

INTERPRETIVE SUMMARY

1
2 **Changes in biomarkers of metabolic stress during late gestation of dairy cows associated with**
3 **colostrum volume and immunoglobulins content.** *By Rossi et al., page XXXX.* We investigated
4 associations between metabolic stress during the last 6 wk of gestation and the volume and
5 immunoglobulin content of the colostrum produced. We observed that cows producing more than
6 6 L of colostrum exhibited increased metabolic activity during late gestation. Also, a greater blood
7 antioxidant activity throughout late gestation was observed in cows with higher yields of
8 colostrum, suggesting that greater availability of antioxidants might support the production of
9 higher volumes of colostrum. Therefore, further studies should evaluate whether supplementation
10 with additional antioxidants supplement during late gestation can improve colostrum yield.

11 METABOLIC STRESS AND COLOSTRUM PRODUCTION
12 **Changes in biomarkers of metabolic stress during late gestation of dairy cows associated**
13 **with colostrum volume and immunoglobulin content**

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21 **ABSTRACT**

22 The objective of this observational study was to compare the metabolic status of dairy cows during
23 the last 6 wk of gestation based on colostrum volume and Ig content. For this, healthy Holstein
24 cows were randomly selected from 3 commercial herds in Michigan. In each farm, four cohorts of
25 21 cows (one per season), stratified by parity, were enrolled (n=228). Cows were blood sampled
26 weekly during the last 6 wk of gestation, and biomarkers related to nutrient utilization, oxidant
27 status, and inflammation were quantified in serum. Cows were milked within 6 h of calving and
28 the volume of colostrum produced was recorded and an aliquot collected. Concentration of IgG,
29 IgA, and IgM were measured by radial immunodiffusion. Cows were grouped into high colostrum
30 producer (**HCP**) or low (**LCP**), high IgG (**HIG**) or low (**LIG**), high IgA (**HIA**) or low (**LIA**), and
31 high IgM (**HIM**) or low (**LIM**). For volume category, we arbitrarily defined 6 L of colostrum (4
32 L for first and 2 L for second feeding of calves) as the cut-off point, whereas for IgG we used the
33 industry standard of $\geq 50\text{g/L}$. To create groups of low and high IgM or IgA, we used the median
34 of these Ig as the cutoff point. Colostrum volume was lowest in winter, but no differences were
35 observed among parity groups. Conversely, colostrum IgG concentration was highest in fall and
36 winter, but colostrum IgM was lowest at these seasons. However, colostrum Ig content only
37 showed a negative weak correlation with volume (Spearman $\rho < -0.28$). Compared to LCP, HCP
38 cows had higher concentrations of antioxidant potential, BHB and lower cholesterol and oxidant
39 status index. HIG cows showed higher concentrations of glucose compared to LIG. HIA cows had
40 higher concentrations of cholesterol, reactive oxygen and nitrogen species, oxidant status index,
41 and total protein, while BHB, and glucose were lower compared with LIA. Biomarkers of
42 metabolic stress were not significantly different between HIM and LIM. Nevertheless, the
43 differences observed did not result in differences in inflammatory status between animals in any

44 of the colostrum variable categories analyzed, suggesting that physiological homeostasis was not
45 disrupted during late gestation in association with the colostrum variables studied. Overall, the
46 great variability observed in colostrum variables suggests that colostrogenesis is a complex and
47 multifactorial process. However, our results suggest that greater availability of antioxidants during
48 late gestation could support the production of higher volumes of colostrum, which needs to be
49 explored in future trials.

50

51 **Keywords:** Antioxidants; Colostrogenesis; Inflammation; Oxidative Stress.

52

INTRODUCTION

53 Despite improvements in dairy calf health management practices over the last decades, preweaning
54 morbidity and mortality incidence risks in US herds are still high, at 33.9% and 5%, respectively
55 (Urie et al., 2018). One of the major contributors to preweaning disease occurrence is failure of
56 transfer passive immunity, a problem that still has a high prevalence of 13% in US dairy herds
57 (Raboisson et al., 2016). Providing insufficient volume and/or low quality of colostrum within the
58 first hours of life is the major contributing factor to failed immunity transfer in calves (Morin et
59 al., 1997). Traditionally, a volume of colostrum of 10 – 12% of the calves' body weight, given in
60 one single meal, was recommended as a feeding strategy to transfer passive immunity successfully
61 (Godden, 2008). However, feeding 4 L of high-quality colostrum within 6 hours of birth and 2 L
62 at 12 h of life has been recently recommended to optimize the transfer of passive immunity and
63 calf health (Hammon et al., 2013; Godden et al., 2019). In fact, calves that received a second
64 colostrum meal within the first 12 h of birth had greater ADG and lower failed immunity transfer
65 and preweaning morbidity risks than calves that only received 1 meal (Abuelo et al., 2021).

66 However, there is considerable variability in colostrum production among cows (Morin et
67 al., 1997; Gavin et al., 2018; Kessler et al., 2020), making it difficult to harvest the volume of
68 colostrum needed to sustain this feeding regime in some cases. Colostrogenesis has been an active
69 area of research and some aspects, such as IgG transfer from bloodstream, have been reviewed
70 extensively (Barrington et al., 2001; Baumrucker and Bruckmaier, 2014). However, the scientific
71 evidence on factors affecting the volume of colostrum being produced is still limited.
72 Colostrogenesis starts 3 to 4 weeks before calving. At this time, cows start experiencing metabolic
73 adaptations in preparation to the onset of lactation. However, cows may develop metabolic stress
74 if they fail to physiologically adapt to the profound increase in nutrient requirements associated

75 with fetal growth and milk production (Sordillo and Mavangira, 2014). Metabolic stress is
76 characterized by excessive lipid mobilization, oxidative stress, and inflammatory dysfunction
77 (Abuelo et al., 2015). The negative effect of metabolic stress on the immune function, health, and
78 production of dairy cattle during this period is well established (Kehrli Jr et al., 1989; Sordillo and
79 Aitken, 2009; Bradford et al., 2015). To the best of our knowledge, however, the association
80 between metabolic stress biomarkers and colostrum production have not yet been examined.
81 Colostrum is more concentrated in nutrients than milk (Godden, 2008), which might result in
82 important nutritional demands for the cow. We, therefore, hypothesized that cows producing high
83 volumes of colostrum and quality as assessed by immunoglobulin content would exhibit increased
84 metabolic activity during late gestation. Thus, the objective of this observational study was to
85 identify the association between biomarkers of metabolic stress during late gestation and colostrum
86 volume and concentration of IgG, IgA, and IgM.

87

88 **MATERIALS AND METHODS**

89 *Animals, Feed, Farms, and Management*

90 All procedures were approved by the Michigan State University Institutional Animal Care and Use
91 Committee (protocol 04/18-065-00) and animals were enrolled with owners' consent. This
92 prospective cohort study was conducted using a convenience sample of three commercial
93 Michigan dairy farms, selected based on location within 50 miles to the university and willingness
94 to participate in the study. The study was designed to have one cohort per season at each farm, a
95 total of four cohorts, to account for the documented changes in colostrum yield associated with
96 season (Gavin et al., 2018; Borchardt et al., 2022). Sampling occurred during the period between
97 June 2019 and September 2020.

