

# The Intertwined Successional Development of the Lamb Gut Microbiota and Immune System

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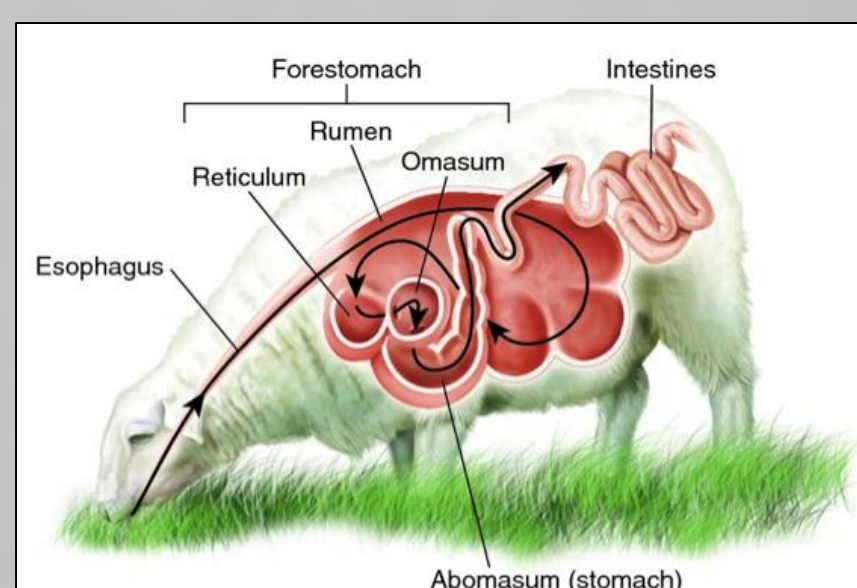


## Abstract

Gastrointestinal tract-dwelling (GIT) microbes play critical roles in host nutrition, health, and immunological development. For adult ruminants, GIT microbes provide ~70% of daily energy requirements. The GIT also houses 70% of the animal's immune system in the form of the Gut-associated Lymphatic Tissue (GALT), which houses 80% of all plasma cells, and depends on microbial stimulation for maturation. Because nutrition and disease are two major factors in the economic sustainability of livestock production, our group set out to characterize the successional development of GIT microbiota and serum immunoglobulin levels. Blood and GIT samples were collected from lambs immediately at birth through one-year of age, and from the dam's vagina, mouth, and rectum at parturition. Blood samples were profiled for serum titers of IgM, IgA and IgG, while microbiota were profiled in GIT samples by 16S rRNA gene sequencing. Lamb GIT microbiota initially resembled the dam's vaginal microbiota but following exposure to the dam, became rapidly more similar to the dam's teat. GIT samples eventually formed stable climax communities similar to that of the dams around 180 days of age. This corresponded to the peak serum titers for each immunoglobulin, which, aside from a peak in IgG at birth (likely maternal transfer), had gradually increased prior to this time. Immunoglobulins peaked and then return to a sub peak level between 180 and 365 days. These results indicate dam vaginal microbiota have a short-lived impact on the neonatal microbiota, with the GIT microbiota going through a dynamic successional development to 180 days when immune function appears to peak. These results support previous data indicating animals with a more mature GIT microbiota have a more mature immune system. Under standard rearing practices, maturing of the GIT microbiota and immune system appear to occur at ~180 days of age. The dam's teat appears to be an important source of early microbes and dietary or microbial interventions targeting the teat may enhance the rate of GIT microbiome and immune system maturation increasing early lamb growth and survivability.

## Introduction

- The neonatal stage is the most critical time for determining future animal performance and, therefore, the economic contribution to future operations<sup>1</sup>.
- Fetuses develop in a sterile environment, and microbial colonization is initiated at parturition by the microbiota in the mother's vaginal tract<sup>2</sup>.
- Internal and external colonization of body surfaces by microbial communities influences animal performance, with effects on reproduction, immune function, gut health, brain function and the ability to provide nutrition to the supporting host<sup>3,9,2</sup>.
- Microbial colonization of the gastrointestinal tract (GI) is crucial for ruminants because it is through microbial fermentation of recalcitrant plant materials in the forestomach (more specifically the rumen) that ~70% of their dietary needs are met<sup>4</sup>.
- The rumen ecosystem, is diverse and contains microbial communities that include: protozoa (10<sup>6</sup>/mL of rumen fluid), fungi (10<sup>7</sup>/mL of rumen fluid), bacteria (10<sup>11</sup>/g of rumen content) and viruses (10<sup>10</sup>/mL of rumen fluid)<sup>5,6,7</sup>.

