean, Meal, solv.44%CP	26
Soybean, Seed, whole roasted	5
Canola Meal, mech. Extract	3
Molasses Beet	3
Calcium Carbonate	0.8
Sodium Bicarbonate	0.7
Salt	0.5
Mineral supplement[1]	1
Vitamin premix[2]	1
Chemical Analysis	
DM (% as fed)	90
Energy (Mcal Kg^{-1})	2.95
NEg (Mcal Kg^{-1})	1.35
Crude Protein (%)	20.7
EE (%)	4
Ash (%)	6.7
NDF (% DM)	13.2
ADF (% DM)	6.93
NFC* (%)	55.4

[1]Contained 4 g Mn, 65 g Ca, 4.5 g Zn, 30 g P, 44 g Mg, 60 g Na, 75 g Cl, 10.5 F, 42 g S, 10 mg Co, 1 g Cu, 24.6 mg I, and 48 mg Se per kg supplement. [2]Contained 1 300 000 IU vit A, 80000 IU vit D_3, 6600 IU vit E, 880 mg vit B_1, 850 mg vit B_2, 1740 mg vit B_5, 1345 mg vit B_5, 870 mg vit B_6, 76 mg vit B_9, 9.4 mg vit B_{12}, 13.4 mg vit H_2, and 16500 mg vit C per kg premix. *NFC = 100 – (%NDF + %CP + %ether extract + %ash).

Colostrum composition, IgG concentration, and bacterial contamination

13-mL colostrum aseptically collected from each batch in sterile 15-mL centrifuge tubes (Jet Biofil, Guangdong Co., China) for colostrum composition, IgG measurement, and microbial contamination.

The concentration of IgG (MonoScreen quantitative C-ELISA; Bio-X diagnostic Co., Belgium), and pH (Portable pH-meter, Cyber Scan, Eutech Co., Germany) were measured in duplicate and mean of each sample was recorded. To measure IgG concentration, calf serum samples were diluted 1:100, and colostrum samples were diluted 1:1000 using by dilution buffer. Briefly, in the dilution microplate wells, 100 µL of the calibration curve dilutions and diluted samples were transferred, and diluted conjugate was added to each well, mixed, and 100 µL of the content were transferred to the original microplate wells. The microplate was incubated at 21-24°C for 1h. samples between all dilution steps vortexed well. Subsequently, the microplate was rinsed 3 times with a washing solution, then 100 µL of chromatogen solution were added to each followed by incubation at 21-24°C and away from light for 10 min. The reaction was stopped by adding 50 µL of stop solution to each well. The optical density of the evaluated samples was determined using an ELISA reader and the Ig concentrations were calculated using Log/Logit computer software.

Colostrum samples were also diluted (vol:vol) using phosphate buffer solution (PBS; pH 7.4) to reduce sample viscosity and prevent technical difficulties usually encountered with undiluted and highly viscous samples (Maunsell, Morin, & Constable, 1998), then samples were analyzed for concentrations of fat and protein (milk-O-scan 133B infra-analyzer, FOSS Electric Co., Denmark).

For microbial investigation, 50 µL of 10-fold serial diluted colostrum samples were cultured into McConkey, blood agar, and Preston enrichment broth medium using an inoculating loop. Then by using biochemical tests listed in the FDA Bacteriological Analytical Manual (BAM) methods the presence or absence of viable *E. coli*, *Salmonella Enteritidis,* and *Campylobacter* spp. in suspected colonies was proved. Plates for identification *E. coli, Salmonella Enteritidis* and *Campylobacter* spp. were incubated at 37°C for 24 to 48h and 41°C, respectively for 24h. To enumerate total plate count (TPC) and total coliform count (TCC) of colostrum batches, samples were also incubated at 32 and 37°C for 48h, respectively. The overall number of bacteria was calculated for each sample and expressed in a TPC and TCC as colony-forming units per milliliter (CFU ml^{-1}).

Health, biochemical parameters, performance, and growth characteristics

All calves were evaluated daily for the respiratory score (from score 1 to 6) and the feces score (from score 1 to 4) based on Larson, Owen, Albright, Appleman and Muller (1977). Respiratory scoring of each calf evaluated based on the type of symptom, as follows: 1: normal; 2: runny nose; 3: heavy breathing; 4: cough-moist; 5: cough-dry; 6: fever accompanied with one of the above-mentioned symptoms. Fecal scoring was also performed daily based on physical appearance, as follows: 1: normal (firm but not hard); 2: soft (piles but spreads slightly); 3: runny (spreads readily to about 6 mm); and 4: watery (liquid consistency, splatters). Fresh feces sample (5 gr) of each calf was collected on sterile sample container at days 3, 7, and 14 post-birth for the total fecal aerobic bacterial count and diarrhea-induced pathogens. All fecal samples were examined for the presence of *E. coli* K99, Rotavirus, Coronavirus and *Cryptosporidium parvum* by a commercial immunochromatography rapid on-farm kit (Rainbow Calf Scour 4, Bio-X Diagnostics Co., Belgium) following manufacturer's instructions and after 10 minutes, the results were read (Klein et al., 2009). In the test red-lined strip, yellow-lined strip, blue-lined strip, green-lined strip were corresponds to Rotavirus, Coronavirus, *E. coli* attachment factor K99, and *C. parvum* respectively. To investigate fecal total aerobic bacterial, 50 µL of homogenized and diluted stool samples by PBS (pH = 7.4) cultured into nutrient agar media and incubated for 24 to 48h at 37°C; then by colony counter number of growth colony in each media counted and recorded.