98 The sample size was calculated using an online calculator (<https://epitools.ausvet.com.au>)
99 to achieve 90% confidence and 5% precision of within-herd prevalence, resulting in 21 animals
100 per cohort per farm (n=252). Healthy Holstein cows were selected using randomization software
101 (<https://www.graphpad.com/quickcalcs/randomSelect1/>) among those expected to calve 6-8
102 weeks after the start of sampling from a list of cows generated by the herd management software.
103 Healthy animals were defined as not being in the sick pen, not currently receiving any medical
104 treatment, and not displaying sick cow behavior based on the subjective interpretation of the
105 research staff. To reflect common demographics of dairy farms, random selection was stratified
106 by parity groups of cows entering their first, second, or third to fifth lactation. Exclusion criteria
107 for enrollment were cows with a successful breeding later than 150 DIM, and body condition score
108 (BCS) under 2 or over 4 on a scale of 1 – 5 (Wildman et al., 1982). Finally, data from 24 enrolled
109 cows were excluded from analyses due to abortions, injuries resulting in euthanasia, deaths prior
110 to calving, or failure to obtain colostrum data (samples not collected or yield not recorded).
111 Therefore, the complete data from 228 cows were included in the analyses.

112 Housing and characteristics of management practices of late-gestation cows of the three
113 farms are reported in Table 1. Cows had ad libitum access to a total mixed ration and water for the
114 entire dry cow period. Farms A and B had two dietary groups for dry cows (far-off and close-up)
115 whereas farm C managed all dry cows in the same diet. Farms A and C had separate groups for
116 heifers and multiparous cows, whereas in farm B heifers and multiparous cows were separated in
117 the far-off group but not the close-up. Diets were formulated by the farms' nutritionist to meet or
118 exceed NRC (2001) recommendations. Samples of all total mixed rations were collected at 2-
119 weeks intervals from the feed bunk at the time of distribution and sent to an external laboratory
120 for chemical composition analysis (Cumberland Valley Analytical Services, Waynesboro, PA).

121 The composition and chemical analysis results of the diets are summarized in Supplementary
122 Tables S1 and S2.

123

124 ***Sample Collection***

125 Blood samples were obtained weekly starting 6 wk before expected parturition, taken
126 approximately at the time of feed delivery via puncture of coccygeal vessels using two-10mL
127 evacuated tubes with serum separator (BD Vacutainer; Becton, Dickinson and Company, Franklin
128 Lakes, NJ). Blood samples that were collected within 2 days of actual calving date were not
129 considered as the -1 wk point to avoid changes in blood biomarkers due to the hormonal changes
130 associated with calving, considering the previous week sample instead. Tubes were transported to
131 the laboratory on ice, separated within 1 h by centrifugation at $2,000 \times g$ for 20 min at 4°C ,
132 aliquoted, snap-frozen in liquid nitrogen, and stored at -80°C pending analysis within 1 month of
133 collection.

134 Colostrum was harvested by trained farm personnel within 6 h of calving following each
135 farm's routine procedures. The volume of colostrum produced was measured using a graduated
136 bucket (10 Quart Measuring Pail with Handle, United States Plastic Corporation, Lima, OH). A
137 50 mL sample of each cow's colostrum was also collected and kept frozen at -20°C for further
138 analysis.

139

140 ***Analytical Determinations***

141 *Serum samples*

142 Oxidant status was assessed following previously reported methods (Abuelo et al., 2016). The
143 concentrations of reactive oxygen and nitrogen species (**RONs**) in serum were determined as

144 indicator of pro-oxidant production using the OxiSelect In Vitro Reactive Oxygen and Nitrogen
145 Species assay kit (Cell BioLabs Inc., San Diego, CA). Briefly, free radicals present in the sample
146 bind to a dichlorodihydrofluorescein probe, converting it to a fluorescing product (2',7'-
147 dichlorodihydrofluorescein). Thus, the fluorescence intensity is proportional to the concentration
148 of RONS in the sample. The fluorescence of dichlorofluorescent dye was determined at excitation
149 wavelengths of 480 nm and emission of 530 nm in a Synergy H1 Hybrid plate reader (Biotek;
150 Winooski, VT, USA). To ensure fluorescence at various concentrations, a standard curve, made
151 by six serial dilutions (0–10,000 nM) of the fluorescence probe 2',7'-dichlorodihydrofluorescein
152 diacetate was included in each plate. All samples and standards were analyzed in duplicate and
153 those with a CV greater than 10% were re-assayed. Background fluorescence was eliminated by
154 subtracting blank values from sample values. Results are reported as the average relative
155 fluorescence units (RFU) between replicates.

156 The antioxidant potential (**AOP**) of serum samples were determined using trolox (synthetic
157 vitamin E analog) equivalents antioxidant capacity, as described previously (Re et al., 1999).
158 Antioxidant components of serum interact, making it difficult to quantify each antioxidant
159 individually. As a result, this method considers the synergism of all antioxidants present in a
160 sample, including albumin, thiols, bilirubin, and superoxide dismutase. In brief, based on the
161 standard curve of 0–25 g/L, a known volume of trolox standard concentration would result in a
162 similar reduction of the radical 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (Sigma-
163 Aldrich, St. Louis, MO). Samples were analyzed in triplicate, and samples with replicates with
164 CVs greater than 10% were re-assayed. Changes in oxidative balance may occur because of shifts
165 in RONS and/or AOP. Thus, the oxidant status index (**OSi**) was calculated as the ratio between

166 RONS and AOP, as this better characterizes shifts in redox balance in periparturient cows (Abuelo
167 et al., 2013).

168 The serum concentration of BHB, blood urea nitrogen (**BUN**), calcium (**Ca**), cholesterol
169 (**Chol**), glucose (**Glu**), magnesium (**Mg**), nonesterified fatty acids (**NEFA**), albumin (**Alb**), and
170 total protein (**TP**) were quantified using commercial reagents from Catachem Inc. (Bridgeport,
171 CT) as biomarkers of nutrient utilization. As biomarker of inflammation, we determined the
172 concentration of the positive acute phase protein Haptoglobin (**Hp**; Phase Haptoglobin Assay TP-
173 801, Tridelta Development Limited, Maynooth, Ireland). All biomarkers related to nutrient
174 utilization and inflammation were determined using a small-scale biochemistry analyzer
175 (CataChemWell-T; Catachem Inc.) previously validated for cattle (Abuelo et al., 2020). The
176 analyzer was calibrated every week using the assay manufacturer's calibrators. Physiological and
177 pathological reference samples were also analyzed at the time of calibration for two-level quality
178 control. The precision of all biomarkers quantified in this analyzer is reported in Supplementary
179 Table S3.

180

181 *Colostrum samples*

182 The concentration of IgG, IgA, and IgM in colostrum samples were measured via radial
183 immunodiffusion (RID) (Bovine IgG, IgA and IgM test; Triple J Farms, Bellingham, WA)
184 following manufacturer's instructions ([https://kentlabs.com/jjj/triple-j-farms-product-
185 information/rid-plate-procedure/](https://kentlabs.com/jjj/triple-j-farms-product-information/rid-plate-procedure/)). The method is based on the precipitation in agarose gel growing
186 in a circle antigen-antibody complexes which develop after 10-20 hours at room temperature and
187 continues to grow until equilibrium is reached. Briefly, the colostrum samples were thawed
188 overnight at 4 °C. Dilutions of each sample were performed in 0.9% NaCl. Samples were diluted

189 at 1:6, 1:9, 1:10 for IgG analyses and at 1:2, 1:4, and 1:5 for IgA and IgM quantification. Standards
190 were included in each plate for reference and ranged from 1.8 to 28.03 (IgG), 0.53 to 3.87 (IgA),
191 and 0.62 to 3.81 (IgM) g/L. The diffusion ring through the agarose gel containing mono-specific
192 antibody after 24 h of incubation at room temperature was measured using a caliper with a
193 precision of 0.1 mm (VWR traceable caliper; Radnor, PA). The values of the sample's ring were
194 read off the standard curve to determine Ig concentrations in g/L. Samples falling outside of the
195 standard curve were re-assayed using a higher or lower dilution as needed.