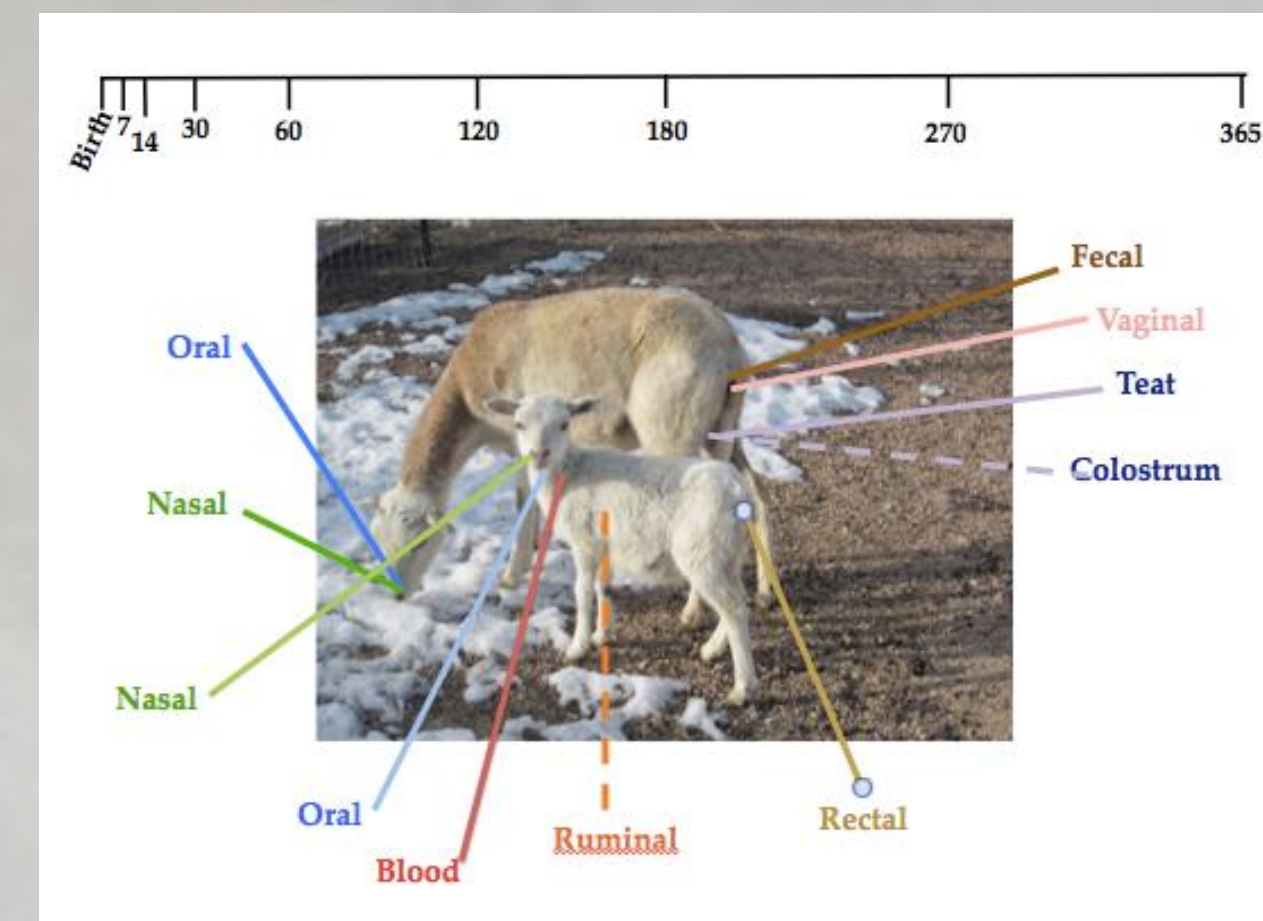


**Figure 1.** Showing basic sheep gastrointestinal anatomy in regards to digesta flow<sup>8</sup>.

- The GIT also houses 70% of the animal's immune system in the form of the Gut-associated Lymphatic Tissue (GALT)<sup>9</sup>.
- Approximately 80% of all plasma cells, which produce immunoglobulins are housed in the GALT<sup>9</sup>.
- The GALT envelopes both isolated and aggregated lymphoid follicles. This also accounts for the important immunological tissues such as the Peyer's Patches in the ileum<sup>9</sup>.
- Neonatal ruminants display two types of Peyer's Patches (PPs), the ileal PP and the jejunal PP. Both types of PP are noted with high lymphopoiesis activity (generation of lymphocytes) and are regarded as primary lymphoid tissues<sup>10</sup>.
- Maturation of the GALT and associated tissues requires microbial stimulation<sup>9</sup>.

