

## SHORT COMMUNICATION

# Transfer and persistence of bovine immunoglobulins in lambs fed a colostrum replacer

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## Abstract

**Background:** Colostrum-derived antibodies are crucial for the protection of newborn lambs from infectious diseases. Several colostrum replacer products that contain bovine antibodies are on the market. We investigated the absorption and persistence of bovine antibodies from a powdered colostrum replacer in newborn lambs.

**Methods:** We tested a lamb colostrum replacer containing bovine serum in lambs that were separated from their dams at birth. Immunoglobulin G (IgG) uptake was analysed by ELISA, and the persistence of antigen-specific antibodies was analysed by parainfluenza 3 virus (PI-3) neutralisation assay.

**Results:** Serum antibody ELISA performed on days 1 and 14 revealed IgG levels of  $17.9 \pm 2.8$  and  $27.5 \pm 2.5$  mg/ml, respectively. PI-3 antibodies derived from the colostrum replacer were present for  $86.3 \pm 10.6$  days.

**Conclusions:** Antibodies derived from bovine serum protein delivered to lambs via a commercial colostrum replacer are readily absorbed and persist for months, suggesting that these products may offer adequate protection.

## KEYWORDS

artificial rearing, colostrum replacer, immunoglobulin, lamb

## INTRODUCTION

Colostrum is the only source of antibodies in newborn lambs, since the ruminant placenta is impermeable to immunoglobulins (Igs).<sup>1</sup> Consumption of sufficient high-quality colostrum is linked to improved lamb survival, growth, gut maturation and immune status.<sup>2-5</sup> Thus, colostrum replacers and supplements play a critical role in ovine production in situations where sufficient high-quality ewe colostrum is not available, such as periparturient death of the dam, multiple births or artificial rearing systems.<sup>6-8</sup> Bovine colostrum has traditionally been used to substitute ewe colostrum.<sup>8-10</sup> Ig genes are highly conserved between cattle and sheep,<sup>11</sup> and a significant number of pathogens target both sheep and cattle, so bovine antibodies are likely to provide relevant protection in lambs. However, bovine colostrum may be contaminated with pathogens, including rotavirus, *Cryptosporidium*, bovine leukosis virus or *Mycobacterium avium* subsp. *paratuberculosis*, thus posing an infection risk.<sup>12-15</sup>

Colostrum replacers are synthetic feed products designed to completely replace maternal colostrum.<sup>16</sup> However, few studies have analysed the impact of feeding colostrum replacers to lambs.<sup>6,17</sup> We analysed Ig uptake and persistence in lambs that were fed a commercial colostrum replacer that contained bovine serum.

## METHODS

### Animals, housing and husbandry

Animal experiments were approved by the Institutional Animal Care and Use Committee of Montana State University, protocol #2019-95. Our investigations were part of a larger study performed between March and October 2020 that involved the generation of a specific-pathogen-free (SPF) research flock.<sup>18</sup> Therefore, a matched control group that did not receive colostrum replacer was not available. Fifteen pregnant Rambouillet/Targhee ewes were purchased and closely monitored prior to lambing and were then

isolated as soon as they displayed signs of labour. Lambs were manually delivered onto clean sheets and immediately transferred to the large animal biosafety level 2 (LABSL-2) facility, where they were raised isolated from other livestock. Lamb rooms had a combination of concrete and slatted flooring and were maintained at a temperature of 16°C with a natural light cycle. Lambs were weighed weekly and monitored by trained animal care staff twice per day to assess food intake, behaviour or any signs of illness. Animals that showed signs of ill health were examined and treated by a veterinarian if indicated.

### Administration of colostrum replacer

Lambs were bottle-fed Rescue Lamb & Kid Colostrum Replacer (Lifeline Nutrition Solutions/APC, Ankeny, IA, USA) at 40 g of powdered colostrum replacer diluted in 189 ml water per kg bodyweight over 24 hours and divided into six meals. Colostrum replacer consumption was recorded after every meal. Per manufacturer's analyses, the colostrum replacer contained 44% crude protein, 24% globulin protein (Ig derived from bovine serum), 24% crude fat and 0.5% crude fiber. Based on this information, the concentration of bovine Ig in the prepared colostrum replacer was 50.8 mg/ml.