196

197 ***Statistical analyses***

198 Data were managed in Excel spreadsheets and exported to the statistical software. All statistical
199 analyses were performed with JMP Pro v.15.2 (SAS Institute Inc., Cary, NC) and the criterion for
200 statistical significance was established at $P < 0.05$. A two-way ANOVA was used to compare
201 colostrum variables among seasons, lactation groups (first, second, or third to fifth), and farms.
202 Associations between colostrum variables were examined using Spearman's correlation
203 coefficient. Cows were grouped *ex-post* into groups based on their colostrum variables to compare
204 the changes in biomarkers of metabolic stress according to the volume and Ig content of their
205 colostrum. Based on the recent recommendation of colostrum volume to sufficiently feed 2 meals
206 of colostrum for one calf (Godden et al., 2019), we considered high colostrum producers (HCP)
207 when cows produced ≥ 6 L while low colostrum producers (LCP) yielded < 6 L. Cows were
208 classified as producing low (LIG) or high IgG (HIG) colostrum based on the industry threshold of
209 50 g/L (Godden, 2008). However, no industry standard exists for IgM and IgA to date. Thus, we
210 used the median of the IgM and IgA values as the cutoff point to create equal sized groups of low
211 and high Ig, LIM or HIM and LIA or HIA, respectively. Linear mixed models with repeated

212 measures were built for the biomarkers Alb, BHBA, BUN, Ca, Chol, Glu, Hp, Mg, NEFA, TP,
213 RONS, AOP and OSi as outcome variables. Fixed effects included time (sampling weeks -6 to -1
214 relative to actual calving), groups (LCP vs HCP, LIG vs HIG, LIM vs HIM, or LIA vs HIA) and
215 their interaction. Cow nested within farm, season, and lactation group were used as random effects.
216 For repeated measures, the covariance structures autoregressive 1, compound symmetry, or
217 residual were tested for each variable, and the one with the lowest Akaike information criterion
218 was chosen. Model assumptions were assessed by evaluation of homoscedasticity and normality
219 of residuals. To satisfy these assumptions, some variables were natural log or square root-
220 transformed and the resulting least squares means estimates were subsequently back transformed
221 and presented as geometric means. All *P*-values given are those controlled for multiple
222 comparisons with Tukey's honestly significant difference test.

223

224

RESULTS

225 *Descriptive results of colostrum variables*

226 The distribution of colostrum volume and concentrations of IgG, IgM and IgA by season and
227 lactation group is depicted in Figure 1. Distribution of cows per groups were 137 LCP vs. 91 HCP,
228 16 LIG vs. 212 HIG, 112 LIA vs. 116 HIA, and 113 LIM vs. 115 HIM. The overall average
229 colostrum yield was 5.5 (range = 0.5 to 15.3) L with the highest average yield recorded in summer
230 and lowest in winter (Table 2). Volume of colostrum did not vary among lactation groups (*P* =
231 0.24) or farms (*P* = 0.15). The overall colostrum average (range) IgG concentration was 118.7 (8.3
232 to 261.2) g/L, with cows calving in fall and winter producing more IgG concentrated colostrum
233 than those calving in summer or spring (Table 2). Also, cows entering their 3rd or greater lactation
234 produced colostrum with greater IgG concentration than those entering their first or second

235 lactation. No differences in IgG concentrations were found between first and second lactation
236 animals. Colostrum IgG concentration was statistically different among all three farms of the study
237 ($P < 0.001$).

238 The mean (range) IgM concentration of colostrum was 4.9 (0.6 to 12.3) g/L, with higher
239 concentrations during summer (Table 2). Interestingly, cows entering the second lactation
240 produced colostrum with significantly lower IgM concentration than those entering the first or
241 third or greater ($P < 0.023$), and there were also differences in colostrum IgM concentration among
242 farms ($P = 0.019$). For IgA, the overall mean (range) concentration was 6.29 (0.33 to 17.5) g/L,
243 with lower concentrations recorded during fall compared to other months (Table 2). IgA
244 concentration in colostrum increased with lactation number ($P = 0.014$), and Farm C's colostrum
245 had higher IgA concentrations than Farm A and B ($P < 0.001$). Colostrum volume showed a
246 negative but weak correlation with the concentrations of IgG and IgA but did not correlate with
247 IgM (Table 3). Conversely, the concentrations of all immunoglobulins showed a positive
248 correlation with each other. Nevertheless, none of the correlations identified showed a correlation
249 coefficient greater than 0.50.

250

251 ***Biomarkers of metabolic stress***

252 The mean (SE) concentration of the cow biomarkers is presented by group and sampling point for
253 the colostrum volume, IgG, IgM, and IgA variables in Tables 4, 5, 6 and 7, respectively. HCP
254 cows had higher concentrations of AOP, BHB, and lower Chol and OSi than LCP. For IgG group,
255 HIG cows showed higher concentrations of Alb and glucose compared to LIG. HIA cows had
256 higher concentrations of Chol, RONS, OSi, and TP, whereas BHB and Glu were lower compared
257 with LIA. There was also a tendency for lower NEFA concentrations ($P = 0.096$) in the HIA group.

258 For the IgM group, biomarkers of the colostrum variables were not significantly different between
259 HIM and LIM. Also, no significant differences were found on the biomarkers of inflammation
260 haptoglobin and albumin for any of the colostrum variable categories analyzed.

261

262

DISCUSSION

263 *Colostrum Variables*

264 The averages and ranges in colostrum yield and Ig isotype concentrations found in our study are
265 in line with previous reports (Kruse, 1970; Larson et al., 1980; Conneely et al., 2013; Quigley et
266 al., 2013; Borchardt et al., 2022). Based on the group distributions, 93% of the cows in this study
267 produced colostrum meeting the industry standard of IgG content (50 g/L), similar to the 96%
268 documented by Conneely et al. (2013). Conversely, only 40% of cows produced sufficient first-
269 milked colostrum to support a second meal of colostrum to calves (6 L total yield). Thus,
270 suggesting that the volume of colostrum produced is a potential bottleneck for optimal colostrum
271 feeding regimes in commercial dairy farms nowadays.

272 Studies reporting factors affecting colostrum volume are scarce in the literature. In
273 agreement with two previous studies (Gavin et al., 2018; Borchardt et al., 2022), we found the
274 lowest colostrum yield during winter. Nevertheless, contrary to previous reports (Conneely et al.,
275 2013; Gavin et al., 2018), we did not note differences on colostrum yield by lactation group. A
276 potential explanation for these differences is the broad range of time interval from calving to
277 milking (0 to 21h) in the previous studies, compared to our study in which all cows were milked
278 within the first 6 h after calving. It is known that colostrum IgG concentration decreases with
279 time to harvest greater than 6-8 h postcalving (Conneely et al., 2013; Quigley et al., 2013). This
280 is believed to be due to the dilution of colostrum due to the start of lactogenesis. Because parous

281 cows produce more milk than primiparous cows, it is possible that this dilution effect is more
282 marked in older cows, which could explain the differences in colostrum yield across parities
283 observed in the Conneely et al. (2013) study as time of colostrum harvest is delayed.
284 Pre-calving nutrition is also known to affect colostrum volume (Mann et al., 2016). Recently,
285 Borchardt et al. (2022) also reported an association between duration in close-up diets and
286 colostrum yield in a large dairy farm. Although we did not intend to evaluate nutrition factors
287 influencing colostrum production, we purposely selected farms with different dry-cow nutrition
288 and management protocols, finding no differences in colostrum yield among study farms.
289 Overall, there was a marked individual variability in colostrum yield within seasons and lactation
290 groups as noted by the broad confidence intervals in Figure 1. Thus, suggesting that many factors
291 might influence colostrum yield production. Therefore, large multi-herd studies that investigate
292 the epidemiology of colostrum production are urgently needed to identify which animals are
293 more likely to produce sufficient amounts of high-quality colostrum.