## Methods

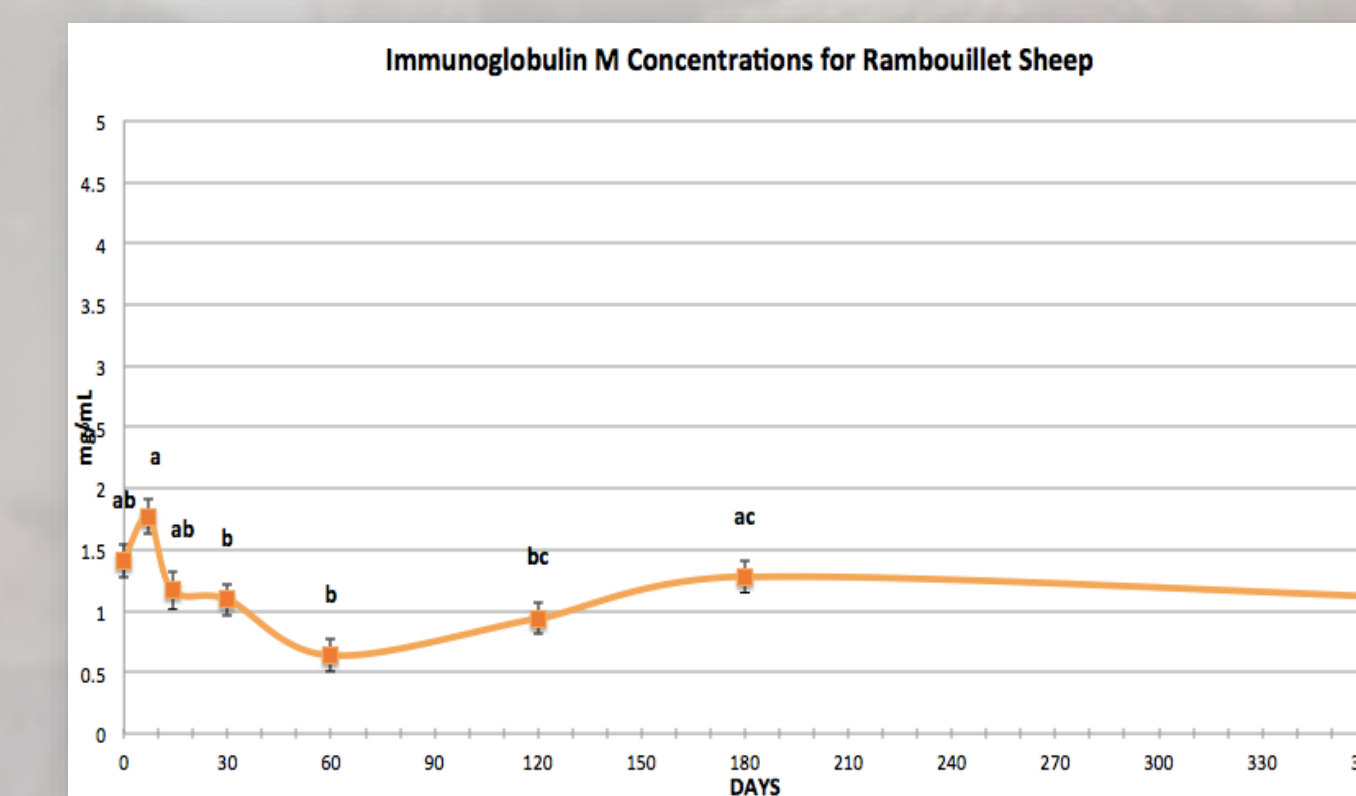
- 54 bred Rambouillet ewes were acquired for this study.