For measurement of biochemical parameters, blood samples were taken by venipuncture and partially evacuated 6 mL sterile tubes without anticoagulant (BD Vacutainer Precision Glide, Becton Dickinson Co., Franklin Lakes, NJ) at 0, 6 and 24h of life. The samples were allowed to clot (15 min.) and sera was separated within one hour of collection by centrifugation at 1,800 $\times$ *g* for 15 min. The serum was harvested in a microtube (Easy-lock, 1.5 mL, FL Medical Technology, Italy) at -20ºC until evaluation. The concentrations of serum total protein (STP) and albumin using commercially available kits (Pars Azmoon Company, Iran) were measured. The concentration of globulin was calculated by subtracting total protein value from albumin, then A:G ratio was assessed. Serum IgG concentration by a commercial C-ELISA kit (MonoScreen quantitative; Bio-X diagnostic Co., Belgium) also, was measured according to the manufacturer's guidelines in duplicates and mean of each sample was recorded.

The efficiency of IgG absorption was determined by multiplying the estimated plasma volume of the calf by its serum IgG concentration and dividing this product by the mass of colostral IgG that was fed based on liter. Plasma volume at 6 and 24h was estimated to be 0.089 × BW (Quigley & Drewry, 1998), and birth BW was used to estimate BW at 6 and 24h.

To evaluate the performance of each calf in the current study, dry matter intake (DMI), feed efficiency rate (FER) and feed conversion rate (FCR) of all calves calculated from the second day of age to weaning time. Calves had their body weight (BW) recorded at birth and every ten days till weaning using by electronic on-farm scale (Sepehr-Alborz Co., Iran) and then, average daily weight gain (ADWG) and total weight gain (TWG) of each calf calculated. Growth characteristics of all calves such as body length (BL), the height of the withers (HW), height of the hip (HH), width of the hip (WH), breast circumference (BC) and abdominal circumference (AC) assessed at day 3, 15, 30, 45 and 60 of suckling period based on the method of Kolkman et al. (2010).

Statistical analyses

Of the 48 enrolled calves, 47 calves (one calf death at 10 days of age from H30 group) were survived at the end of study, and respective data were used for statistical analyses. Data were expressed as mean±standard error of the mean (SEM) and $p < 0.05$ was considered significant. Repeated measures ANOVA was used to detect the main effects of treatment, time, and the interaction between treatment and time for evaluation of skeletal growth characteristics and performance parameters of calves during the suckling period. The relative and absolute numbers of calves between different groups was compared by the Chi-square test and Fisher's exact coefficient. All analyses were carried out using statistical software programs (Statistical Analysis System [SAS], 2015). The statistical model also used was

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

where, Y_{ij} is the outcome variable (SPC, TCC, IgG, STP, ...), μ is overall mean, α_i is the effect of treatment, and e_{ij} is residual.

Results

Colostrum composition, IgG concentration, and bacterial contamination

Composition and bacterial count of colostrum batches after the various time (H30, H60, H90) at 60°C in heat-treated and unheated (H0) treatments are presented in Table 2. The concentrations of fat, protein, and F:P ratio of heat-treated colostrum batches were not shown any significant change after heat-treatment at different duration in comparison to raw colostrum ($p > 0.05$). The pH of colostrum batches after heat-treatment increased slightly (from 6.77 ± 0.003 to 6.79 ± 0.003), although this increment was not statistically significant ($p > 0.05$).

IgG concentration of H0, H30, H60, and H90 colostrum batches was equal to 67.63 ± 0.08, 66.17 ± 0.08, 63.07 ± 0.08, and 59.53 ± 0.09 g L^{-1} which indicated a significant difference between H90 compared to other batches ($p = 0.02$). Colostral IgG concentration decreased significantly ($p = 0.01$) by 2.16 ± 0.62, 6.74 ± 0.78, and 11.98 ± 1.03% when batches heat-treated for 30, 60, and 90 min., respectively.

Counts for TPC and TCC declined as time and temperature increased (Table 2) compared with bacterial counts in unheated colostrum, but after 30 min. treatment of batches at 60°C *E.coli* was destructed and not detected. Colostral TPC decreased significantly ($p = 0.02$) up to 18.59 ± 0.66, 49.36 ± 0.9, and 62.18 ± 0.11% when batches heat-treated for 30, 60, and 90 min, respectively. Besides, the TCC reduced significantly ($p = 0.04$) after heating at 60°C for 30, 60, and 90 min. to 26.15 ± 0.54, 45.67 ± 1.2, and 62.65 ± 1.03%, respectively.

Results of pathogen viability determined all batches were infected with *Salmonella enteritidis*, *E. coli,* and *Campylobacter* Spp. naturally. For all batches, *Salmonella enteritidis*, *Campylobacter* Spp. were consistently not found in any of the examined colostrum batch samples collected upon reaching the target temperature of 60°C, which indicating this organisms had not survived after the 60-min heat-up phase (Table 2). *E. coli* was consistently not detected from any colostrum batches after heating at 60°C for 30 min., which indicating the organism had not survived in colostrum samples treated for 30 min.

