Heat treatment of colostrum at 60°C decreases colostrum immunoglobulins but increases serum immunoglobulins and serum total protein: A meta-analysis

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ABSTRACT

Calves are born hypogammaglobulinemic; thus, the newborn calf's immune defense relies on the ingestion and absorption of colostrum, which provides energy, immunoglobulins, immune cells, and cytokines to the newborn calf. A heat treatment applied to colostrum for 60 min at 60°C has been found to be effective at reducing the total bacterial count while preserving the colostrum IgG levels. The objective of this work was to perform a meta-analysis on the association between the characteristics of heat-treated colostrum and the concentration of colostrum IgG, serum IgG concentration, and serum total protein (STP). A meta-analysis was carried out based on existing peer-reviewed literature. Publications comparing colostrum IgG, serum IgG, and STP for heat-treated or raw frozen colostrum were included. The different heating temperatures applied to the colostrum were divided into 2 subgroups: high temperature (HT; >60°C) and low temperature (LT; ≤60°C). Twelve studies, including 21 trials, met the inclusion criteria for colostrum IgG concentration. The results indicated decreases in colostrum IgG by 20.6 g/L [95% confidence interval (CI) = 11.8–29.4] for HT and 5.38 g/L (95% CI = 2.9–7.8) for LT when colostrum was heat-treated compared with raw or frozen colostrum. Heterogeneity was high to moderate (I^2 = 82% for HT and 65% for LT). The heat treatment of colostrum was also associated with a nonsignificant decrease in serum IgG by 3.40 g/L for HT (95% CI = 7.54–0.74) but a significant increase in serum IgG by 2.65 g/L for LT (95% CI = 1.51–3.79). The regression model indicated that heterogeneity was not explained by any moderators. The heat treatment of colostrum was also associated with a significant increase in STP by 0.21 g/dL for LT (95% CI = 0.07–0.35). In conclusion, the present work demonstrated that the heat

treatment of colostrum ≤60°C decreased colostrum IgG by 5.38 g/L for LT and increased serum IgG by 2.65 g/L and STP by 0.21 g/dL. When compared with the range of values observed in the field for serum IgG, the present results are of high interest for the cattle industry. Because immune colostrum benefits also include cytokines and immune cells, further work is required to evaluate the effect of colostrum heat treatment on these 2 immune components of colostrum.

Key words: dairy cow, frozen colostrum, heat-treated colostrum, passive immunity, passive transfer

INTRODUCTION

Calves are born hypogammaglobulinemic due to syndesmoplacentation (Noakes, 2009). Thus, the newborn calf immune defense relies on ingestion and absorption of colostrum immunoglobulins. Thanks to its specific composition, colostrum provides energy, immunoglobulins, immune cells, and cytokines to the newborn calf. In the field, the passive immunity provided by colostrum to the calf is evaluated as immunoglobulin concentration in the calf blood serum after 7 to 10 d of colostrum feeding (Godden 2008). Calf serum total proteins (STP) are also useful to evaluate the passive transfer of immunity (Thornhill et al., 2015). Failure of passive immunity transfer is associated with higher risks of mortality, respiratory diseases, diarrhea, and septicemia (Raboisson et al., 2016). Similarly, half of all neonatal mortality can be directly attributed to failure to acquire passive immunity at birth (Tyler et al., 1999). One case of failure of passive immunity transfer has been assessed to cost between €60 and €120 per calf (Raboisson et al., 2016).

Colostrum management is one of the most important management factors that determine calf health and survival (McGuirk and Collins, 2004). Despite the nutritional and immunogenic effects of colostrum, colostrum feeding can also provide the first exposures to pathogenic microorganisms such as *Mycobacterium avium* ssp. *paratuberculosis* (Streeter et al., 1995), *Sal-*

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monella spp. (McEwen et al., 1988), *Mycoplasma* spp., *Listeria monocytogenes* (Farber et al., 1988), *Campylobacter* spp. (Steele et al., 1997), and *Escherichia coli* (Clarke et al., 1989). These pathogenic microorganisms can occur from infected dams or any other cow providing colostrum or may be from contamination during colostrum harvesting, storage, or handling (Stewart et al., 2005). Heat treatment applied to colostrum for 60 min at 60°C has been found to be effective at reducing the total bacterial count while preserving the colostrum IgG levels (Godden et al., 2006). Heat-treated colostrum helps to improve passive immune transfer while preventing the transmission of disease from the dam to the calf. In the United States, up to 15% of farmers use heat-treated colostrum (ISU, 2013). The heating of colostrum is labor-intensive, and a pasteurizer may cost \$5,000 to \$15,000 (Quigley, 2011). The other potential adverse effects of heat treatment are deterioration of colostral IgG and bioactive components in colostrum (Lakritz et al., 2000; Godden et al., 2003). Heat treatment also reduced the concentrations of 45 types of proteins compared with raw colostrum (Tacoma et al., 2017), and it decreased colostral insulin (22%), IGF-I, and IgA levels (Mann et al., 2020).

Discrepancies regarding heating temperature applied to colostrum are observed from the literature, with reports of treatments at 76°C (Lakritz et al., 2000), 63°C (Lakritz et al., 2000; Godden et al., 2003; Teixeira et al., 2013), and 60°C (Godden et al., 2012; Gelsinger et al., 2014, 2015a). The positive effect of heat-treated colostrum has called for much research in the last 2 decades, but the results of heat-treated colostrum and its effect on immune calf status remain inconsistent. To address this gap, the objective of this work was to perform a meta-analysis on the association between the characteristics of heat-treated colostrum and the concentrations of colostrum IgG, serum IgG, and STP.