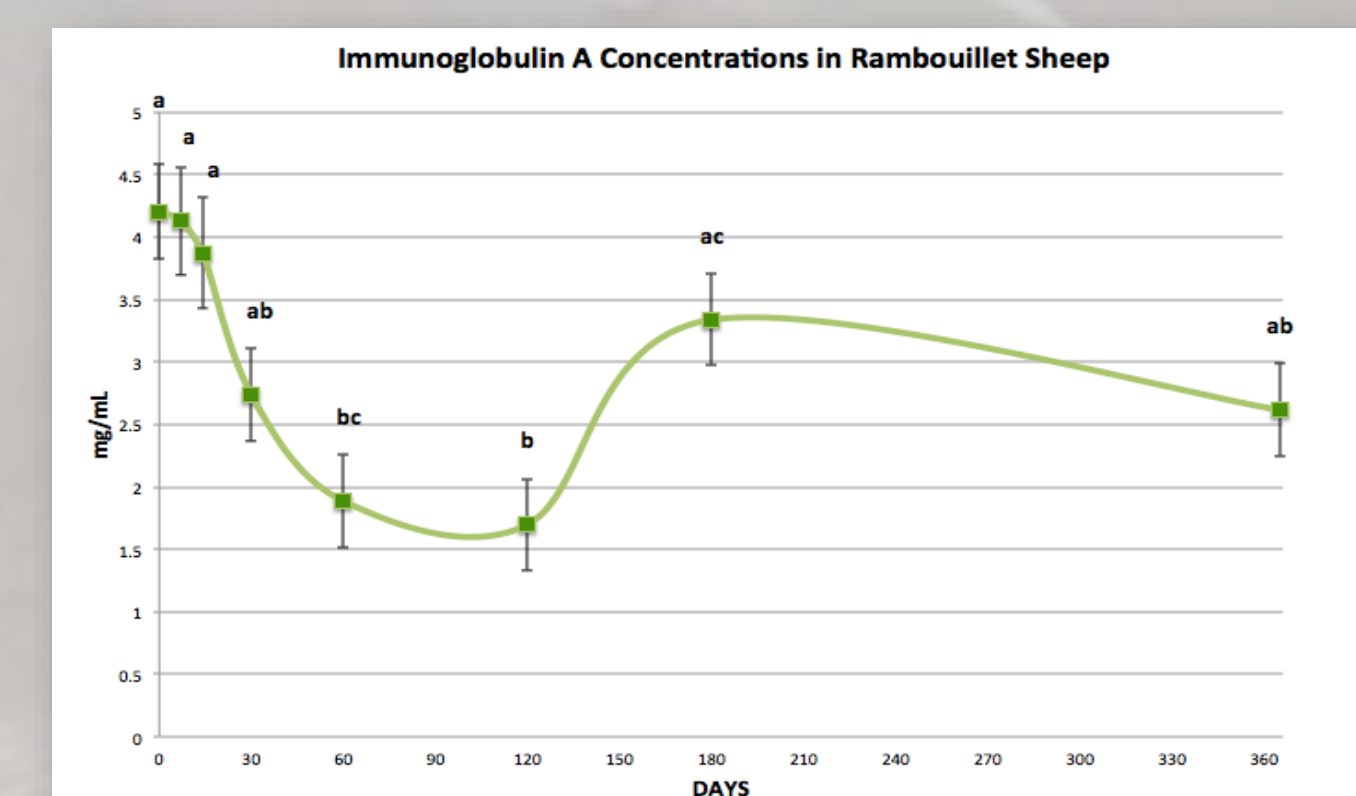
**Figure 2.** Locations of samples collected from both the dams and lambs on trial.

- Dams were swab sampled for oral, nasal, fecal, and teat microbes following parturition. Dams were also milked for colostrum.
- Lambs were swabbed immediately following birth and periodically thereafter for the oral, nasal, and rectal orifices. Blood samples were collected via jugular venipuncture in the lambs.
- Blood sera, swabs and lavage samples were stored at -20°C until processing.
- DNA was extracted and the V3-V4 regions of the 16S rRNA subunit amplified from all samples.
- Amplicons were sequenced by Illumina MiSeq.
- Contigs were assembled using Pandaseq<sup>11</sup>, curated with mothur<sup>12</sup> and all figures were created using PRIMER<sup>13</sup> and Microsoft Excel.
- Blood serum samples were profiled for antibody titers in triplicate using enzyme-linked immunosorbent assays (ELISAs) specific for sheep IgM, IgA and IgG.
- For the calculation of immunoglobulin concentrations all standard curves were weighted with a relative weighting of 1/y<sup>2</sup>.
- Immunoglobulin concentration data was analyzed using ANOVA with repeated measures of SAS.
- Differences in means were separated by Bonferroni (t-test adjustment) of SAS.