Table 2. The effect of different heat-treatment methods on colostrum composition, bacterial count and pathogens viability (Mean ± SEM). Totally 240 L of pooled colostrum divided to one of four treatment batches; unheated colostrum (H0; 60 L); heat-treated colostrum at 60°C for 30 min (H30; 60 L); heat-treated at 60°C for 60 min (H60; 60 L); heat-treated at 60°C for 90 min (H90; 60 L).

	Treatment			
Colostrum composition	H0	H30	H60	H90
Volume (L)	60 (3 × 20L)	60 (3 × 20L)	60 (3 × 20L)	60 (3 × 20L)
IgG (g L^{-1})	67.63 ± 0.08^{a}	66.17 ± 0.08^{a}	63.07 ± 0.2^{a}	59.53 ± 0.09^{b}
Loss of IgG (%)	-	2.16 ± 0.62^{a}	6.74 ± 0.78^{a}	11.98 ± 1.03^{b}
Fat (%)	4.15 ± 0.003	4.14 ± 0.005	4.16 ± 0.006	4.18 ± 0.003
Protein (%)	17 ± 0.06	17 ± 0.1	17.03 ± 0.07	17 ± 0.1
Fat:Pro	0.24 ± 0.001	0.24 ± 0.003	0.24 ± 0.003	0.24 ± 0.003
pH	6.77 ± 0.003	6.78 ± 0.002	6.78 ± 0.006	6.79 ± 0.003
Total palate count (log_{10} Cfu ml^{-1})	6.24 ± 0.05^{a}	5.08 ± 0.1^{a}	3.16 ± 0.07^{b}	2.36 ± 0.03^{b}
Total palate count decrement (%)	-	18.59 ± 0.66^{a}	49.36 ± 0.9^{b}	62.18 ± 0.11^{b}
Total coliform count (log_{10} Cfu ml^{-1})	5.89 ± 0.03^{a}	4.35 ± 0.23^{a}	3.2 ± 0.2^{b}	2.2 ± 0.1^{b}
Total coliform count decrement (%)	-	26.15 ± 0.54^{a}	45.67 ± 1.2^{b}	62.65 ± 1.03^{b}
Salmonella enteritidis	G	G	ND	ND
E. Coli	G	ND	ND	ND
Campylobacter Spp	G	G	ND	ND

$^{a\text{-}b}$Means within each row with a dissimilar letter(s) are significantly different from each other at 5% level ($p < 0.05$). G: growth; ND: not detected.

Health status, biochemical parameters, performance and growth characteristics

Observation of fecal and respiratory score based on every ten days and an overall score of each treatment showed calves that were fed heat-treated colostrum for 90 min. (H90) had significantly lower ($p = 0.02$) fecal score (1.37 ±0.02; firm feces) than calves fed unheated colostrum (1.56 ±0.02; soft feces) and heat-treated for 30 min. (1.59 ±0.02; soft feces) during the suckling period. Although from point of respiratory score was not found any significant difference ($p > 0.05$) between treatments. The analyses of the fecal samples with the immunochromatographic rapid test to the detection of diarrhea-induced pathogens were positive in 42.55% of the fecal samples in studied calves. In the H0 group all enteropathogens were detected as days 3, 7, and 14, but in groups of H60 and H90 prevalence of diarrhea-induced pathogens was lower (Table 3) when compared to other treatments.

Table 3. The effect of consumption of unheated (H0) and heat-treated (H30, H60, and H90) colostrum on definitive and relative frequencies of infection by various diarrhea-induced pathogens by using immunochromatography method and total aerobic bacterial count of feces during first 2 weeks of neonatal period. Group H0 received unheated colostrum; Group H30 received heat-treated colostrum at 60°C for 30 minutes; Group H60 received heat-treated colostrum at 60°C for 60 minutes; Group H90 received heat-treated colostrum at 60°C for 90 minutes.

	Day 3 (N = 48)				Day 7 (N = 48)				Day 14 (N = 47)			
	Treatment											
Infective agents	H0	H30	H60	H90	H0	H30	H60	H90	H0	H30	H60	H90
C. Parvum (%)	2 (16.66%)	1 (8.33%)	-	-	5 (41.66%)	1 (8.33%)	1 (8.33%)	-	6 (50%)	3 (25%)	1 (8.33%)	1 (8.33%)
Coronavirus	2 (16.66%)	2 (16.66)	2 (16.66%)	-	3 (25%)	3 (25%)	2 (16.66%)	1 (8.33%)	4 (33.33%)	3 (25%)	1 (8.33%)	-
Rotavirus	3 (25%)	3 (25%)	1 (8.33%)	-	4 (33.33%)	2 (16.66%)	1 (8.33%)	-	6 (50%)	2 (16.66%)	1 (8.33%)	-
E. coli K99	3 (25%)	3 (25%)	2 (16.66%)	-	1 (8.33%)	2 (16.66%)	-	-	1 (8.33%)	1 (8.33%)	-	-
Total infected	10 (20.83%)	9 (19.15%)	5 (10.42%)	-	13 (27.08%)	8 (17.02%)	4 (8.33%)	1 (4.8%)	17 (35.42%)	9 (19.15%)	3 (6.25%)	1 (4.8%)
TAB count (log_{10} cfu mL^{-1})	-	-	-	-	8.50^{a} ± 0.36	7.21^{b} ± 0.39	6.35^{bc} ± 0.39	5.97^{c} ± 0.36	9.05^{a} ± 0.41	7.13^{b} ± 0.38	7.27^{b} ± 0.38	7.10^{b} ± 0.41