MATERIALS AND METHODS

A comprehensive literature search was conducted for the creation of a data set using the PUBMED (<https://www.ncbi.nlm.nih.gov/pubmed/>), Agricola (<https://agricola.nal.usda.gov/>), CABI (<https://www.cabi.org/publishing-products/animal-science-database/>), and Google Scholar (<https://scholar.google.com/>) databases. The number of articles that appeared for each key word was noted for all databases. For PubMed, the following key words were used: “heat treated colostrum” (48), “heat treated colostrum and calves” (20), “heat treatment colostrum” (125), “heat treatment colostrum and calves” (46), “pasteurized colostrum” (62), “passive transfer in calves” (328), and “passive immunization and calves” (228). For Agricola, we used the follow-

ing key words: “heat treated colostrum” (34), “heat treated colostrum and calves” (20), “heat treatment colostrum” (70), “heat treatment colostrum and calves” (27), “pasteurized colostrum” (34), “passive transfer in calves” (190), “passive immunization and calves” (357). In CABI, we used the following key words: “heat treated colostrum” (119), “heat treated colostrum and calves” (61), “heat treatment colostrum” (220), “heat treatment colostrum and calves” (86), “pasteurized colostrum” (179), “passive transfer in calves” (474), “passive immunization and calves” (378). For Google Scholar, we used the following key words: “heat treated colostrum” (200), “heat treated colostrum and calves” (100), “heat treatment colostrum” (100), “heat treatment colostrum and calves” (100), “pasteurized colostrum” (70), “passive transfer in calves” (100), “passive immunization and calves” (100). The reference list of each selected article was further screened to identify other potentially relevant articles. The Google Scholar search was stopped after 30 consecutive articles were judged not to be related to the research question.

Inclusion and Exclusion Criteria

To be included, studies needed have a control group of calves fed fresh or frozen colostrum and a treated group fed heat-treated colostrum (Figure 1). They also had to be peer-reviewed articles and written in English. At least 1 of the 3 following criteria needed to be present: (1) assessment of the colostrum IgG for calves of the 2 groups, (2) assessment of the serum or plasma IgG for calves of the 2 groups, or (3) assessment of the STP for calves of the 2 groups. The raw data for studies included in the meta-analysis for colostrum IgG, serum IgG, and STP are presented in Tables 1, 2, and 3, respectively. The references of all selected publications were screened to identify potential new relevant publications. All publications were manually screened to identify duplicates.

Data Extraction

The 21 selected publications were thoroughly studied by 2 authors, and data were extracted and implemented in the data set. When available, the information retained included (1) heating temperature applied to colostrum during the heating process, (2) duration of heat treatment applied to colostrum, (3) the sample sizes for the 2 groups of each trial, (4) the method for IgG analysis [ELISA, radial immunodiffusion (RID), turbidimetric immunoassay (TIA)], (5) the mean colostrum IgG concentration (and its variance, SD or SEM), (6) the blood sampling time after colostrum feeding, (7) the mean serum IgG and STP concentrations (and

Figure 1. The flow diagram of data screening from initial search to final selection of publications to be included in the meta-analysis.

their variances, SD or SEM), (8) the techniques carried out to analyze the colostrum IgG, serum IgG (RID, ELISA, TIA), and STP (Brix refractometer), and (9) sample size. All the responses of interest were standardized to the same unit. For missing data related to IgG or STP means or variance, emails were sent to corresponding authors of the respective studies. For those who had not replied after 7 d, a second reminder

email was sent to collect missing information. For the studies that were found to have multiple treated and one control group comparison, the data from these experiments were extracted, and calculations were carried out using RevMan 5.4 (The Cochrane Centre, The Cochrane Collaboration). Only 2 studies were found to have more than 1 treated group and 1 control; Hesami et al. (2021) had 1 control and 3 heat-treated colostrum

Table 1. Summary of trials included in the meta-analysis for colostrum IgG concentration

Study ¹	Heat-treated colostrum IgG (g/L; mean $\pm$ SD)	Raw or frozen colostrum IgG (g/L; mean $\pm$ SD)	Sample size (n)	Heating temperature (°C)	Heating time (min)	IgG analysis Method ²
Godden et al. (2003)	42.70 $\pm$ 10.39	58.90 $\pm$ 11.95	27	>60	30	RID
McMartin et al. (2006)	77.10 $\pm$ 12.69	73.60 $\pm$ 10.88	6	$\leq$ 60	120	TIA
McMartin et al. (2006)	70.40 $\pm$ 11.19	71.60 $\pm$ 13.89	6	$\leq$ 60	120	TIA
McMartin et al. (2006)	66.10 $\pm$ 9.48	73.00 $\pm$ 18.08	6	>60	120	TIA
McMartin et al. (2006)	58.50 $\pm$ 12.0	73.30 $\pm$ 14.6	6	>60	120	TIA
McMartin et al. (2006)	43.70 $\pm$ 10.88	76.10 $\pm$ 14.7	6	>60	120	TIA
McMartin et al. (2006)	74.50 $\pm$ 24.26	76.40 $\pm$ 26.45	30	$\leq$ 60	120	TIA
McMartin et al. (2006)	46.00 $\pm$ 13.20	77.00 $\pm$ 26.18	30	>60	120	TIA
Johnson et al. (2007)	67.30 $\pm$ 11.50	72.60 $\pm$ 13.5	25	$\leq$ 60	60	TIA
Elizondo-Salazar and Heinrichs (2009)	66.17 $\pm$ 4.556	69.55 $\pm$ 4.55	6	$\leq$ 60	30	RID
Donahue et al. (2012)	59.20 $\pm$ 21.20	60.70 $\pm$ 21.2	266	$\leq$ 60	60	TIA
Godden et al. (2012)	61.10 $\pm$ 20.40	63.90 $\pm$ 19.2	266	$\leq$ 60	60	TIA
Gelsinger et al. (2015a), LB	71.40 $\pm$ 12.49	67.30 $\pm$ 7.10	6	$\leq$ 60	30	RID
Gelsinger et al. (2015a), HB	67.30 $\pm$ 7.593	71.80 $\pm$ 14.94	6	$\leq$ 60	30	RID
Gelsinger et al. (2015b)	69.47 $\pm$ 18.78	77.94 $\pm$ 18.78	45	$\leq$ 60	30	RID
Gelsinger et al. (2015b)	20.23 $\pm$ 18.78	40.08 $\pm$ 18.78	45	$\leq$ 60	30	ELISA
Kryzer et al. (2015)	77.70 $\pm$ 10.95	78.93 $\pm$ 10.93	56	$\leq$ 60	60	RID
Rafiei et al. (2019)	57.56 $\pm$ 0.939	60.59 $\pm$ 0.939	5	$\leq$ 60	30	RID
Saldana et al. (2019), LQ	46.00 $\pm$ 2.058	52.30 $\pm$ 2.058	6	$\leq$ 60	60	RID
Saldana et al. (2019), MQ	54.90 $\pm$ 2.058	65.70 $\pm$ 2.058	6	$\leq$ 60	60	RID
Saldana et al. (2019), HQ	87.60 $\pm$ 2.058	98.10 $\pm$ 2.058	6	$\leq$ 60	60	RID