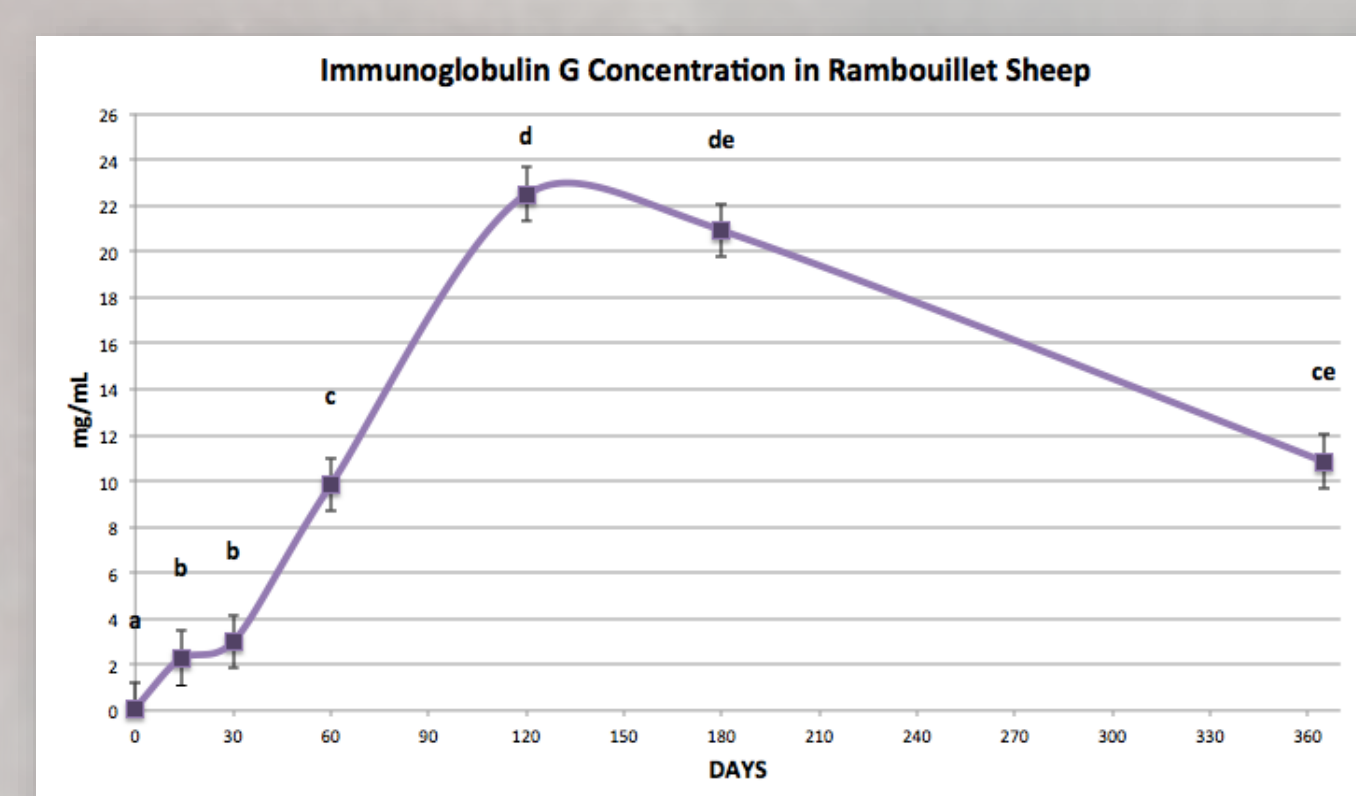
## Results



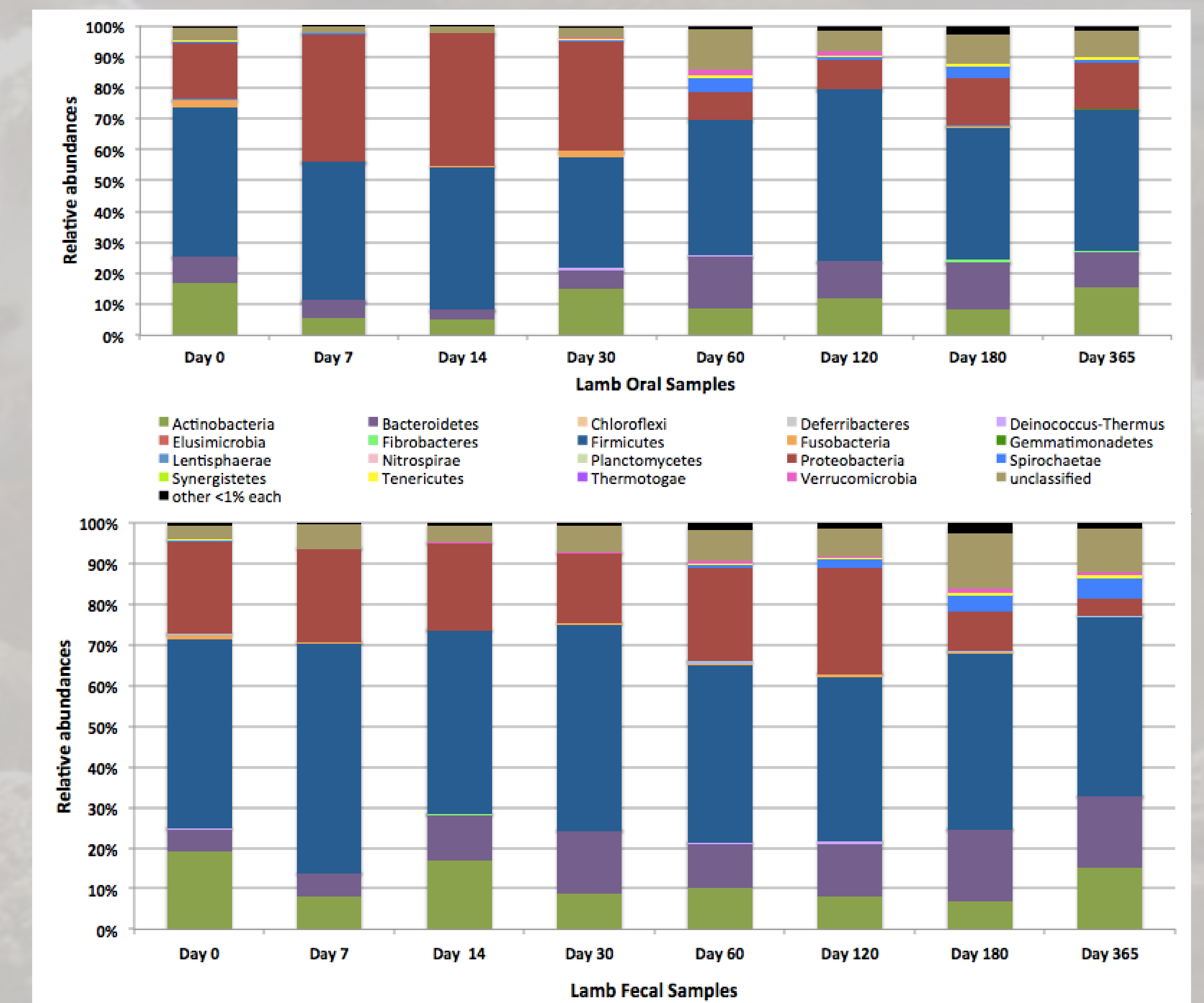
**Figure 3.** Least square means of immunoglobulin M (IgM) blood sera concentrations (mg/mL) for lambs at days 0, 7, 14, 30, 60, 120, 180 and 365. Means with different letters differ ( $P < 0.05$ ). Pooled SEM= 0.31 mg/mL.