$^{a\text{-}c}$:Means within each row with a dissimilar letter(s) are significantly different from each other at 5% level ($p < 0.05$). Fecal sample was not collected for bacterial count at day 3. TAB: total aerobic bacterial

Serum total protein and albumin concentrations of all calves varied from 3.6 to 5.9 and 0.09 to 2.9 mg dL^{-1}, respectively. Values above 5.00 mg dL^{-1} of STP were reached by 18 (38.3%) of these calves at hours 6 and by 45 (95.7%) at hours 12 post-birth. Nine animals (19.1%) at hours 6 and one animal (4.7%) at hours 24 had total protein values of ⩾ 5.00 mg dL^{-1}, that implying a moderately successful passive transfer. The remaining were 20 animals (42.5%) at hours 6 and one animal at hours 24 (4.7%) showed insufficient STP

concentrations (≤ 4.9 mg dl^{-1}). STP concentration of calves before colostrum consumption were relatively low and equal to 4.3, 4.21, 4.27, and 4.23 mg dL^{-1} in groups H0, H30, H60, and H90, respectively which were not statistically different ($p > 0.05$; Table 4). Calves in groups H60 ($p = 0.03$) and H90 ($p = 0.02$) had higher concentrations of STP than calves were received untreated (H0) and heat-treated colostrum (H30) at hour 6 of the experiment. The results showed from point of STP concentration were not found the difference between calves fed H0 and calves fed H30 at 6 ($p = 0.07$) and 24h ($p = 0.08$).

A negligible amount of serum IgG was found at birth (0 hour) in calves before colostrum feeding but was not found a significant difference between groups ($p > 0.05$). Feeding calves by heat-treated colostrum (H90) resulted in marked increment in serum IgG concentrations when compared to calves that were fed H30 and H60 ($p = 0.03$). Calves that were consumed heat-treated colostrum (H60) had higher levels of IgG at 6 ($p = 0.02$) and 24h ($p = 0.01$) than calves that were fed heat-treated (H30) and raw colostrum (H0).

As displayed in Table 4, the apparent efficiency of absorption (AEA) of serum IgG was calculated at 6 and 24h to assess the success of the passive transfer of immunity. Calves in group H60 had a higher ($p = 0.03$) means of AEA rates than group H0 (19.99 ± 1.06 vs 16.19 ± 0.73). Similar AEA rates were noted for group H0 at hours 6 and 24 ($p > 0.05$). However, group H30 (18.61 ± 0.84) had a lower AEA rate compared with calves in groups H60 (19.85 ± 1.06) and H90 (22.06 ± 0.9) 6 hours post colostrum consumption. Calves in group H90 had the highest AEA rate on hours 6 ($p = 0.02$) and 24 ($p = 0.02$) in the current study which was significantly different compared to other treatments.

Table 4. The effect of consumption of unheated (H0) and heat-treated (H30, H60, and H90) colostrum on serum concentrations of albumin, globulin, A:G ratio, serum total protein, IgG and AEA rate of calves at 0, 6, and 24h after colostrum feeding (Mean ± SEM). Group H0 received unheated colostrum; Group H30 received heat-treated colostrum at 60°C for 30 minutes; Group H60 received heat-treated colostrum at 60°C for 60 minutes; Group H90 received heat-treated colostrum at 60°C for 90 minutes.

	Treatment			
Parameters/Time	H0	H30	H60	H90
Albumin (mg dL^{-1})				
0	1.49^{b} ± 0.056	1.46^{b} ± 0.054	1.43^{b} ± 0.047	1.3^{a} ± 0.073
6	1.65^{c} ± 0.035	1.75^{b} ± 0.037	1.81^{b} ± 0.035	1.96^{a} ± 0.035
24	2.12^{b} ± 0.065	2.16^{b} ± 0.068	2.20^{b} ± 0.065	2.55^{a} ± 0.065
Globulin (mg dL^{-1})				
0	2.9^{bc} ± 0.1	3.03^{ab} ± 0.096	2.83^{c} ± 0.11	2.92^{bc} ± 0.11
6	3.17 ± 0.051	3.19 ± 0.053	3.22 ± 0.051	3.10 ± 0.051
24	3.09 ± 0.068	3.14 ± 0.072	3.13 ± 0.069	3.08 ± 0.068
A:G (%)				
0	0.52 ± 0.024	0.5 ± 0.027	0.52 ± 0.033	0.46 ± 0.043
6	0.53^{b} ± 0.016	0.54^{b} ± 0.018	0.57^{b} ± 0.016	0.64^{a} ± 0.016
24	0.71^{b} ± 0.037	0.69^{b} ± 0.039	0.71^{b} ± 0.037	0.83^{a} ± 0.037
STP (mg dL^{-1})				
0	4.3 ± 0.077	4.51 ± 0.084	4.27 ± 0.093	4.23 ± 0.075
6	4.83^{b} ± 0.038	4.84^{b} ± 0.042	5.07^{a} ± 0.038	5.15^{a} ± 0.038
24	5.21^{c} ± 0.043	5.28^{bc} ± 0.047	5.33^{b} ± 0.043	5.65^{a} ± 0.043
IgG (g L^{-1})				
0	0.008 ± 0.008	0.088 ± 0.07	0	0.12 ± 0.05
6	8.67^{b} ± 0.49	9.63^{ab} ± 0.52	9.68^{ab} ± 0.49	10.1^{a} ± 0.49
24	13.7^{c} ± 0.59	15.2^{bc} ± 0.62	16.2^{b} ± 0.59	18.1^{a} ± 0.59
AEA (%)				
6	16.36^{c}±1.13	18.61^{b}±0.84	19.85^{b}±1.06	22.06^{a}±0.9
24	16.19^{b}±0.73	17.9^{b}±0.96	19.99^{c}±0.84	23.32^{a}±0.57
FPTI (*n*)				
6	8 (66.6%)	6 (54.5%)	7 (58.3%)	6 (50%)
24	1 (12%)	-	-	-