¹HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum.

²RID = radial immunodiffusion; TIA = turbidimetric immunoassay.

groups, and Kryzer et al. (2015) had 1 control and 2 heat-treated colostrum groups.

Statistical Analysis

A meta-analysis was carried out using R Studio (R version 4.0.4) with the R Packages Meta (Schwarzer, 2007) and DMETAR (Mathias et al., 2021). A random-effects model was conducted with the concentrations of colostrum IgG, serum IgG, and STP as outcome variables to assess the effect size, its 95% confidence interval, and the statistical significance. Additionally, a 3-level meta-analytic random-effects model was applied to find out the dependency between effect sizes in the data set that was to be analyzed. Applying a 3-level structure to a meta-analytic model (Cheung, 2014) considers 3 different variance components distributed over the 3 levels of the model as follows: sampling variance of the extracted effect sizes at level 1, variance between effect sizes extracted from the same study at level 2, and variance between studies at level 3.

The effect size was calculated as the mean difference (MD) for each outcome variable between calves receiving heat-treated colostrum and raw or frozen colostrum (outcome for heat-treated colostrum – outcome for raw or frozen colostrum; Borenstein et al., 2009). The MD were weighted using the generic inverse variance of the random-effect meta-analysis (DerSimonian and Laird, 2015). Subgroup analysis was carried out for

heat treatments >60°C (high temperature; **HT**) and $\leq$ 60°C (low temperature; **LT**). There was no subgroup analysis in STP because all the studies that met the inclusion criteria were in the LT subgroup. An overall analysis (HT and LT) of all studies was also carried out for each outcome.

Variations among studies were assessed by the χ^2 test or the Q test (Cochrane's test of heterogeneity), as well as with I^2 (Higgins et al., 2003) and τ^2 values. The I^2 value was defined as $I^2 = (Q - df/Q) \times 100$, where Q is the χ^2 statistic and df is its degree of freedom. Values of I^2 at 0 to 40% were considered possibly not important, 30 to 60% were considered moderate, 50 to 90% were considered substantial, and 75 to 100% were considered considerable heterogeneity (Higgins et al., 2003).

Forest Plot, Bias, and Influence Analysis

The effects of heat treatment and raw or frozen colostrum on the concentrations of colostrum IgG, serum IgG, and STP are displayed separately in respective forest plots using the estimated MD. A contour-enhanced funnel plot for each outcome was created to assess the risk of bias in the studies included in the meta-analysis. Asymmetrical distribution of studies around the calculated random-effect size (MD) indicated no risk of bias, or an asymmetrical distribution around effect size (MD) was an indication of the potential risk of bias. The presence of bias was identified by Egger's test (Eg-

Table 2. Summary of trials included in the meta-analysis for serum IgG concentration