294 The colostrum immunoglobulin concentration also showed important individual
295 variability for all isotypes measured, but the ranges were similar to previous studies (Newby et
296 al., 1982; Conneely et al., 2013). Immunoglobulin content varied by season. In agreement with
297 previous reports, the lowest IgG concentration was documented in the spring (Conneely et al.,
298 2013). The referenced study was conducted in a pasture-based dairy system and the authors
299 speculated that the observed differences could be attributed to differences in dry period lengths
300 or diet composition. However, we observed the same differences in IgG concentrations in farms
301 that followed year-round calving patterns with a targeted dry period length and similar total
302 mixed ration composition throughout the year. Thus, indicating that other underlying factors are
303 likely involved, and further research is needed to elucidate the changes in IgG concentration

304 associated with season of calving. Interestingly, during the fall and winter seasons, when
305 colostrum showed the highest IgG concentrations, the concentrations of IgM were the lowest
306 (Table 2) despite observing an overall positive correlation between IgG and IgM in colostrum
307 samples (Table 3). Also, colostrum produced in the fall showed lower IgA content than at other
308 seasons. To our knowledge, this is the first report evaluating changes in colostrum IgM and IgA
309 concentrations across seasons and no research is available on factors affecting the colostrum
310 concentration of these Ig isotypes.

311 Colostrum immunoglobulin composition also varied by parity. Cows entering their third
312 to fifth lactation produced colostrum with greater IgG concentration compared to cows entering
313 their first and second lactation. Interestingly, no differences were observed between parity 1 and
314 2 animals. Despite earlier studies recommending to discharge colostrum from first lactation
315 heifers due to low IgG content (Selman et al., 1971), the results from this and previous studies do
316 not support this (Conneely et al., 2013). The mean IgG concentration of colostrum for heifers in
317 this study was twice that considered to be the threshold for good quality colostrum (50 g/L), and
318 only 5% of the colostrum samples obtained from heifers were below that threshold. The higher
319 IgG concentration in older (parity 3-5) cows is consistent with the existing literature (Kruse,
320 1970; Pritchett et al., 1991). Older cows are likely to have been exposed to a greater number of
321 antigens in their lifetime, resulting in greater antibodies in serum and, subsequently, in colostrum
322 (Larson et al., 1980). Although while IgG are transferred from the bloodstream across the
323 mammary barrier into colostrum and IgA and IgM are largely derived from local synthesis by
324 plasma cells in the mammary gland (Larson et al., 1980), differences in antigenic stimulation
325 associated with age might also justify the observed increase in IgA concentration as parity
326 increases. However, colostrum from parity 2 cows had lower IgM content than colostrum from

327 parities 1 or 3-5. We cannot explain this finding based on the current evidence available of IgM
328 content in colostrum. Colostrum Ig composition varied also by farm. However, these differences
329 could be attributed to differences in management across farms (diet, housing, dry period length,
330 etc.) as these factors are known to influence IgG content of colostrum (Godden, 2008).

331 Correlation analyses revealed a weak yet statistically significant negative association
332 between colostrum volume and IgG ($\rho = -0.28$) and A ($\rho = -0.15$) concentrations. The negative
333 association between colostrum yield and IgG concentration had also been previously reported
334 (Quigley et al., 1994; Conneely et al., 2013; Mann et al., 2016). Nevertheless, the variation in
335 colostrum volume only explained and 7.8 and 2.3% of the variation in colostrum IgG and IgA
336 concentrations, respectively (Table 3). Thus, indicating a weak association between colostrum
337 volume and immunoglobulin concentration. This suggests that the processes of synthesis and
338 transfer of IgG and IgA to colostrum might be largely independent of the volume of colostrum
339 produced. For example, colostral IgG are derived from those circulating in plasma and are actively
340 taken up by the mammary gland through binding to FcRn receptors (Zhang et al., 2009); whereas
341 the volume of produced milk depends on the osmotic equilibrium of the blood–milk barrier,
342 regulated mainly by lactose (Costa et al., 2019). We speculate that the amount of absorbed water
343 in the mammary gland alveoli during colostrogenesis, and thus, the colostrum volume is also
344 dependent on this equilibrium, which is affected by many other solutes beyond Igs.

345 Traditionally, colostrum quality has been evaluated based solely on the concentration of
346 IgG as this is the most abundant immunoglobulin isotype in bovine colostrum (Larson et al.,
347 1980). However, we found weak correlations (defined as Spearman's coefficient ≤ 0.50) among
348 the different isotypes of immunoglobulins in colostrum. Thus, optimization of passive immunity
349 transfer for IgG might not necessarily result in optimal transfer of other immunological factors of

350 colostrum, such as other Ig isotypes. However, the relevance of the transfer of IgA and IgM to
351 the calf via colostrum on calf health and productivity remains unexplored to date and more
352 research is needed to unravel the impact that all colostrum immunological components have on
353 calf health.

354

355 *Nutrient Utilization and Colostrum Variables*

356 We evaluated the association between colostrum variables and biomarkers of energy (NEFA,
357 BHB, Glu, Chol), protein (BUN, TP), and macromineral (Ca, Mg) status in blood samples
358 collected throughout the last 6 wk of gestation. The higher BHB concentration exhibited by HCP
359 cows compared to LCP indicates that cows producing > 6 L of colostrum had greater nutrient
360 demands than those producing less than 6 L, as BHB is commonly used as an indicator of energy
361 deficit (McArt et al., 2013). However, the lack of differences in NEFA concentration between
362 colostrum volume groups suggests that cows were able to cope with these increased energy
363 demands without a marked increase in lipid mobilization. Furthermore, HCP cows exhibited lower
364 Chol concentrations than LCP cows, which can be associated with decreased hepatic Chol export
365 due to increased ketogenesis (Kessler et al., 2014; Gross et al., 2021), and supports the finding of
366 increased energy demands in association with the volume of colostrum produced, despite no
367 marked lipid mobilization.