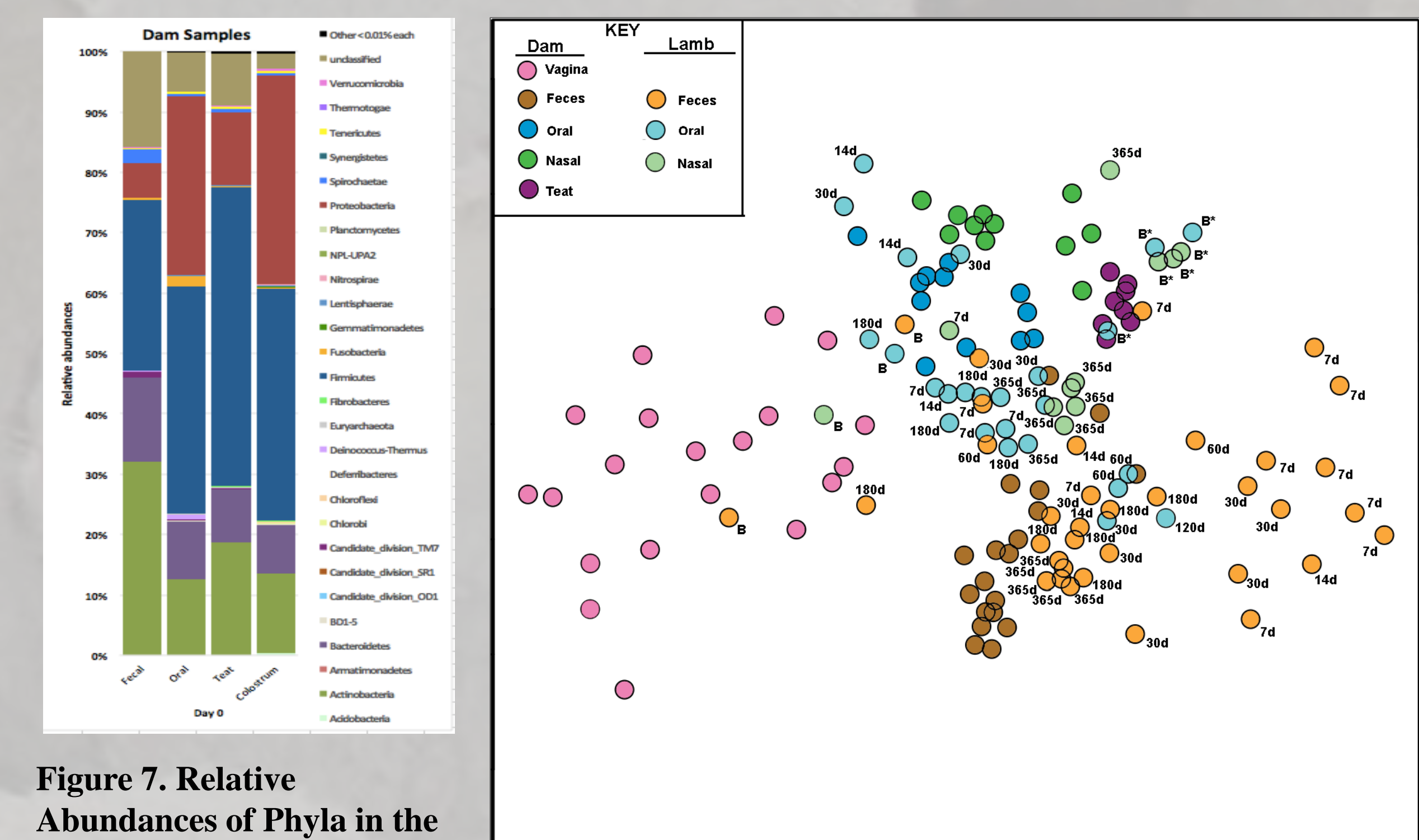
**Figure 4.** Least square means of immunoglobulin A (IgA) blood sera concentrations (mg/mL) for lambs at days 0, 7, 14, 30, 60, 120, 180 and 365. Means with different letters differ ( $P < 0.05$ ). Pooled SEM= 3.15 mg/mL.