Study ¹	Heat-treated colostrum		Raw or frozen colostrum		Heating temperature (°C)	Blood sampling time (h)	IgG analysis method ²	Heating time (min)	Colostrum quantity offered to calves (L)
	Serum IgG (g/L; mean ± SD)	Sample size (n)	Serum IgG (g/L; mean ± SD)	Sample size (n)					
Lakritz et al. (2000)	7.95 ± 17.56	4	19.46 ± 17.72	7	>60	24	RID	15	4.0
Tyler et al. (2000)	4.19 ± 5.47	5	13.55 ± 13.55	5	>60	24	RID	15	4.0
Tyler et al. (2000)	3.9 ± 1.42	5	4.96 ± 2.17	5	>60	24	RID	15	4.0
Godden et al. (2003)	10.2 ± 5.19	75	17.6 ± 9.00	48	>60	48	RID	15	4.0
Johnson et al. (2007)	22.34 ± 4.55	24	18.07 ± 5.95	25	<60	24	RID	60	3.8
Elizondo-Salazar and Heinrichs (2009)	25.3 ± 4.45	10	19.1 ± 4.45	10	<60	24	RID	30	3.8
Elizondo-Salazar and Heinrichs (2009)	23.6 ± 4.45	10	18.4 ± 4.45	10	<60	24	RID	30	3.8
Godden et al. (2012)	18.4 ± 34.63	533	15.4 ± 34.13	518	<60	84	TIA	60	3.8
Teixeira et al. (2013)	23.1 ± 15.18	309	22.5 ± 15.32	281	>60	72	ELISA	60	4.0
Gelsinger et al. (2014), HQ	27.5 ± 15.80	26	23.3 ± 16.10	27	<60	48	RID	30	3.8
Gelsinger et al. (2014), MQ	22.5 ± 16.0	25	18.1 ± 15.67	24	<60	48	RID	30	3.8
Gelsinger et al. (2014), LQ	14.8 ± 15.12	21	13.3 ± 15.00	22	<60	48	RID	30	3.8
Gelsinger et al. (2015a), LB	20.97 ± 8.31	27	20.19 ± 8	25	<60	48	RID	30	3.78
Gelsinger et al. (2015a), HB	8.79 ± 8.15	26	10.72 ± 8	25	<60	48	RID	30	3.78
Gelsinger et al. (2015a), LB	14.87 ± 9.35	27	12.13 ± 9	25	<60	48	ELISA	30	3.78
Gelsinger et al. (2015a), HB	8.79 ± 9.17	26	6.78 ± 9	25	<60	48	ELISA	30	3.78
Kryzer et al. (2015)	40.6 ± 5.02	28	35.29 ± 0.96	56	<60	24	RID	60	3.8
Cummins et al. (2017)	34.2 ± 12.74	15	41.4 ± 10.26	15	<60	24	RID	60	2.89
Gelsinger and Heinrichs (2017)	20.5 ± 6.85	13	23.3 ± 6.85	13	<60	36	RID	60	3.3
Gelsinger and Heinrichs (2017)	20.4 ± 6.48	13	21.7 ± 6.48	13	<60	36	ELISA	60	3.3
Raffei et al. (2019)	15.37 ± 4.15	20	12.53 ± 4.15	20	<60	24	RID	30	4.3
Raffei et al. (2019)	12.95 ± 4.15	20	11.37 ± 4.15	20	<60	72	RID	30	4.3
Saldana et al. (2019), LQ	21.6 ± 10.73	12	19.4 ± 10.73	12	<60	24	RID	60	3.8
Saldana et al. (2019), MQ	26.3 ± 10.73	12	18.2 ± 10.73	12	<60	24	RID	60	3.8
Saldana et al. (2019), HQ	27.2 ± 10.73	12	21.4 ± 10.73	12	<60	24	RID	60	3.8
Mann et al. (2020)	37.5 ± 16.58	11	42 ± 16.58	11	<60	24	RID	60	4.0
Hesami et al. (2021)	16.64 ± 2.28	36	13.7 ± 2.04	12	<60	24	ELISA	60	5.0

¹HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum.

²RID = radial immunodiffusion; TIA = turbidimetric immunoassay.

Table 3. Summary of trials included in the meta-analysis for serum total protein concentration¹

Study	Heat-treated colostrum		Raw or frozen colostrum		Blood sampling time (h)	Heating time (min)	Colostrum quantity (L)
	STP (g/dL; mean $\pm$ SD)	Sample size (n)	STP (g/dL; mean $\pm$ SD)	Sample size (n)			
Johnson et al. (2007)	6.34 $\pm$ 0.5	25	5.92 $\pm$ 0.68	24	24	60	3.8
Elizondo-Salazar and Heinrichs (2009)	6.25 $\pm$ 0.72	10	5.70 $\pm$ 0.72	10	24	30	3.8
Elizondo-Salazar and Heinrichs (2009)	6.14 $\pm$ 0.72	10	5.68 $\pm$ 0.72	10	48	30	3.8
Gelsinger et al. (2014), HQ	7.90 $\pm$ 5.09	26	8.00 $\pm$ 5.19	27	48	30	3.8
Gelsinger et al. (2014), MQ	7.50 $\pm$ 5.0	25	7.40 $\pm$ 4.89	24	48	30	3.8
Gelsinger et al. (2014), LQ	7.40 $\pm$ 4.58	21	6.80 $\pm$ 4.69	22	48	30	3.8
Gelsinger et al. (2015a), LB	8.32 $\pm$ 0.52	27	8.24 $\pm$ 0.50	25	48	30	3.78
Gelsinger et al. (2015a), HB	7.22 $\pm$ 0.51	26	7.38 $\pm$ 0.50	25	48	30	3.78
Kryzer et al. (2015)	6.80 $\pm$ 0.47	28	6.48 $\pm$ 0.09	56	24	60	3.8
Armengol and Fraile (2016)	7.34 $\pm$ 9.35	300	7.27 $\pm$ 9.65	287	84	60	4
Rafiei et al. (2019)	6.80 $\pm$ 0.26	20	6.31 $\pm$ 0.26	20	24	30	4.3
Rafiei et al. (2019)	6.61 $\pm$ 0.26	20	6.21 $\pm$ 0.26	20	72	30	4.3
Saldana et al. (2019), LQ	5.20 $\pm$ 0.34	12	5.30 $\pm$ 0.34	12	24	60	3.8
Saldana et al. (2019), MQ	5.70 $\pm$ 0.34	12	5.60 $\pm$ 0.34	12	24	60	3.8
Saldana et al. (2019), HQ	6.10 $\pm$ 0.34	12	6.10 $\pm$ 0.34	12	24	60	3.8

¹HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum; STP = serum total protein.

ger et al., 1997), and $P < 0.05$ indicated the presence of bias in the funnel plot.