### Lamb diet and weaning

After the first 24 hours, lambs received SuperLamb Milk Replacer (Milk Specialties Global, New Holstein, WI, USA), which contained 24% crude protein, 30% crude fat and 0.1% crude fibre. The protein in the milk replacer contained dried whey and whey protein concentrate. Lambs were bottle fed for the first week, followed by bucket feeding. Gradual weaning onto a mixture of corn, oats and barley with molasses as well as Lamb Starter Pellets (Payback, Sioux Falls, SD, USA) was initiated at around 45 days. Hay, water and a salt/mineral supplement were freely available throughout the study from birth.

### Measurement of serum IgG, total serum protein and parainfluenza 3 virus neutralisation titres

Blood samples (3–7 ml) were collected from the external jugular vein using a Vacutainer system and centrifuged to obtain serum. Day 1 blood samples were collected between 23 and 25 hours after birth. Serum IgG concentrations were measured using a bovine IgG ELISA kit (MyBioSource, San Diego, CA, USA). Total serum protein concentrations were measured using the Pierce Bicinchoninic Acid Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Parainfluenza 3 (PI-3) serum virus neutralisation titres were measured by the Washington Animal

Disease Diagnostic Lab (Pullman, WA, USA) using bovine turbinate cells and the Reisinger SF-4 strain of PI-3 virus. The titre was determined as the highest dilution showing complete neutralisation with no evidence of a cytopathic effect.

### Statistical analysis

Data were analysed using GraphPad Prism 9.3.0 and are shown as the mean  $\pm$  standard deviation. Normality of distribution was determined using a Shapiro-Wilk test ( $\alpha = 0.05$ ). Differences between time points were analysed using a Wilcoxon matched-pairs signed rank test or ANOVA with Tukey's multiple comparisons test and were considered significant at  $p \leq 0.05$ . A simple linear regression analysis was performed to assess relationships between different parameters.

## RESULTS

### Characteristics and survival rates of artificially reared lambs

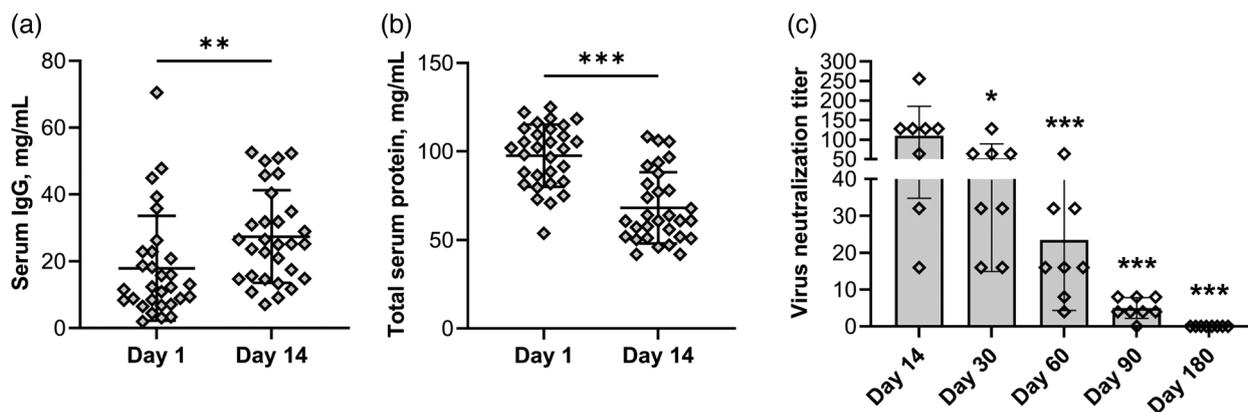
Out of 32 live births, 30 lambs (93.8%) survived and were included in the data analysis. One lamb was euthanised 2 days after birth due to lethargy and inappetence, and a second lamb born with neurological issues was euthanised 6 days after birth. The study group was composed of 12 males and 18 females, with two single lambs, 12 twins, 10 triplets and six quadruplets. The average daily gains were  $0.36 \pm 0.04$  kg/day during the first 8 weeks of life. Detailed health records showed that six out of 30 lambs developed mild diarrhoea during the study that generally resolved within 1 day, but no other signs of infectious disease were observed.

### IgG and total serum protein concentrations in colostrum-replacer-fed lambs

Lambs weighed  $4.9 \pm 1.1$  kg at birth and thus received colostrum replacer with a total of  $47.0 \pm 10.6$  g of IgG over 24 hours. Serum IgG levels of  $17.9 \pm 15.7$  mg/ml were detected 1 day after birth. On day 14, IgG levels were significantly higher at  $27.5 \pm 13.9$  mg/ml ( $p \leq 0.01$ ; Figure 1a). Conversely, total serum protein levels were  $97.6 \pm 17.6$  mg/ml 1 day after birth but decreased significantly by day 14 ( $68.1 \pm 20.0$  mg/ml;  $p \leq 0.001$ ; Figure 1b).