[a-c]Means within each row with a dissimilar letter(s) are significantly different from each other at 5% level ($p < 0.05$).

The BW and ADWG changs of animals during the suckling period shown in Figures 1a and b. Results indicated that the consumption of heat-treated colostrum did not have any negative effect on daily and total weight gain during the suckling period, but was not found a significant difference between heat-treated and unheated groups ($p > 0.05$) at weaning time. The mean and SD of FCR at weaning time showed that in H0, H30, H60, and H90 groups were equal to 1.55 ± 0.05, 1.62 ± 0.05, 1.74 ± 0.05, and 1.64 ± 0.05%, respectively, which indicated a significant difference between heat-treated and unheated groups ($p = 0.04$), but was not found a significant difference from point of FER in various treatments

($p > 0.05$; Figures 1c and d). Also, between treatments during the suckling period from point of DMI was not seen as a statistically significant difference ($p > 0.05$). Furthermore, groups H60 and H90 have significantly higher performance in comparison to calves that were fed unheated colostrum. Skeletal growth characteristics demonstrated WH of calves that were received heat-treated colostrum at 60°C for 90 min. (23.0 ± 0.24 cm) was significantly higher ($p = 0.03$) than calves were fed raw colostrum (22.6 ± 0.24 cm) or heat-treated colostrum at 60°C for 30 min. (22.0 ± 0.24 cm), although from point of HW ($p > 0.05$), AC ($p > 0.05$), and BC ($p > 0.05$) calves in group H90 have higher growth rate at weaning time, this difference was not significant (Figures 2a, b, c, and d). The performed linear regression tests demonstrated that no significant relationship existed between serum total protein concentration and birth weight ($R^2 = 0.07$), BW ($R^2 = 0.04$), BL ($R^2 = 0.05$), BC ($R^2 = 0.06$), HW ($R^2 = 0.06$), FCR ($R^2 = 0.1$), FER ($R^2 = 0.3$) and ADWG ($R^2 = 0.06$). While, was found a significant relationship between serum IgG concentration and overall BL ($R^2 = 0.09$), BC ($R^2 = 0.08$), and HW ($R^2 = 0.08$) of studied calves during the suckling period.