If evidence of heterogeneity was found, a meta-regression analysis was carried out to explore the sources of heterogeneity. The moderators to test for colostrum IgG were heating time applied to colostrum and colostrum IgG analysis methods. For serum IgG, the moderators to test were heating time applied to colostrum, blood sampling time, colostrum volume fed to calves, and serum IgG analysis methods. For STP, the moderators to test were blood sampling time, colostrum volume, and heating time applied to colostrum. The τ^2 values of the models were compared with or without a moderator to evaluate the decrease in heterogeneity. Influence analysis for heterogeneity identification was carried out for the response of interest (colostrum IgG, serum IgG, and STP) using Baujat diagnostics. The Baujat diagnostics (Baujat et al., 2002) identified the respective contribution of each study. Combining the influence (Baujat diagnostics) and heterogeneity (Cochran's QQ) analyses allowed us to highlight studies contributing too much to the overall heterogeneity while having very low influence on the effect size. Studies with a lower number of observations may contribute too much heterogeneity while having a small effect size on the pooled effect size.

RESULTS

Colostrum IgG

The flow diagram of study inclusion and exclusion (Figure 1) led to 21 studies being included. The data set is provided in Table 1. Twelve studies and 21 trials were used for colostrum IgG concentration (Figure 2), with 2 studies, 5 trials, and 75 observations for HT, and 10 publications, 16 trials, and 786 observations for LT. The heterogeneity for colostrum IgG concentration in the HT subgroup was moderate ($I^2 = 65\%$, $\tau^2 = 61.58$, and Q statistics: $\chi^2 = 11.43$). The heterogeneity for IgG concentration in the LT colostrum subgroup was also high ($I^2 = 82\%$, $\tau^2 = 14.94$, and Q statistics: $\chi^2 = 84.46$). The overall (LT and HT) heterogeneity ($I^2 = 85\%$, $\tau^2 = 23.40$, and Q statistics: $\chi^2 = 134.34$) was also high (Table 4).

The influence analysis (Table 5) for colostrum IgG concentration indicated that McMartin et al. (2006) highly contributed to heterogeneity, followed by Rafiei et al. (2019) and Saldana et al. (2019). The meta-regression models showed that the moderator heating time was not significantly associated with the colostrum IgG concentration ($P = 0.185$). The moderator analysis method (RID, ELISA, TIA) was significantly associated ($P < 0.0001$) with IgG colostrum concentra-

Figure 2. Forest plot of the random-effects meta-analysis for colostrum IgG concentration. Subgroup analysis was carried out for (1) heating temperature applied to colostrum $\geq 60^\circ\text{C}$ (HT) and (2) heating temperature applied to colostrum $\leq 60^\circ\text{C}$ (LT). The effect size was calculated as the mean difference (MD). The points to the left of the solid line indicate a decrease in the outcome, whereas the points to the right of the solid line indicate a positive response in outcome. The horizontal lines connected with diamonds represent the upper and lower confidence intervals (95%). The blue diamonds indicate the average effect sizes of the subgroups and the cumulative analysis. The vertical solid line represents the line of no effect or MD zero. HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum.

tion. The estimates for the concentration of colostrum IgG corresponding to the class ELISA, RID, and TIA were -19.85 , -6.41 , and -8.53 g/L, respectively. The meta-regression was not significant when the moderator temperature (HT or LT) and analysis method were included, and the subgroup analyses were retained as the final results (Figure 2). The effect size (MD) indicated (Figure 2) decreases in colostrum IgG by 20.6 g/L (95% CI = 11.8 – 29.4), 5.38 g/L (95% CI = 2.9 – 7.8), and 7.67 g/L (95% CI = 5.0 – 10.3) when heat treatment was compared with raw or frozen colostrum for HT, LT, and overall (HT and LT), respectively. The funnel plot for colostrum IgG concentration (Figure 3) showed a slight bias, but Egger's test was nonsignificant ($P =$

0.174) for the presence of funnel plot asymmetry (95% CI = 0.45 – 2.75).

Serum IgG

For serum IgG concentration, 16 studies and 27 trials met the inclusion criteria (Figure 4), and raw data are provided in Table 2. In the HT subgroup, there were 4 studies in 5 trials and 398 and 346 observations for heat-treated and raw or frozen colostrum, respectively. In the LT subgroup, 12 studies and 22 trials were included, and the numbers of observations were 947 and 932 for heat-treated and raw or frozen colostrum, respectively. The heterogeneity of the serum

Table 4. Comparison of effect size and heterogeneity (I^2) between random-effects meta-analysis model and multilevel random-effects meta-analysis model for colostrum IgG, serum IgG, and serum total protein (STP)

Parameter ¹	Random effect	Multilevel random effect
Colostrum IgG		
Heterogeneity (I^2)	85.0	81.49
HT ES (g/L)	-20.63	-21.31
LT ES (g/L)	-5.38	-4.41
Serum IgG		
Heterogeneity (I^2)	73.0	66.10
HT ES (g/L)	-3.40	-2.78
LT ES (g/L)	2.65	2.59
STP		
Heterogeneity (I^2)	59.0	64.53
LT ES (g/dL)	0.21	0.24

¹HT = heating temperature >60°C; LT = heating temperature applied to colostrum ≤60; ES = effect size.

IgG concentration in the HT colostrum subgroup was high ($I^2 = 81\%$, $\tau^2 = 13.42$, Q statistic: $\chi^2 = 21.0$). The heterogeneity for serum IgG concentration in the LT subgroup was low ($I^2 = 38\%$, $\tau^2 = 2.20$, Q statistic: $\chi^2 = 33.9$). The overall (HT and LT) heterogeneity was high ($I^2 = 73\%$, $\tau^2 = 9.00$, Q statistic: $\chi^2 = 96.42$).