368 Changes in nutrient utilization biomarkers were also identified between the colostrum IgG
369 and IgA groups but not for IgM. Colostral IgG are derived from those circulating in plasma and
370 are taken up by the mammary gland via action of receptors (Zhang et al., 2009). Changes in
371 colostrum IgG concentrations could therefore be caused by changes in circulating blood
372 concentrations in the dam, a change in transfer capability, a difference in the rate of water

373 inclusion, or a combination of these. Cows producing colostrum above the 50 g/L IgG cut-off
374 showed greater Glu concentrations than those producing colostrum below this threshold, but no
375 other differences in energy-related metabolites were identified. In a recent study, Immler et al.
376 (2021) also found no relationship the Brix value of the colostrum (an estimate of IgG
377 concentration) and the biomarkers Chol or NEFA. However, these authors did not include Glu in
378 their panel of biomarkers. Glu is the primary fuel for immune cells (Calder et al., 2007), and
379 therefore it is plausible that greater availability of glucose as energy source allowed blood
380 lymphocytes to synthesize more IgG that would have been subsequently transported into the
381 mammary gland for colostrum synthesis. In fact, a positive correlation between plasma glucose
382 and IgG has been documented in goats (Hefnawy et al., 2010). However, studies in human and
383 bovine lymphocytes described a reduction in lymphocyte function under high concentrations of
384 glucose in vitro (Franklin et al., 1991; Jennbacken et al., 2013). However, these studies used
385 concentrations of glucose (11.1 mM) exceeding normal blood concentrations in cattle (~ 3.2 – 4.4
386 mM), limiting the translation of their findings to the live animal. To our knowledge, the biological
387 significance of blood glucose concentrations on immunoglobulin synthesis has not been
388 investigated in cattle to date. Furthermore, we did not assess blood IgG concentrations in the cows
389 in the present study, which limits our ability to discern if the changes in IgG colostrum content is
390 associated with differences in blood IgG concentrations or due to other steps involved in the
391 translocation of IgG from bloodstream into colostrum.

392 Cows with higher IgA colostrum concentration showed a metabolic profile suggestive of a
393 better energy status (lower concentrations of BHB and a tendency towards lower NEFA, and
394 higher concentrations of Chol and TP). Unlike IgG, IgA are primarily synthesized locally in the
395 mammary gland and not translocated from bloodstream (Larson et al., 1980). However, it is

396 possible that the greater energy balance would allow B lymphocytes in the mammary gland to
397 secrete more IgA, as producing IgA is a considerable energy expense for the body of mammals
398 (Woof and Kerr, 2006). However, it could also be possible that the greater IgA concentration in
399 colostrum was due to increased recruitment of B cells into the mammary gland and/or increased
400 class switch to IgA⁺ cells. Lower serum Glu concentrations were also found in cows with higher
401 IgA colostrum. Because the mammary gland uptakes 60-85% of blood glucose (Annison and
402 Linzell, 1964; Rigout et al., 2002), lower glycemia might be indicative of higher availability of
403 glucose for the mammary gland IgA-producing immune cells, as Glu is the main fuel used by these
404 cells (Calder et al., 2007). We are unaware of any research in the bovine species studying the
405 impact of energy status on the colostrogenesis mechanisms, and, therefore, more research is
406 needed in this area to be able to modulate colostrum production in the dairy cow.

407 We detected no association between any of the colostrum variables studied and
408 concentrations of the macrominerals Ca or Mg. Immler et al. (2021), however, found a negative
409 association between serum Ca concentration and colostrum Brix value was, but were unable to
410 clarify the physiological background behind this observation. Thus, the differences between their
411 study and ours underscores the need for further research in this area, given that different Ca
412 management nutritional strategies resulted in differences in colostrum IgG concentrations (Diehl
413 et al., 2018).

414

415 ***Inflammatory Status and Colostrum Variables***

416 Based on the biomarkers Hp and Alb, positive and negative acute phase proteins respectively, we
417 observed no differences in the inflammatory status of cows based on colostrum volume or Ig
418 content. This suggests that the cows' physiological homeostasis was not disrupted during late

419 gestation in association with the colostrum variables studied. Although exacerbated inflammation
420 is often seen in transition cows (Bradford et al., 2015), and is one of the hallmarks of metabolic
421 stress (Abuelo et al., 2019), changes in biomarkers of inflammation usually occur after parturition
422 (Abuelo et al., 2014; Burfeind et al., 2014; Pohl et al., 2015). Thus, even though we are unable to
423 determine cause-effect relationships in this observational study, the lack of association between
424 acute phase proteins and colostrum yield or Ig isotype content let us to speculate that the
425 colostrogenesis process might not increase inflammation in the pre-partum cow.

426

427 ***Oxidant Status and Colostrum Variables***

428 Oxidative stress is also a common feature of the transition period of dairy cattle (Abuelo et al.,
429 2015), known to impair functional capabilities of immune cell populations, including the
430 lymphocytes responsible for Ig synthesis (Lacetera et al., 2005; Sordillo and Aitken, 2009; Cuervo
431 et al., 2021). Thus, we anticipated that lower colostrum Ig content or volume would be associated
432 with a more pro-oxidant systemic redox balance. In line with our hypothesis, LCP cows showed
433 greater OSi values than HCP cows. This was due to differences in AOP as RONS remained similar
434 between colostrum yield groups (Table 4). Therefore, it is possible that higher AOP might have
435 supported HCP cows to produce more colostrum, rendering availability of antioxidants a potential
436 limiting factor in colostrum volume production for cows. Our study is, to our knowledge, the first
437 one to examine relationships between cow oxidant status and colostrum production, but given the
438 observed relationship between lower AOP and colostrum volume, the extent to which increasing
439 antioxidant capacity in late-gestation cows enhances colostrum yield warrants further research.

440 There were no differences in oxidant status between cows in the IgG or IgM groups.
441 However, HIA cows showed higher concentration of RONS and greater OSi values compared to

442 LIA. This finding is contrary to our initial hypothesis linking a pro-oxidant status to lower Ig
443 content. How systemic redox balance might influence the production of the mucosa-derived IgA
444 but not IgG or IgM remains unexplained and asserts the complexity of redox regulation of
445 biological processes. Certainly, the role of oxidative balance in the colostrogenesis process needs
446 to be elucidated further.

447

448 ***Study Limitations***

449 Given the observational nature of this study, we were only able to show associations among
450 colostrum variables and biomarkers of metabolic stress and did not explore cause-effect
451 relationships. However, this is the first study to investigate changes in biomarkers of metabolic
452 stress in association with colostrum variables and the associations identified can be examined
453 further via controlled interventional studies. Another limitation of the study is that using the
454 industry standard of colostrum IgG concentration (50 g/L) resulted in unbalanced groups sizes
455 (212 vs. 16 cows in HIG and LIG), which might have influenced our ability to detect differences.
456 In fact, post hoc power calculations revealed that only powers of 0.13, 0.21, 0.11, and 0.19 for
457 detecting differences in NEFA concentrations between groups of colostrum volume, IgG, IgM,
458 and IgA, respectively. Thus, we cannot exclude associations between metabolic stress and IgG
459 content that we were identified in this study.

460

461

461 **CONCLUSIONS**

462 This study evaluated, for the first time, the metabolic status of dairy cows during the last 6 wk of
463 gestation based on the volume and Ig concentration of colostrum. We observed marked individual
464 variability in colostrum yield and Ig isotype concentration, suggesting that colostrum production

465 is a complex and multifactorial process. Also, we detected differences in nutrient utilization and
466 oxidant status biomarkers in association with the volume and IgG and IgA concentration of
467 colostrum. Among all changes detected, our results suggest that increasing availability of
468 antioxidants during late-gestation could support the production of higher volumes of colostrum,
469 which warrants further investigation through supplementation trials.