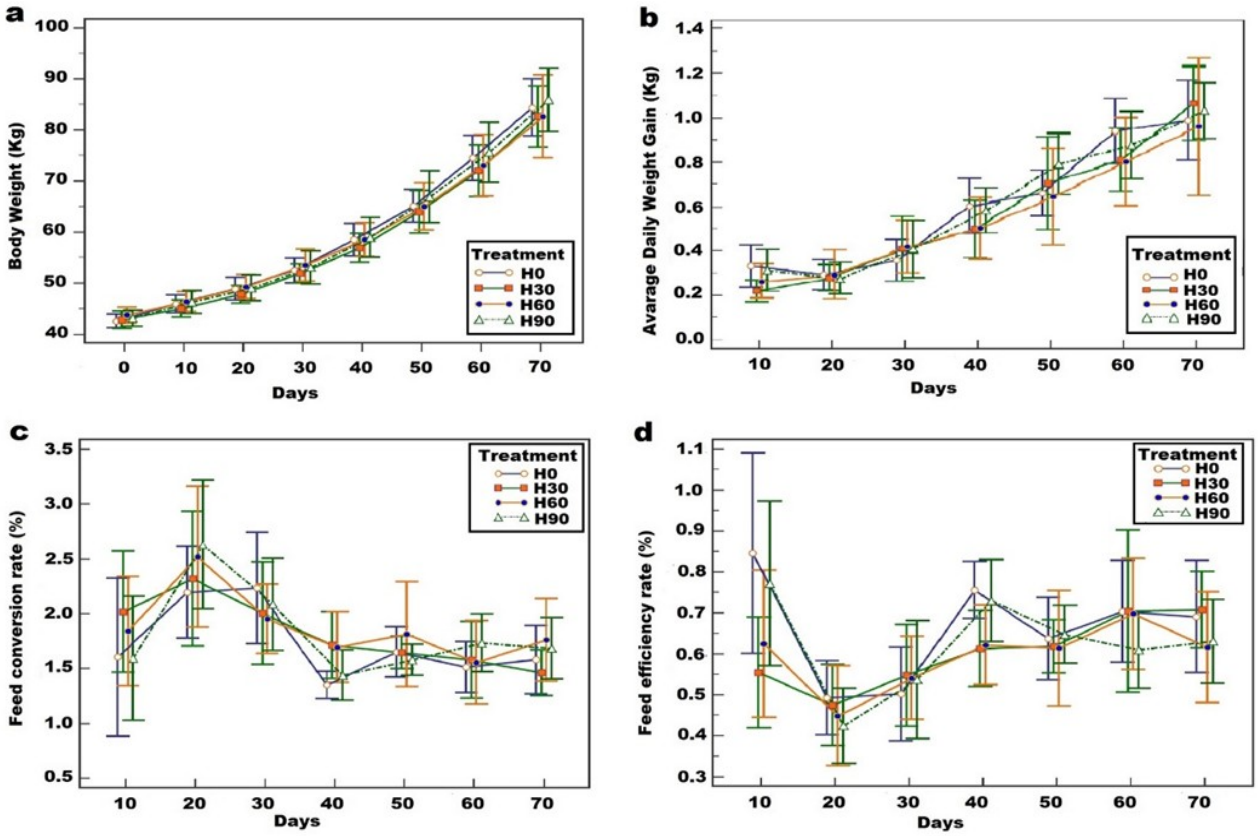

Figure 1. Calculated body weight (BW, a), average daily weight gain (ADWG, b), feed conversion rate (FCR, c) and feed efficiency rate (FER, d) of 48 Holstein calves every 10 days from birth to weaning time. Calves were randomly assigned to one of four treatment groups of 12 animals; group H0 fed unheated colostrum; group H30 fed heat-treated colostrum at 60°C for 30 min.; group H60 fed heat-treated colostrum at 60°C for 60 min.; group H90 fed heat-treated colostrum at 60°C for 90 min. Data is presented as mean ± SD.

Discussion

Colostrum composition, IgG concentration, and bacterial contamination

Results showed that colostrum heat-treatment at 60°C till 90 min. have not a significant effect on fat and protein concentrations, although IgG of the unheated batches with 67.63 g L^{-1} after 30, 60 and 90 min. heat-treatment was reduced in 2.16, 6.74 and 11.98%, respectively. The proteins of milk are probably the constituents most affected by heating (Fox & McSweeney, 1998). Typically, decreased whey protein solubility is founded when milk is heated for 40 to 60°C. Over this range, whey proteins denature due to disruption of noncovalent bonds stabilizing the secondary and tertiary structure (Pelegrine & Gasparetto, 2005). In a study, Tacoma et al. (2017) evaluated the effects of heat-treatment (at 60°C) for 0, 30, 60, and 90 min. on colostrum protein profile.

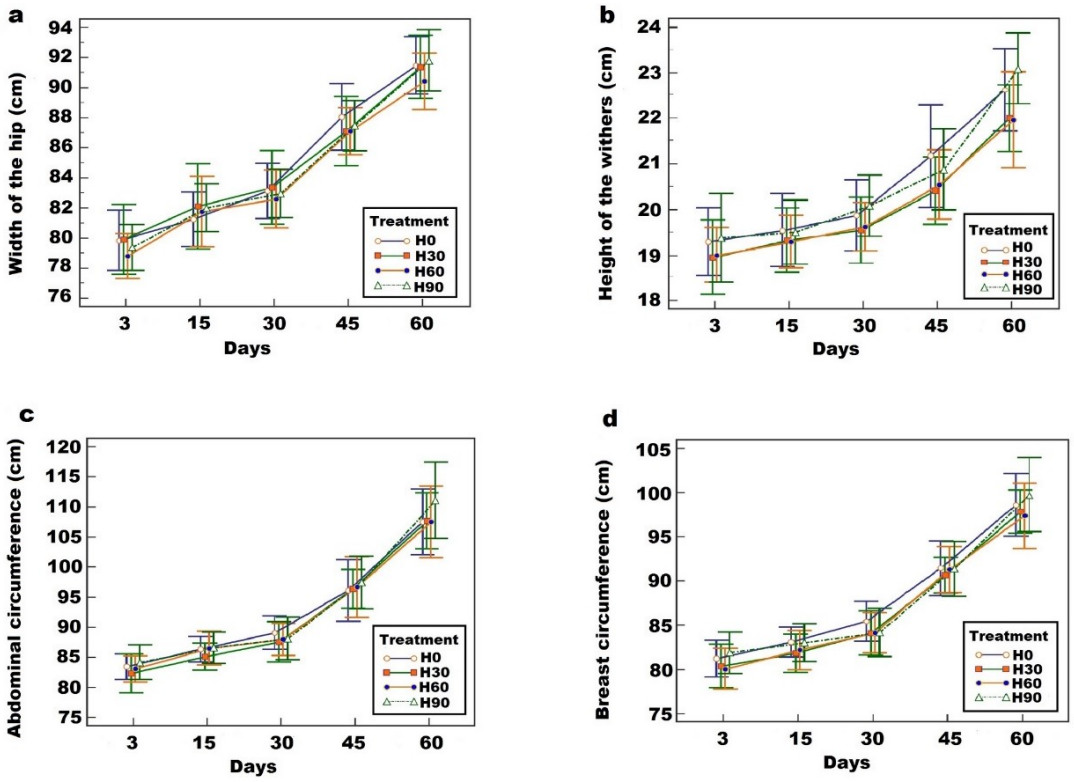

Figure 2. Calculated width of the hip (WH, a), height of the withers (HW, b), breast circumference (BC, c), and the abdominal circumference (AC, d) assessed at day 3, 15, 30, 45 and 60 of suckling period based on the method of Kolkman et al. (2010). Fourteen-eight Holstein calves were randomly assigned to one of four treatment groups of 12 animals; group H0 fed unheated colostrum; group H30 fed heat-treated colostrum at 60°C for 30 min.; group H60 fed heat-treated colostrum at 60°C for 60 min; group H90 fed heat-treated colostrum at 60°C for 90 min. Data is presented as mean ± SD.