The influence analysis (Table 6) for serum IgG concentration indicated that Godden et al. (2003) was the only main contributor to heterogeneity. The regression model indicated that the moderator heating time ($P = 0.108$), blood sampling time ($P = 0.649$), colostrum quantity ($P = 0.236$), and IgG analysis method (RID, ELISA, TIA; $P = 0.396$) were nonsignificantly associated with serum IgG concentrations. The effect size (MD) indicated a nonsignificant ($P > 0.05$) decrease in serum

Table 5. Influence analysis of colostrum IgG concentration with Baujat diagnostics (sorted by heterogeneity contribution) and weighted effect sizes of the trials included in the meta-analysis

Study ¹	Heterogeneity	Weighted influence of effect size (%)
McMartin et al. (2006), 63°C	22.44	2.327
Rafiei et al. (2019)	19.29	7.747
Saldana et al. (2019), MQ	18.87	7.417
Saldana et al. (2019), HQ	16.72	7.417
Gelsinger et al. (2015a), ELISA	12.89	4.709
McMartin et al. (2006), 63°C	12.85	3.536
Godden et al. (2003)	12.00	5.630
Donahue et al. (2012)	5.069	6.872
Kryzer et al. (2015)	3.171	6.232
Gelsinger et al. (2015a), LB	2.756	3.183
Godden et al. (2012)	2.733	6.984
McMartin et al. (2006), 59°C	1.795	2.631
McMartin et al. (2006), 62°C	1.410	2.219
Elizondo-Salazar and Heinrichs (2009)	0.738	6.070
Gelsinger et al. (2015b), RID	0.511	4.790
McMartin et al. (2006), 60°C	0.372	2.408
McMartin et al. (2006), 60°C	0.326	2.774
Saldana et al. (2019), LQ	0.309	7.417
Gelsinger et al. (2015a), HB	0.028	2.621
McMartin et al. (2006), 61°C	0.023	1.990
Johnson et al. (2007)	0.009	5.115

¹HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum; RID = radial immunodiffusion.

IgG/mL serum in HT colostrum-fed calves; however, in the LT subgroup, a significant ($P < 0.05$) increase of 2.65 g/L of serum IgG in calves fed heat-treated colostrum compared with those fed raw or frozen colostrum was observed. The presence of asymmetry in the funnel plot was absent, and Egger's test did not ($P = 0.315$)

Figure 3. A contour-enhanced funnel plot created between heat-treated versus raw or frozen colostrum for all studies included in the meta-analysis for colostrum IgG concentration. The asymmetrical presentation of studies around the mean difference (MD) indicates publication bias for colostrum IgG concentration.

indicate the presence of funnel plot asymmetry (95% CI = 0.64–2.04; Figure 5).

Serum Total Protein

There were 8 studies and 15 trials in the STP meta-analysis (Figure 6), corresponding to 574 and 586 observations of heat-treated colostrum and raw or frozen colostrum, respectively; only data for the LT subgroup were available. The heterogeneity for STP concentration in LT colostrum was moderate ($I^2 = 59\%$, $\tau^2 = 0.033$, Q statistic: $\chi^2 = 34.32$).

The influence analysis (Table 7) for STP concentration indicated that Gelsinger et al. (2015a) and Rafiei et al. (2019) both contributed to the heterogeneity. The meta-regression models showed that the moderator heating time ($P = 0.378$) and blood sampling time ($P = 0.943$) were not significantly associated with the STP concentration, but the association was significant for colostrum quantity fed to calves ($P = 0.004$; Figure 7). Out of 15 studies, only 3 studies showed a decrease in STP (g/dL) in calves fed LT heat-treated colostrum. The effect size (MD) indicated that there was a minor increase (STP = 0.21 g/dL; 95% CI = 0.07–0.35) in

Figure 4. Forest plot of random-effects meta-analysis for serum IgG concentration. Subgroup analysis was carried out for (1) heating temperature applied to colostrum $\geq 60^\circ\text{C}$ (HT) and (2) heating temperature applied to colostrum $\leq 60^\circ\text{C}$ (LT). The effect size was calculated as the mean difference (MD), and the solid vertical line represents the line of no effect or the zero line. The dotted vertical line represents the average effect size of heat-treated colostrum on serum IgG concentration; a negative value indicates a decrease in IgG concentration, and a positive value indicates an increase in IgG concentration in serum in calves fed heat-treated colostrum. The blue diamonds indicate the average effect sizes of the subgroups and the cumulative analysis. HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum. HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum; RID = radial immunodiffusion.

Table 6. Influence analysis of serum IgG with Baujat diagnostics (sorted by heterogeneity contribution) and weighted effect sizes of the trials included in the meta-analysis

Study ¹	Heterogeneity	Weighted influence of effect size (%)
Godden et al. (2003)	41.78	5.392
Kryzer et al. (2015)	12.98	6.006
Tyler et al. (2000)	6.295	1.153
Elizondo-Salazar and Heinrichs (2009), 24 h	4.747	4.592
Cummins et al. (2017)	4.595	2.220
Gelsinger and Heinrichs (2017), RID	3.002	3.674
Tyler et al. (2000)	2.944	5.756
Elizondo-Salazar and Heinrichs (2009), 48 h	2.813	4.592
Gelsinger et al. (2015a), HB, RID	2.799	4.220
Johnson et al. (2007)	2.556	5.282
Hesami et al. (2021)	2.388	6.276
Saldana et al. (2019), MQ	2.029	2.112
Gelsinger and Heinrichs (2017), ELISA	1.537	3.850
Lakritz et al. (2000)	1.464	0.455
Teixeira et al. (2013)	0.996	5.630
Saldana et al. (2019), HQ	0.810	2.112
Mann et al. (2020)	0.808	1.011
Rafiei et al. (2019), 24 h	0.560	5.553
Gelsinger et al. (2014), MQ	0.316	2.022
Godden et al. (2012)	0.291	4.414
Gelsinger et al. (2014), HQ	0.286	2.112
Gelsinger et al. (2015a), LB, RID	0.226	4.220
Gelsinger et al. (2015a), LB, ELISA	0.121	3.850
Rafiei et al. (2019), 72 h	0.044	5.553
Gelsinger et al. (2014), LQ	0.006	1.978
Saldana et al. (2019), LQ	0.006	2.112
Gelsinger et al. (2015a), HB, ELISA	0.004	3.850

¹HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum; RID = radial immunodiffusion.