470

471

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477

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6 TABLES

7 **Table 1: Description of herd, housing, and management practices for late-gestation cows in the study farms.**

	Farm A				Farm B			Farm C		
	Close up cows	Close up heifers	Far-off cows	Far-off heifers	Close up cows / heifers	Far-off cows	Far-off heifers	Close-up cows / heifers	Far-off cows	Far-off heifers
Pen design	Free stall	Free stall	Free stall	Free stall	Bedded pack	Free stall	Free stall	Bedded pack	Free stall	Free stall
Heat abatement	137 cm diameter fans on stall area		None	None	91 cm diameter fans on bedding areas		None	61 cm diameter fans on bedding areas		
Target dry period length (d)		60			60			45		
Target duration of close-up diet		21			21			Same diet for whole dry period		
Rolling yearly milk yield per cow (kg)		15,049			14,508			14,011		
Rolling yearly average of cows in milk (n)		3,712			1,054			1,608		
Min-Max ambient temperature (°C) ¹										
<i>Summer</i>		17.2 to 30.1			17.8 to 29.4			15.2 to 30.5		
<i>Fall</i>		5.5 to 14.8			5.5 to 15.1			11.1 to 22.2		
<i>Winter</i>		-6.8 to 1.0			-6.7 to 1.1			-6.1 to 2.8		
<i>Spring</i>		1.3 to 11.0			1.1 to 12.2			0.8 to 12.8		

8 ¹ Data from the National Environmental Satellite, Data, and Information Service station closest to each farm (<https://www.ncei.noaa.gov/access/past-weather/>). Reported as the average of the daily minimum and maximum temperatures during the sampling period at each farm.

0

1 **Table 2: Distribution of colostrum variables across seasons, lactation groups, and study farms.**

	Volume (L)	IgG (g/L)	IgM (g/L)	IgA (g/L)
Season				
<i>Summer</i>	6.9 (6.6 – 7.3) ^a	110.7 (105.5 – 115.9) ^a	5.8 (5.5 – 6.0) ^a	6.4 (6.0 – 6.8) ^a
<i>Fall</i>	5.4 (5.1 – 5.8) ^b	127.7 (122.6 – 132.8) ^b	4.5 (4.2 – 4.7) ^b	5.6 (5.2 – 6.0) ^b
<i>Winter</i>	4.3 (4.0 – 4.6) ^c	130.8 (125.9 – 135.6) ^b	4.6 (4.4 – 4.8) ^{bc}	6.4 (6.0 – 6.8) ^a
<i>Spring</i>	5.4 (5.0 – 5.7) ^b	104.9 (100.0 – 110.0) ^a	5.0 (4.8 – 5.2) ^c	6.8 (6.4 – 7.2) ^a
Lactation group				
<i>1st</i>	5.3 (5.0 – 5.6) ^a	115.9 (111.4 – 120.4) ^a	5.2 (5.0 – 5.5) ^a	4.4 (4.0 – 4.8) ^a
<i>2nd</i>	5.4 (5.1 – 5.7) ^a	108.5 (104.8 – 112.2) ^a	4.3 (4.1 – 4.5) ^b	6.3 (6.0 – 6.6) ^b
<i>3rd to 5th</i>	5.7 (5.4 – 6.0) ^a	135.1 (130.8 – 139.4) ^b	5.4 (5.2 – 5.6) ^a	8.1 (7.8 – 8.4) ^c
Farm				
<i>A</i>	6.1 (5.7 – 6.9) ^a	119.0 (114.7 – 123.4) ^a	5.2 (5.0 – 5.4) ^a	5.8 (5.5 – 6.2) ^a
<i>B</i>	5.7 (5.4 – 6.0) ^a	105.6 (101.0 – 110.1) ^b	4.7 (4.5 – 4.9) ^b	5.9 (5.5 – 6.2) ^a
<i>C</i>	5.4 (5.1 – 5.8) ^a	130.0 (125.8 – 134.2) ^c	4.9 (4.7 – 5.1) ^{ab}	7.1 (6.8 – 7.4) ^b

2 Colostrum was harvested within 6 h of calving by farm staff. Results are expressed as means (95% confidence
3 intervals). Data were analyzed through two-way ANOVAs and means compared with Tukey honest significant
4 difference test. ^{a,b,c} Within each group and variable, means with different superscript letters are statistically
5 different ($P < 0.05$).

6 **Table 3: Correlations among colostrum volume and concentrations of IgG, IgM, and IgA.**

Variable	Volume (L)	IgG (g/L)	IgM (g/L)	IgA (g/L)
Volume				
ρ	1.0	-0.28	0.03	-0.15
(95% CI)		(-0.38 to -0.15)	(-0.10 to 0.17)	(-0.28 to -0.02)
<i>P</i> -value		< 0.0001	0.62	0.021
IgG				
ρ		1.0	0.26	0.50
(95% CI)			(0.13 to 0.38)	(0.40 to 0.60)
<i>P</i> -value			< 0.0001	< 0.0001
IgM				
ρ			1.0	0.30
(95% CI)				(0.17 to 0.41)
<i>P</i> -value				< 0.0001
IgA				
ρ				1.0
(95% CI)				
<i>P</i> -value				

7 Results expressed as Spearman's correlation coefficient (ρ) with 95% confidence intervals (95% CI) and
8 associated *P*-values.

9 **Table 4: Estimated means and significance of the main effects from the generalized linear mixed models of blood biomarkers of late-**
0 **gestation dairy cows comparing groups of high colostrum producers (HCP, n=91) and low colostrum producers (LCP, n=137).**

Outcome (units)	Estimated means of at the different time points relative to calving												SE	P value		
	Wk -6		Wk -5		Wk -4		Wk -3		Wk -2		Wk -1			Group	Time	G×T
	HCP	LCP	HCP	LCP	HCP	LCP	HCP	LCP	HCP	LCP	HCP	LCP				
NEFA (mM) ¹	0.22	0.24	0.21	0.21	0.20	0.22	0.19	0.22	0.19	0.21	0.23	0.23	0.08	0.491	0.027	0.555
BHB (mg/dL) ¹	4.23	4.09	4.39	4.05	4.32	4.03	4.51	3.90	4.65	3.78	5.15	4.17	0.30	< 0.001	< 0.001	0.001
Cholesterol (mg/dL) ¹	176	198	160	175	143	154	131	139	112	125	99	110	1.02	0.002	< 0.001	0.214
Glucose (mg/dL)	73	76.6	74.3	74.8	72.6	73.0	72.8	73.3	72.0	74.5	71.7	73.2	1.01	0.222	0.003	0.087
Total protein (g/dL) ¹	7.54	7.41	7.62	7.37	7.45	7.24	7.40	7.23	7.14	7.10	6.97	6.82	0.29	0.153	< 0.001	0.268
BUN (mg/dL)	11.4	10.8	11.8	11.00	11.72	11.22	11.46	11.85	11.99	12.32	12.94	12.86	0.37	0.646	< 0.001	0.081
Albumin (g/dL) ¹	4.21	4.13	4.23	4.05	4.09	3.96	4.13	3.98	4.06	3.99	4.04	3.95	0.27	0.051	< 0.001	0.343
Haptoglobin (g/L) ¹	0.50	0.46	0.49	0.48	0.47	0.45	0.50	0.43	0.48	0.44	0.46	0.42	0.05	0.229	0.019	0.205
Calcium (mg/dL)	9.29	9.16	9.27	9.18	9.03	9.02	9.28	9.06	9.10	9.17	9.21	9.11	0.11	0.533	0.016	0.213
Magnesium (mg/dL)	2.87	2.76	3.00	2.81	3.02	2.80	2.94	2.84	3.01	2.86	2.97	2.89	0.07	0.065	0.323	0.613
AOP (TE/μL) ¹	8.19	7.49	8.42	7.35	8.18	7.23	8.20	7.32	7.97	7.11	7.67	6.77	0.33	0.002	< 0.001	0.690
RONS (RFU) ¹	44.8	49.3	46.8	49.8	48.2	51.5	52.6	55.3	54.9	59.2	53.7	61.7	1.05	0.239	< 0.001	0.572
OSi (arbitrary units) ¹	5.38	6.53	5.46	6.73	5.79	7.07	6.31	7.50	6.78	8.27	6.88	9.05	0.06	0.006	< 0.001	0.583

1 Generalized linear mixed models with repeated measurements were built for the blood biomarkers as outcomes with the Group, Time, their interaction
2 (G×T) as fixed effects. Wk-6 through Wk -1 represent sample points relative to actual calving. HCP: High colostrum producers (≥ 6 L); LCP: Low
3 colostrum producers (< 6 L). SE: Standard error. TE: Trolox equivalent; RFU: Relative fluorescence units; NEFA: non-esterified fatty acids; BHB:
4 Beta-hydroxybutyrate; BUN: Blood urea nitrogen; AOP: Antioxidant potential; RONS: Reactive oxygen and nitrogen species; OSi: Oxidant status
5 index. ¹ Variable natural log transformed for statistical analysis.