They indicated that the length of heat-treatment changed the composition of high and low abundance proteins within bovine colostrum, and the majority of low abundance proteins affected by heat were involved in cellular and immune processes. deWit and Klarenbeek (1984) studied the effects of heat-treatment on the structure and solubility of the Ig fraction of whey. They demonstrated that Ig are among the most heat-stable whey proteins, which was attributed to the high content of disulfide bonds and whey components such as fats, lactose, carbohydrates, salts, and other proteins that help stabilize antibodies during thermal treatment (Indyk, Williams, & Patel, 2008). Fat is the main energy providing component of milk. The mean percentage of fat in colostrum is 6.7%, compared to 4.0% in mature milk (Foley & Otterby, 1978). Proteins of the milk fat globule membrane start to denature at temperatures above 70°C. This damage to the milk fat globule membrane leads to the formation of free (non-globular) fat. However, of milk's principal constituents, lipids are the least affected by heat (Fox & McSweeney, 1998).

With denaturation temperatures between 65 and 70°C (Topel, 2004), immunoglobulins (IgG, IgG, IgM, and IgA) are very thermolabile whey proteins (dewit and Klarenbeek, 1984). IgG, which accounts for 85 to 90% of the total immunoglobulin mass in bovine colostrum (Larson, Heary, & Devery, 1980), exhibits two transitions when exposed to heat. The isolated Fab fragment shows a transition at 61°C, while the Fc fragment displays a transition at 71°C, which means that these individual transitions represent the denaturation of the IgG's Fab and Fc domains, respectively (Vermeer & Norde, 2000). Donahue et al. (2012) findings indicated that when IgG change was expressed as an absolute change (mg mL^{-1}), batches of colostrum with ⩾ 70 to 79.9 mg mL^{-1} initial IgG concentration experienced a 6.7 mg mL^{-1} or 8.8% loss of IgG after heat-treatment for 60 min., and batches of colostrum with ⩾ 80 mg mL^{-1} initial IgG concentration experienced a 9.8 mg mL^{-1} or 9.8% loss of IgG after heat-treatment. Therefore, results of the present study were in agreement with Donahue et al. (2012) and Gelsinser, Jones and Heinrichs (2015) where IgG concentration of unheated batches showed a high decrement rate at 60°C for 60 min. but were in contrast to the results of Godden et al. (2006), McMartin et al. (2006), Johnson, Godden, Molitor, Ames and Hagman, (2007); Elizondo-Salazar, and Heinrichs (2009), where colostrum heat-treated at 60°C up to 120 min. was not expressed any significant change in IgG concentration.

Minimizing bacteria counts in colostrum requires the knowledge of conditions and practices that cause these elevations of counts. TPC and TCC are standard methods to survey the quality of dairy products. Fresh/raw colostrum fed to calves should contain less than 100,000 cfu mL^{-1} TPC and less than 10,000 cfu mL^{-1} TCC (McGuirk & Collins, 2004). However, goals for bacteria levels in heat-treated colostrum are TPC less than 20,000 cfu mL^{-1} and coliform count less than 100 cfu mL^{-1}, respectively (Godden et al., 2019). In an observational study that tested 827 colostrum samples from 67 farms, almost 43% of samples had TPC greater than 100,000 cfu mL^{-1}, and 17% of samples had greater than 1 million cfu mL^{-1} (Walz et al., 1997). The results of the study Donahue et al. (2012) shown heat-treatment of colostrum at 60°C for 60 min. decreased TPC by 2.25 log_{10} and TCC by 2.49 log_{10} cfu mL^{-1}. Accordingly, the results of our study indicated unheated colostrum with 6.24 log_{10} TPC and 5.89 log_{10} cfu mL^{-1} TCC after 30, 60, and 90 min. heat-treatment, was observed 18.59, 49.36 and 62.18% loss of TPC and 25.16, 45.67 and 62.65% of TCC, respectively. The findings of the present study were also verified in 4 clinical trials, each conducted on individual dairies by university personnel (Johnson et al., 2007; Elizondo-Salazar & Heinrichs, 2009; Kent-Dennis, 2014; Rafiei et al., 2019). Godden et al. (2006) reported that after 15 and 30 min. of heat treatment at 60°C, *E.coli* and *Salmonella enteritidis* were not detected at colostrum batches, respectively. Our study was in agreement with Godden et al. (2006) findings and not detected aformentioned colostrum-borne pathogens at 60°C for 60 min.