STP concentration in calves fed LT heat-treated colostrum compared with those fed raw or frozen colostrum. The studies included in the STP meta-analysis indicated a symmetrical distribution around the funnel plot (Figure 8), and Egger's test was nonsignificant ($P = 0.465$).

DISCUSSION

The present results highlighted that heat treatment of colostrum decreases colostrum IgG by 20.6 g/L for HT and by 5.38 g/L for LT ($P < 0.05$, Figure 2). The subgroups HT ($>60^{\circ}\text{C}$) and LT ($\leq 60^{\circ}\text{C}$) were defined in accordance with McMartin et al. (2006) because heating temperature $>60^{\circ}\text{C}$ may decrease the concentration of IgG, whereas LT ($\leq 60^{\circ}\text{C}$) has no adverse effects on IgG concentration. The decrease in the concentration of IgG in heat-treated colostrum is associated with denaturation of IgG by high temperature (McMartin et al., 2006).

The heat treatment of colostrum was also associated with a nonsignificant ($P > 0.05$) decrease in serum IgG for HT but a significant ($P < 0.05$) increase in serum IgG by 2.65 g/L for LT (Figure 4). The serum IgG in HT heat-treated colostrum has been described as reduced or unchanged, and the present meta-analysis concluded that there was no significant decrease in serum IgG for the HT subgroup. Only 5 trials were available and included in the meta-analysis of the HT subgroup. Heat treatment of colostrum $>60^{\circ}\text{C}$ has been reported to destroy or alter colostral IgG and colostral proteins, leading to decreased availability or absorption of IgG from colostrum to the bloodstream

Figure 5. A contour-enhanced funnel plot created between heat-treated vs. raw or frozen colostrum for all studies included in the meta-analysis for serum IgG concentration. The slight asymmetrical presentation of studies around the mean difference (MD) indicates that slightly biased publications are included in this meta-analysis.

Figure 6. Forest plot of the random-effects meta-analysis for serum total protein meta-analysis. In all studies, the heating temperature applied to colostrum was $\leq 60^\circ\text{C}$. The effect size was calculated as the mean difference (MD), and the solid vertical line represents the line of no effect or the zero line. The dotted vertical line represents the average effect size of heat-treated colostrum on serum total protein concentration, a negative value indicates a decrease in serum total protein concentration, and a positive value indicates an increase in serum total protein concentration in calves fed heat-treated colostrum. The blue diamond indicates the average effect size of the meta-analysis. HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum. HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum.

(Lakritz et al., 2000; Godden et al., 2003; McMartin et al., 2006). In contrast, the increase in serum IgG in the LT subgroup could be linked to increased absorption of IgG, and antibodies in colostrum have been shown to bind pathogens present in the gut before absorption can occur. By reducing the number of pathogens in heat-treated colostrum, more antibodies are potentially free for absorption (Elizondo-Salazar and Heinrichs, 2009). Another potential explanation for the increase

in serum IgG is the lack of bacterial interference at the receptors that are responsible for IgG absorption. Bacteria can bind nonspecific receptors on neonatal

Table 7. Influence analysis of serum total protein with Baujat diagnostics (sorted by heterogeneity contribution) and weighted effect sizes of the trials included in the meta-analysis

Study ¹	Heterogeneity	Weighted influence of effect size (%)
Gelsinger et al. (2015a), HB	8.778	9.356
Rafiei et al. (2019)	7.412	12.31
Saldana et al. (2019), LQ	6.444	9.356
Saldana et al. (2019), HQ	3.354	9.356
Rafiei et al. (2019)	2.762	12.31
Gelsinger et al. (2015a), LB	1.602	9.356
Saldana et al. (2019), MQ	1.264	9.356
Johnson et al. (2007)	0.876	7.929
Elizondo-Salazar and Heinrichs (2009), 24 h	0.800	3.586
Kryzer et al. (2015)	0.451	11.99
Elizondo-Salazar and Heinrichs (2009), 48 h	0.382	3.586
Gelsinger et al. (2014), HQ	0.064	0.245
Gelsinger et al. (2014), LQ	0.058	0.245
Armengol and Fraile (2016)	0.058	0.768
Gelsinger et al. (2014), MQ	0.013	0.245

¹HB = high bacterial count; LB = low bacterial count; LQ = low-quality colostrum; HQ = high-quality colostrum; MQ = medium-quality colostrum.