6 **Table 5: Estimated means and significance of the main effects from the generalized linear mixed models of blood biomarkers of late-**
7 **gestation dairy cows comparing groups of high immunoglobulin G (HIG, n=212) and low immunoglobulin G (LIG, n=16).**

Outcome (units)	Estimated means of at the different time points relative to calving												SE	P value		
	Wk -6		Wk -5		Wk -4		Wk -3		Wk -2		Wk -1			Group	Time	G×T
	HIG	LIG	HIG	LIG	HIG	LIG	HIG	LIG	HIG	LIG	HIG	LIG				
NEFA (mM) ¹	0.23	0.13	0.22	0.15	0.21	0.15	0.20	0.18	0.20	0.18	0.23	0.24	0.09	0.304	0.328	0.415
BHB (mg/dL) ¹	4.12	3.75	4.19	3.52	4.13	4.19	4.13	3.88	4.11	3.90	4.51	5.08	0.71	0.605	0.004	0.249
Cholesterol (mg/dL)	196	198	176	154	154	141	139	129	124	110	109	93.3	8.4	0.369	< 0.001	0.583
Glucose (mg/dL) ¹	75.5	68.7	74.9	66.9	73.4	64.7	73.4	69.6	73.9	66.4	72.9	67.4	1.11	0.013	0.464	0.548
Total protein (g/dL) ¹	7.48	7.20	7.51	7.02	7.35	6.98	7.32	7.13	7.14	6.76	6.91	6.51	0.18	0.165	< 0.001	0.649
BUN (mg/dL)	11.0	12.1	11.2	12.8	11.3	13.7	11.5	13.8	12.1	13.6	12.8	13.8	1.14	0.089	0.101	0.766
Albumin (g/dL) ¹	4.17	3.84	4.13	3.85	4.02	3.80	4.04	3.88	4.02	3.86	4.00	3.58	0.02	0.043	0.018	0.172
Haptoglobin (g/L) ¹	0.48	0.56	0.48	0.45	0.46	0.45	0.46	0.54	0.45	0.43	0.44	0.45	0.10	0.816	0.242	0.367
Calcium (mg/dL)	9.23	8.92	9.22	9.09	9.04	8.60	9.14	9.05	9.15	8.87	9.15	9.00	0.21	0.430	0.263	0.820
Magnesium (mg/dL) ²	2.77	2.87	2.86	2.92	2.86	2.96	2.85	2.86	2.88	3.04	2.91	2.57	0.15	0.941	0.270	0.122
AOP (TE/μL) ¹	7.85	7.09	7.84	7.21	7.67	6.82	7.75	6.85	7.52	6.73	7.21	6.18	0.36	0.213	0.001	0.914
RONS (RFU) ¹	47.7	34.1	48.7	41.4	50.5	37.8	54.3	47.2	57.5	50.3	58.2	57.2	2.11	0.282	< 0.001	0.186
OSi (arbitrary units) ¹	6.01	4.81	6.15	5.73	6.51	5.53	6.94	6.89	7.57	7.47	8.00	9.26	1.13	0.771	< 0.001	0.155

8 Generalized linear mixed models with repeated measurements were built for the blood biomarkers as outcomes with the Group, Time, their
9 interaction (G×T) as fixed effects. Wk-6 through Wk -1 represent sample points relative to actual calving. HIG: High immunoglobulin G producers
0 (≥ 50 g/L IgG); LIG: Low immunoglobulin G producers (< 50 g/L IgG). SE: Standard error. TE: Trolox equivalent; RFU: Relative fluorescence
1 units; NEFA: non-esterified fatty acids; BHB: Beta-hydroxybutyrate; BUN: Blood urea nitrogen; AOP: Antioxidant potential; RONS: Reactive
2 oxygen and nitrogen species; OSi: Oxidant status index. ¹ Variable natural log transformed for statistical analysis. ² Variable square root transformed
3 for analysis.

4 **Table 6: Estimated means and significance of the main effects from the generalized linear mixed models of blood biomarkers of late-**
5 **gestation dairy cows comparing groups of high immunoglobulin M (HIM, n=115) and low immunoglobulin M (LIM, n=113).**

Outcome (units)	Estimated means of at the different time points relative to calving												SE	P value		
	Wk -6		Wk -5		Wk -4		Wk -3		Wk -2		Wk -1			Group	Time	G×T
	HIM	LIM	HIM	LIM	HIM	LIM	HIM	LIM	HIM	LIM	HIM	LIM				
NEFA (mM) ¹	0.23	0.23	0.23	0.20	0.21	0.21	0.21	0.20	0.19	0.21	0.23	0.23	0.08	0.925	0.040	0.477
BHB (mg/dL) ¹	4.01	4.23	4.11	4.22	4.20	4.09	4.27	4.00	4.17	4.07	4.47	4.60	0.29	0.966	< 0.001	0.206
Cholesterol (mg/dL) ¹	187	188	167	170	150	147	135	135	118	121	105	105	2.15	0.885	< 0.001	0.461
Glucose (mg/dL) ¹	75.3	74.8	74.3	74.5	73.1	72.6	72.2	73.9	73.1	73.6	71.8	73.2	1.01	0.709	0.004	0.437
Total protein (g/dL) ¹	7.73	7.50	7.50	7.45	7.35	7.3	7.32	7.29	7.13	7.10	6.94	6.83	0.59	0.752	< 0.001	0.558
BUN (mg/dL)	11.0	11.1	11.4	11.1	11.8	10.9	12.1	11.2	12.4	12.0	12.9	12.8	0.36	0.315	< 0.001	0.272
Albumin (g/dL) ¹	4.19	4.14	4.14	4.10	4.02	4.00	4.01	4.05	4.01	4.02	3.99	3.98	1.01	0.841	< 0.001	0.643
Haptoglobin (g/L) ¹	0.46	0.51	0.48	0.48	0.45	0.47	0.48	0.45	0.45	0.45	0.43	0.45	0.23	0.714	0.007	0.098
Calcium (mg/dL)	9.12	9.31	9.13	9.29	8.97	9.08	9.06	9.22	9.08	9.20	9.08	9.20	0.41	0.251	0.025	0.992
Magnesium (mg/dL)	2.81	2.79	2.93	2.85	3.01	2.78	2.96	2.80	2.99	2.86	2.99	2.86	0.07	0.089	0.260	0.394
AOP (TE/μL) ¹	7.97	7.66	7.81	7.84	7.66	7.61	7.76	7.66	7.55	7.43	7.23	7.11	0.03	0.706	< 0.001	0.775
RONS (RFU) ¹	50.0	44.0	51.7	45.1	53.4	46.6	56.8	51.2	57.1	56.9	60.6	55.6	1.45	0.156	< 0.001	0.072
OSi (arbitrary units) ¹	6.19	5.71	6.53	5.73	6.88	6.08	7.23	6.65	7.47	7.61	8.28	7.77	0.06	0.323	< 0.001	0.081

6 Generalized linear mixed models with repeated measurements were built for the blood biomarkers as outcomes with the Group, Time, their interaction
7 (G×T) as fixed effects. Wk-6 through Wk -1 represent sample points relative to actual calving. HIM: High immunoglobulin M producers (≥ 4.6 g/L
8 IgG); LIM: Low immunoglobulin M producers (< 4.6 g/L IgM). SE: Standard error. TE: Trolox equivalent; RFU: Relative fluorescence units; NEFA:
9 non-esterified fatty acids; BHB: Beta-hydroxybutyrate; BUN: Blood urea nitrogen; AOP: Antioxidant potential; RONS: Reactive oxygen and nitrogen
0 species; OSi: Oxidant status index. ¹ Variable natural log transformed for statistical analysis.