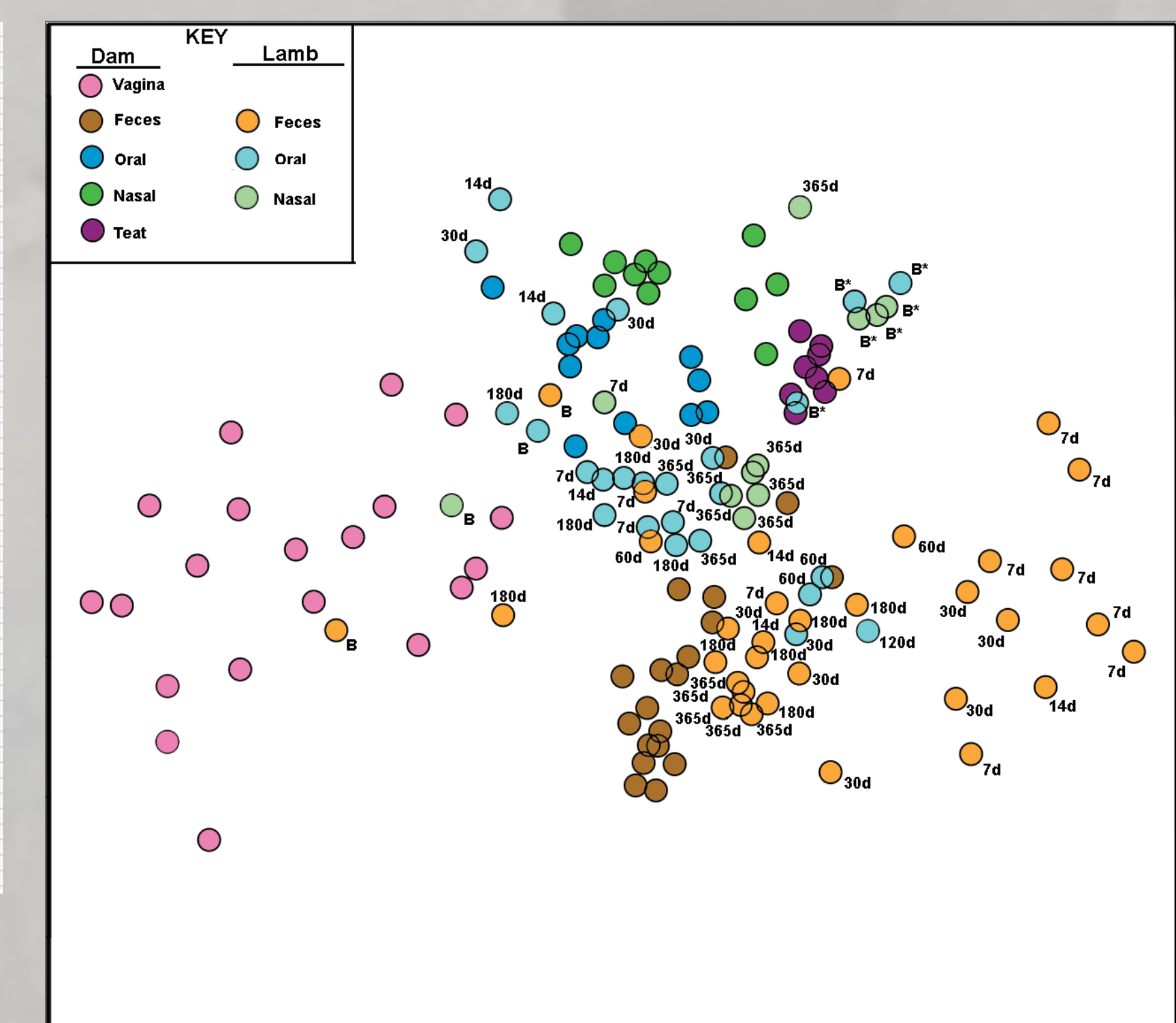
**Figure 5.** Least square means of immunoglobulin M (IgM) blood sera concentrations (mg/mL) for lambs at days 0, 14, 30, 60, 120, 180 and 365. Means with different letters differ ( $P < 0.05$ ). Pooled SEM= 1.26 mg/mL.



**Figure 6.** Relative Abundances of Successional Phyla in the Lamb Oral and Rectal Orifices. Stacked bars show a trend towards an gradual overall decrease in the Phylum Proteobacteria with a concomitant increase in the Phylum Bacteroidetes, which begins at birth and continues on through one year of age. The Phylum Firmicutes stays relatively constant from birth to one year of life in all lambs.



**Figure 7.** Relative Abundances of Phyla in the dam's fecal, oral, teat and colostrum. Shows phylum-level composition of potential sources of microbes.



**Figure 8.** NMDS Plot of Dam and Temporal Lamb Samples Showing successional change with early colonization from vaginal microbiota towards the resemblance of an adult ovine in the oral, nasal and rectal orifices.

## Conclusion

- Antibody titer levels are consistent with previously reported serum levels in sheep. Immunoglobulins M, A and G show similar trends. Graphs of all three antibody levels display a gradual increase in concentration until reaching peaks levels by day 180 and six months later, at day 365, all samples show reduced levels in comparison to d180.
- Microbial phyla patterns are similar to those seen in humans and cows. Relative phyla abundances show successional transition from facultative anaerobes, principally Proteobacteria (example: *E. coli*) towards a strictly anaerobic population (Bacteroidetes & Firmicutes). Also, all lambs follow this pattern and become more similar suggesting more variation in early microbiota that eventually tends towards a stable and diverse climax microbiota.
- The impact of the initial colonization of the neonatal lamb from the dam's vaginal tract microbiota is brief, with the GIT microbiota going through a dynamic successional development when immune function appears to peak.