Figure 7. Graph of colostrum quantity fed (x-axis) to calves and mean effect size [y-axis = mean difference (MD); serum total protein] of the individual studies; spheres below the 0 on the y-axis indicate the decrease in effect size and vice versa. The round spheres represent studies.

enterocytes, thus reducing the number of receptors available for immunoglobulin uptake (Johnson et al., 2007). Subsequently, by reducing the number of pathogens in heat-treated colostrum, more antibodies are potentially free for absorption (Staley and Bush, 1985). The effect size observed in the present meta-analysis (+2.65 g/L of serum IgG for LT compared with raw or frozen colostrum) can be seen as very important when compared with expected values for serum IgG in the field. A higher concentration of serum IgG has positive effects on the immune status of calves (Godden et al., 2012), even after crossing the threshold level (>10 g/L) of passive transfer of immunity (Weaver et al., 2000). Failure of passive transfer varies with serum IgG level, and IgG <4 was at greater risk than 4 to <10 g/L, with 6.3% and 12.9%, respectively (Beam et al., 2009). The mortality rate of calves with serum IgG concentrations <10 g/L was more than twice as high as that of calves with IgG concentrations >10 g/L (Wells et al., 1996). The results for STP for the LT subgroup (Figure 8) are in agreement with the results of serum IgG. The measurement of STP by refractometry is an indirect estimation of the immune status of calves, which is highly correlated with serum IgG contents and is more practical for on-farm management (Thornhill et al., 2015).

Considering the analysis performed on the HT and LT subgroups, the present results highlighted the usefulness of colostrum heat treatment and its expected effect on calf immune status in the field. The present meta-analysis faced difficulties in substantially reducing the heterogeneity of the data set, and, unfortunately, no moderator except temperature of colostrum treatment was significantly associated with the outcome.

The multilevel random-effects model was also included in our analysis; the findings suggested that marginal differences for overall heterogeneity (I^2) and effect size between the random-effect model and the multilevel random-effects model. A comparison between the random-effects model and multilevel random-effects model for heterogeneity and effects size (colostrum IgG, serum IgG, and STP) is presented in Table 4. The distribution of heterogeneity at various levels (Figure 9) indicated that sampling variance (level 1, I^2) of the extracted effect sizes were 18.51%, 33.92%, and 35.47% for colostrum IgG, serum IgG, and STP, respectively. The variance between effect sizes extracted from the same study (level 2, I^2) were 34.61%, 0%, and 0% for colostrum IgG, serum IgG, and STP, respectively. The variance between studies (level 3, I^2) were 46.88%, 66.08%, and 64.53% for colostrum IgG, serum IgG, and STP, respectively. Some studies were identified as major contributors to heterogeneity (Tables 5 and 6). The major reason for the higher contribution to heterogeneity is the smaller sample size (≤ 6) in all these studies. Smaller studies are more heterogeneous than larger studies, as also reported earlier (Int'Hout et al., 2015). This means that the limits of the heterogeneity reduction arise from the raw data structure and not from the meta-analysis implementation. The analyses indicate substantial heterogeneity (I^2 ; 50–90%; Higgins et al., 2003) for colostrum and serum IgG concentration. The commonly recommended approaches to address heterogeneity are subgroup analysis, random-effect meta-analysis, and meta-regression, as done in the present analysis (Higgins et al., 2019). Meta-regression (i.e., adding moderators for colostrum IgG such as heating time) revealed

Figure 8. A contour-enhanced funnel plot created between heat-treated vs. raw or frozen colostrum for all studies included in the meta-analysis for serum total protein concentration. The slight asymmetrical presentation of studies around the mean difference (MD) indicates slight publication bias in this meta-analysis.

Figure 9. Panel a represents the variance at different levels for colostrum IgG; b represents variance at different levels of serum IgG concentration; and c represents variance at different levels for serum total protein. The variance components are identified by multilevel random effects in a meta-analysis. Level 1 = sampling variance of the extracted effect sizes, level 2 = variance between effect sizes extracted from the same study, level 3 = variance between studies.

that heating time did not influence effect size. However, the colostrum IgG analysis method (RID, ELISA, TIA, categorical moderator) was associated with the colostrum IgG concentration. The heterogeneity was not reduced in the bivariate meta-regression for IgG colostrum concentration. The heterogeneity for serum IgG was not explained by the addition of any moderator. The observed heterogeneity was likely to be clinical or methodological rather than statistical, as suggested by the lack of variation in the direction of effect sizes; for instance, 18 out of 20 comparisons showed decreases in IgG concentration in colostrum compared with raw or frozen colostrum. Clinical heterogeneity could be associated with colostrum quality, animal breed (Maunsell et al., 1999), parity of colostrum donors (Muller and Ellinger, 1981), the season of calving (Cabral et al., 2016), and dam nutrition (Feyera et al., 2019). These data were not provided in most of the raw publications included here. Importantly, the main result of interest and its field application arising from the present work is the increase in serum IgG (+2.65 g/L) in LT as compared with raw or frozen colostrum (Figure 4). The heterogeneity for the LT subgroup for serum IgG is low ($I^2 = 38\%$), demonstrating the robustness of the present results.

Visual interpretation of funnel plots is a common method for the identification of publication bias. In ad-

dition, Egger's test was nonsignificant ($P > 0.05$) for all parameters for colostrum IgG, serum IgG, and STP. A possible explanation for the asymmetrical funnel plot for colostrum IgG concentration was the exclusion of gray literature (proceedings, abstracts, theses, technical reports, and dissertations). To avoid publication bias in favor of studies with positive outcomes, studies reported in the gray literature would need to be included in meta-analyses (Borenstein et al., 2009). Such types of studies may yet adopt a poor peer review process compared with those published in peer-reviewed journals (Weisz et al., 1995).

CONCLUSIONS

The present work demonstrated that heat treatment of colostrum $<60^\circ\text{C}$ decreased colostrum IgG by 5.38 g/L and increased serum IgG by 2.65 g/L and STP by 0.21 g/dL. When compared with the range of values observed in the field for serum IgG, the effect size was of high interest for the cattle industry, and these results clearly demonstrated the added value of colostrum heat treatment in the field. Because immune benefits in colostrum also include cytokines and immune cells, further work is required to evaluate the effect of colostrum heat treatment on these 2 immune components of colostrum.

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