### Antibodies derived from bovine colostrum persist for several months

Our unique study design, where lambs were raised artificially in an ABL-2 facility, enabled us to analyse the persistence of antigen-specific antibodies derived from the colostrum replacer. To that end,



**FIGURE 1** Serum concentrations of immunoglobulin G (IgG) and total protein in artificially raised lambs. Serum samples from 30 lambs that received colostrum replacer were collected 1 and 14 days after birth. (a) The concentration of total bovine IgG in lamb serum was determined by bovine IgG ELISA. (b) Total protein concentrations in lamb serum on days 1 and 14 after birth were determined by bicinchoninic acid assay. (a and b) Individual data points, mean  $\pm$  standard deviation are shown, Wilcoxon matched-pairs signed rank test,  $**p \leq 0.01$ ,  $***p \leq 0.001$ . (c) Virus neutralisation titres for parainfluenza 3 virus on days 14, 30, 60, 90 and 180 after birth. Days 30, 60, 90 and 180 were compared to day 14 using ordinary one-way ANOVA with Tukey's post hoc test,  $*p \leq 0.05$ ,  $***p \leq 0.001$

we measured PI-3 neutralising antibodies in nine randomly selected lambs. Since PI-3 infections or vaccinations are extremely common in bovine herds,<sup>19</sup> reactive antibodies were expected to be present in the colostrum replacer, which contains bovine serum, and our management practices prevented natural PI-3 infection with resulting antibody production in the lambs. The PI-3 neutralising activity of lamb sera was measured on days 14, 30, 60, 90 and 180. A 24-hour sample was not analysed due to insufficient sample volumes. The sera of all lambs contained PI-3 neutralising antibodies, with average titres of  $110.0 \pm 75.3$  at 14 days after birth. Titres were highest on day 14 and decreased steadily until they dropped below the limit of detection at day 180 (Figure 1c). In seven out of eight lambs (87.5%), PI-3 neutralising activity was still observed at 90 days. The average persistence of PI-3 neutralising activity was  $86.3 \pm 10.6$  days.

### Colostrum consumption did not correlate with IgG or total serum protein concentrations

The actual uptake of colostrum replacer by the lambs varied between 63% and 155% of the volume recommended by the manufacturer (mean: 94.7%). However, there was no significant correlation between colostrum replacer consumption and either IgG or serum protein concentrations measured at 24 hours after birth (Figure 2a,b). Unexpectedly, there was also no correlation between serum IgG and protein concentrations (Figure 2c).

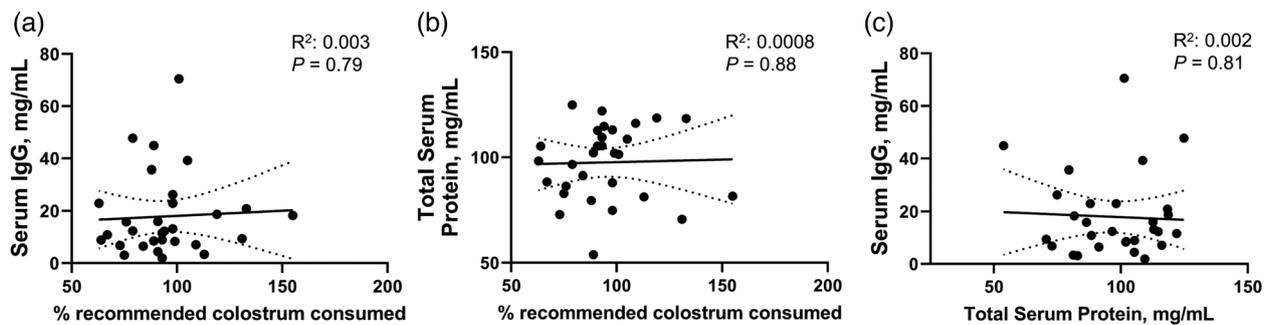
## DISCUSSION

Our data indicate that bovine serum antibodies in a commercial lamb colostrum replacer are readily absorbed into the bloodstream of lambs and persist

for up to 3 months. Moreover, we did not observe any negative effects of colostrum replacer administration on lamb health and performance, indicating that colostrum replacers with bovine serum are a viable replacement for ewe colostrum.