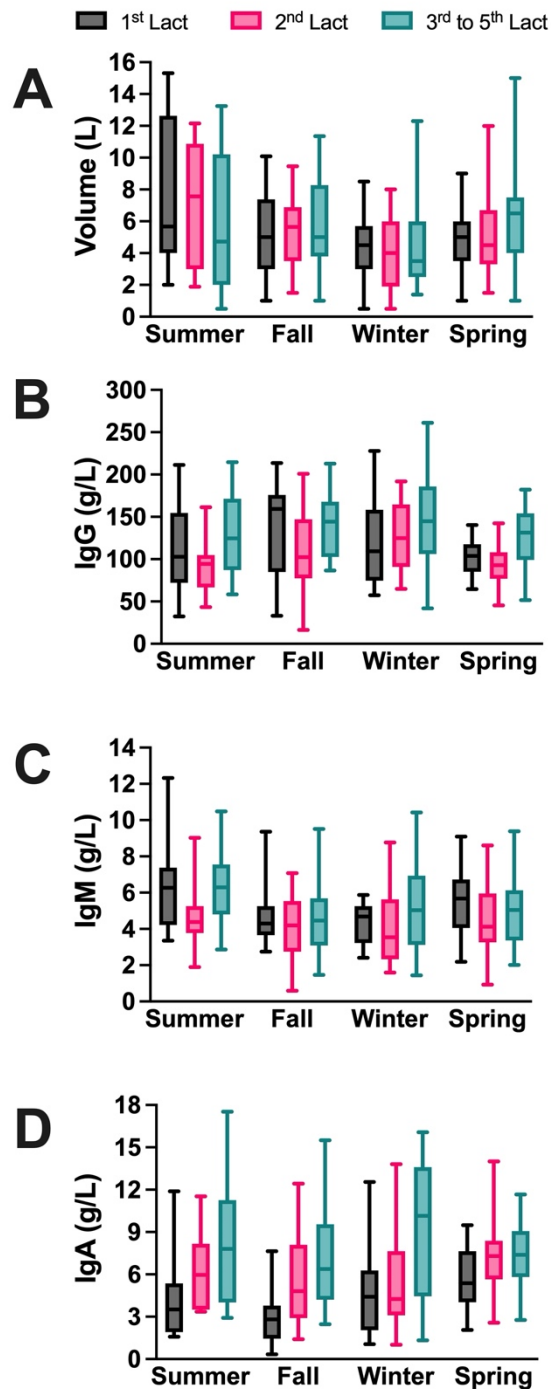
Table 7: Estimated means and significance of the main effects from the generalized linear mixed models of blood biomarkers of late-gestation dairy cows comparing groups of high immunoglobulin A (HIA, n=116) and low immunoglobulin A (LIA, n=112).

Outcome (units)	Estimated means of at the different time points relative to calving												SE	P value		
	Wk -6		Wk -5		Wk -4		Wk -3		Wk -2		Wk -1			Group	Time	G×T
	HIA	LIA	HIA	LIA	HIA	LIA	HIA	LIA	HIA	LIA	HIA	LIA				
NEFA (mM) ¹	0.23	0.24	0.19	0.25	0.19	0.23	0.18	0.23	0.18	0.22	0.23	0.23	0.08	0.096	0.030	0.046
BHB (mg/dL) ¹	3.89	4.29	4.02	4.25	3.99	4.23	4.15	4.05	3.73	4.47	4.12	4.93	0.03	0.007	< 0.001	< 0.001
Cholesterol (mg/dL) ¹	203	169	179	156	154	141	137	131	122	115	107	101	2.02	0.002	< 0.001	< 0.001
Glucose (mg/dL) ¹	73.8	76.3	73.1	75.7	71.0	74.8	70.8	75.2	72.0	74.8	70.8	74.2	1.13	0.005	0.003	0.663
Total protein (g/dL) ¹	7.66	7.24	7.69	7.22	7.46	7.11	7.39	7.13	7.29	6.86	7.01	6.66	0.09	< 0.001	< 0.001	0.090
BUN (mg/dL)	11.1	10.9	11.5	11.0	11.7	11.1	12.0	11.3	12.6	11.8	12.9	12.7	0.36	0.222	< 0.001	0.682
Albumin (g/dL) ¹	4.18	4.14	4.13	4.11	4.01	4.01	3.99	4.07	4.04	3.99	4.02	3.95	0.01	0.782	< 0.001	0.061
Haptoglobin (g/L) ¹	0.49	0.47	0.49	0.48	0.43	0.49	0.45	0.47	0.44	0.46	0.43	0.44	0.05	0.628	0.011	0.040
Calcium (mg/dL)	9.20	9.23	9.21	9.21	9.02	9.03	9.09	9.19	9.17	9.12	9.18	9.11	0.10	0.967	0.026	0.758
Magnesium (mg/dL)	2.77	2.79	2.82	2.92	2.84	2.89	2.84	2.87	2.88	2.91	2.83	2.96	0.02	0.409	0.189	0.747
AOP (TE/μL) ¹	7.94	7.67	7.71	7.92	7.79	7.47	7.75	7.65	7.59	7.37	7.24	7.08	0.05	0.6274	< 0.001	0.083
RONS (RFU) ¹	54.2	40.8	56.0	41.8	58.4	42.6	62.4	47.0	65.3	50.1	66.7	50.9	1.05	< 0.001	< 0.001	0.928
OSi (arbitrary units) ¹	6.75	5.29	7.19	5.25	7.42	5.66	7.97	6.09	8.51	6.76	9.12	7.13	0.06	< 0.001	< 0.001	0.739

Generalized linear mixed models with repeated measurements were built for the blood biomarkers as outcomes with the Group, Time, their interaction (G×T) as fixed effects. Wk-6 through Wk -1 represent sample points relative to actual calving. HIA: High immunoglobulin A producers (≥ 5.52 g/L IgA); LIG: Low immunoglobulin A producers (< 5.52 g/L IgA). SE: Standard error. TE: Trolox equivalent; RFU: Relative fluorescence units; NEFA: non-esterified fatty acids; BHB: Beta-hydroxybutyrate; BUN: Blood urea nitrogen; AOP: Antioxidant potential; RONS: Reactive oxygen and nitrogen species; OSi: Oxidant status index. ¹ Variable natural log transformed for statistical analysis.

688 **FIGURE CAPTIONS**

689 **Figure 1: Distribution of colostrum (A) volume, (B) IgG concentration, (C) IgM**
690 **concentration, and (D) IgA concentration per season and lactation group. The whiskers of**
691 **the Box Plots represent the 5-95% percentiles.**



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