Health, biochemical parameters, performance and growth characteristics

Absorption of IgG from colostrum is higher when the gastrointestinal tract have a proper health condition and this led to reduction of diarrhea incidence (Corley, Staley, Bush, & Jones, 1977; English et al., 2007; Yang, Zou, Wu, Li, & Cao, 2015) and respiratory diseases. Elizondo-Salazar et al. (2010) indicated calves that were received heat-treated colostrum at 60°C for 60 min. have lower feces score and higher health status than calves consumed unheated colostrum at birth. The results of the study Rafiei et al. (2019) indicated the rate of incidence to pneumonia during suckling period between treatments that were fed unheated colostrum had greater rate than calves fed unheated colostrum. Our findings showed that consumption of heat-treated colostrum was not significant effect on respiratory score, while fecal score of calves that were received heat-treated colostrum (especially H60 and H90) was lower when compared to those that received unheated colostrum during suckling period. Higher IgG concentration of serum during first days of calf life resulted in general and local immunity against *E.coli*, *Rotavirus*, *Coronavirus* and *Cryptosporidium parvum* because these are important agents that led to diarrhea in neonatal ruminants (Acres, 1985; Arthington, Cattell, & Quigley, 2000; Crouch et al., 2000; Constable et al., 2016). Decreasing bacterial exposure to calves through colostrum while maintaining colostrum quality (IgG concentrations) should result is a healthier calf due to the higher AEA rate of IgG. The result of current study showed when bovine colostrum heated for up to 90 min. approximately most of the infective agents omitted from colostrum, thereupon the risk of transmitting of diarrhea-induced pathogens reduced within the first 2 weeks of age or so after and prevalence of neonatal diseases in calves of heat-treated groups was lower than those were received unheated colostrum. Returning to the question posed at the beginning of this study, it is now possible to state that colostrum heat-treatment with reduction of microbial count leads to the increment of AEA rate of Ig and health of newborn calves.

Different studies showed calves that were fed heat-treated colostrum at 60°C for 30 or 60 min. have higher STP and IgG concentrations during first 24h of age than calves fed unheated colostrum (Johnson et al., 2007, Elizondo-Salazar & Henrichs, 2009; Gelsinger et al., 2015; Rafiei et al., 2019). It is generally accepted that FTPI is indicated when a blood Ig and STP concentration is less than 10 g L^{-1} or 5.2 g dL^{-1} at 48h of age, respectively (Elsohaby & Keefe, 2015; Elsohaby et al., 2019). Tyler (personal communication, 2002) proposed that a successful passive transfer program was one in which 90% of sampled calves test 5.0 to 5.2 g dL^{-1} or higher of STP. In the current study 92, 87, 76, and 71% of calves in H90, H60, H30, and H0 had STP concentration of 5.0 g dL^{-1} or higher at 24h of age, respectively. Calves with FTPI are more susceptible to infectious diseases and have higher morbidity and mortality (Windeyer et al., 2014). Pathogenic bacteria may be bound and neutralized by colostral Ig in the lumen of the small intestine, thereby decreasing the total mass of fed IgG available for absorption. Pathogenic bacteria (e.g., *E.coli*) may attach to and damage intestinal epithelial cells, thereby reducing permeability to IgG molecules. Mean AEA rate from maternal colostrum typically averages 20 to 35% and the concentration of IgG in the colostrum may influence AEA

(Stott & Fellah, 1983; Quigley & Drewry, 1998), but in our study AEA rate was between 10 to 27.32%. Also, the AEA rate of calves in group H0 was significantly lower than calves in groups H30, H60, and H90 at hours 6, that was indicated the effects of consumption of heat-treated colostrum on IgG absorption. This finding was in agreement with other studies (Elizondo-Salazar & Henrichs, 2009; Moazeni, Rasooli, Nouri, Ghorbanpoor, & Mosavari, 2017; Rafiei et al., 2019).

Rebelein (2010) showed that calves fed raw colostrum at first feeding had an average weight gain of 0.79 ± 0.12 kg per day, while calves fed heat-treated colostrum gained 0.87 ± 0.12 kg per day. Results of Kent-Denis (2014) indicated that consumption of heat-treated colostrum at 60°C for 60 min. led to higher ADWG and total gaining at suckling period than calves fed unheated colostrum. Different studies (Elizondo-Salazar & Henrichs, 2009; Rafiei et al., 2019) confirmed that consumption of heat-treated colostrum have not a negative effect on body weight and skeletal growth of calves in the suckling period. Results of our study were in agreement and confirmed the finding of previous studies (Elizondo-Salazar & Heinrichs, 2009; Rafiei et al., 2019) and showed that consumption of heat-treated colostrum improves growth characteristics and average daily weight gain during suckling period which indicated that heat-treatment of colostrum at 60°C up to 90 min. have not any damage on essential compositions of colostrum such as hormones, growth factors, and minerals.

Conclusion

To put it in a nutshell, the current study proved that the best duration for colostrum heat-treatment was 90 min. Since it not only did not significantly reduced the pH, fat and protein contents of colostrum, but also significantly increased Alb, STP, and IgG concentrations of blood and AEA rate of calves that were fed H90. This research has thrown up many questions in need of further investigation. It would be interesting to assess the effects of heat-treatment at 60°C for 60 and 90 min. on macro and micro mineral compositions and also active immunologic substances of colostrum and serum of calves that have consumed this colostrum.

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