We showed that the commercial lamb colostrum replacer used resulted in significant and sufficient IgG uptake into the lambs' circulation. The average concentration of serum IgG 24 hours after birth was  $17.9 \pm 15.7$  mg/ml, which is above the threshold of 15 mg/ml that is used to indicate failure of passive transfer<sup>5</sup> and comparable to the IgG concentrations reported for 24-hours-old lambs fed ewe colostrum (19.6 mg/ml, Barta<sup>20</sup> and 24.6 mg/ml, Massimini et al.<sup>21</sup>). We thus confirm previous reports of bovine IgG absorption in lambs.<sup>6,17,22</sup> The precolostral IgG concentration in lambs was reported to be only  $0.22 \pm 0.01$   $\mu$ g/ml,<sup>23</sup> strongly suggesting that the IgG we measured was derived from the colostrum replacer. Although the milk replacer product used contained some whey-derived Ig, absorption of IgG from the gut ceases within 24 hours in lambs.<sup>24</sup> Therefore, the increased IgG concentrations detected on day 14 compared to 1 day after birth likely reflect the initiation of antibody production by the lambs, consistent with previous studies.<sup>25,26</sup>

Interestingly, there was no significant correlation between the volume of colostrum replacer consumed and IgG or serum protein concentrations. While the serum protein concentrations measured here were similar to those reported elsewhere,<sup>27,28</sup> they did not correlate with IgG concentrations. This was unexpected, since serum protein is frequently used to assess colostrum intake.<sup>5,29</sup> However, serum protein concentrations vary widely depending on hydration status, liver function, genetics and other factors. Moreover, since all lambs in our study were supplied with an adequate amount of colostrum with sufficient IgG, it is possible that IgG transport across the gut wall was saturated and that a correlation between serum protein



**FIGURE 2** Serum immunoglobulin G (IgG) and total protein in artificially raised lambs do not correlate with colostrum replacer intake. (a) Relationship between the concentration of total bovine IgG in lamb serum 24 hours after birth and the percentage of recommended colostrum consumed. (b) Relationship between total serum protein and percentage of recommended colostrum consumed at 24 hours after birth. (c) Relationship between total bovine IgG and protein concentration in lamb serum 24 hours after birth. (a–c) Data were analysed using a simple linear regression model, and dotted lines indicate the 95% confidence interval.  $R^2$  denotes the square of the correlation coefficient

and IgG would only be detectable with suboptimal colostrum intake.

Our unique experimental setup, where lambs were raised in isolation under SPF conditions, enabled us to monitor the degradation of antigen-specific antibodies over time without interference from developing natural immune responses in the lambs. We showed that PI-3 neutralising antibodies from bovine serum incorporated into a powdered colostrum replacer maintained a physiological half-life in the lambs' sera and remained functional for up to 3 months. The observed kinetics are consistent with the expected rate of Ig degradation in lambs.<sup>30</sup>

Notably, lamb colostrum replacers have been associated with variable effects on lamb survival and performance.<sup>6,22,31,32</sup> This may be due to differences in the IgG content of different lamb colostrum supplements products and lots<sup>16</sup> and other study parameters. In our study, the SPF conditions and intensive management likely improved animal health, survival and growth, so our observations need to be interpreted with caution. Additional limitations of our study were the lack of control groups that received ewe colostrum or no colostrum, the lack of pre-colostrum antibody and protein data, and the lack of PI-3 neutralisation data at 24 hours. Overall, our findings of sufficient antibody uptake, long-term antibody persistence and excellent lamb health corroborate other publications<sup>17,22</sup> that support the use of high-quality colostrum replacers with a bovine Ig source in lambs.

#### ACKNOWLEDGEMENTS

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#### CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### ETHICS STATEMENT

Animal experiments were approved by the Institutional Animal Care and Use Committee of Montana State University, protocol #2019-95.

#### AUTHOR CONTRIBUTIONS

*Conceptualisation, methodology, investigation and writing—original draft:* Thea Johnson. *Software, formal analysis, investigation and writing—review and editing:* Bryan Tegner Jacobson. *Conceptualisation, investigation, resources, supervision and project administration:* Kerri Jones. *Investigation:* Cassie Mosdal, Steve Jones, Maia Vitkovic and Sam Kruppenbacher. *Investigation and formal analysis:* Andy Sebrell. *Conceptualisation, formal analysis, writing—original draft, review and editing, visualisation, supervision, project administration and funding acquisition:* Diane Bimczok.

#### DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available from the corresponding author on reasonable request.

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