Reduced α-diversity of bacteria in refrigerated compared to non-refrigerated colostrum from primi and multiparous dairy cows

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HoloRuminant Understanding microbiomes of the ruminant holobiont

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Introduction and Objectives:

Colostrum is essential for calf health. Beyond immunoglobulins, there are a multitude of bioactive components that contribute to the immune and physiological development of the calf. Microbes in colostrum have previously been attributed to environmental contamination, however, recent evidence supports the existence of an entero-mammary pathway and a natural colostral microbiome. In addition to this, colostral microbes have also been theorized to act as pioneering microbes associated with seeding and colonization of the calf gut microbiome. A paucity of work has been done on the characterization of the colostral microbiota, their functional role and the effect of common colostrum management practices may have on this community. Thus, the study objective was to characterize the archaeal and bacterial components of colostrum collected from a single, spring-calving dairy herd comprised of primiparous and multiparous Holstein-Friesian and Jersey cows. A secondary objective was to investigate the potential implications that refrigeration and re-heating may have on the colostral microbiota.

Materials and Methods

Animal Model

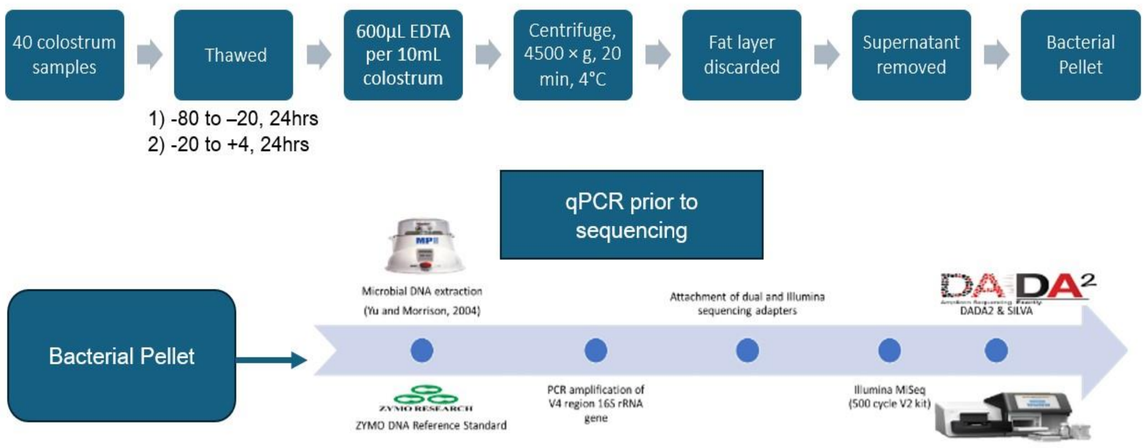
Single herd — Spring-calving; 2022 calving season

40 cows — 25 Holstein-Friesian, 15 Jersey; 13 primi, 27 multiparous

Collection/Storage — 27 fresh (Dam); 13 refrigerated (Fridge)

Colostrum samples were either 1), collected from all four mammary gland quarters within 2h of parturition (**Dam**; n=27) and fed to the calf or 2), within 6h, refrigerated at 4°C (≤24h) and re-heated to 38°C in a water bath (**Fridge**; n=13) prior to feeding of calves. All calves were manually fed colostrum within 2h of birth, at which point colostrum aliquots (10mL × 3) were collected and immediately snap frozen and stored at -80°C.

Extraction, Sequencing and Analysis



Data underwent PERMANOVA, α- and β-diversity analyses using *R* packages *DADA2*, *Phyloseq*, *Microbiome* and *Vegan*. Taxonomy was assigned using the SILVA database (v. 138.1). Data were filtered by colostrum source, with all analyses performed on each subset separately.

Conclusion:

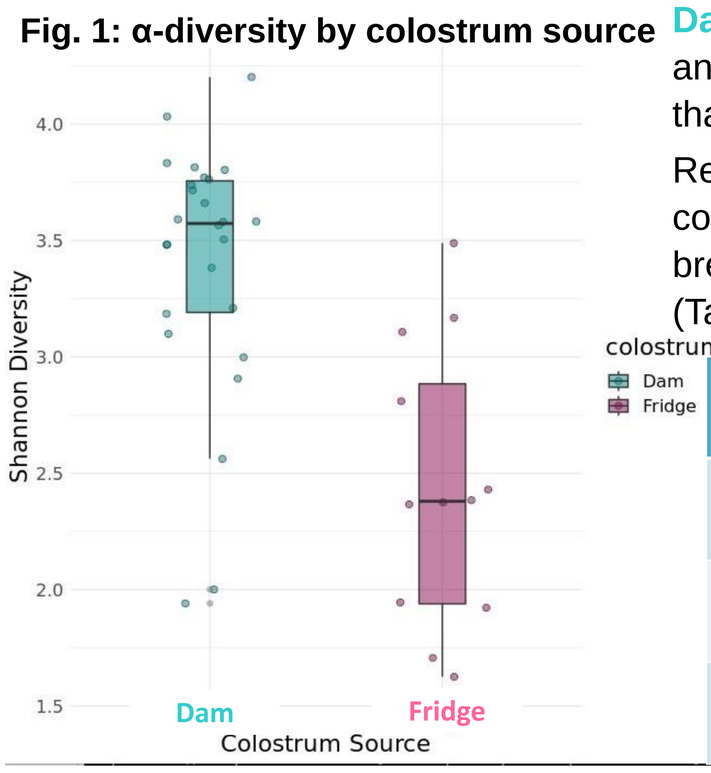
Colostrum that was collected from the **Dam** had low bacterial loads, high α-diversity and homogenous microbial composition across samples. Colostrum that was collected and refrigerated (**Fridge**) was observed to have higher bacterial loads, reduced α-diversity and heterogeneous bacterial composition across samples. The bacterial diversity observed in **Dam** colostrum went beyond taxa, and included aerobes, facultative and obligate anaerobes, which are critical to the transition of the calf hindgut from aerobic to anaerobic post-birth. Many of the genera identified, have also previously been identified as common bovine gut commensals, including *Methanobrevibacter*, lending support to the theory of an entero-mammary pathway in ruminants. Further work is warranted to determine effect of common colostrum management practices on the colostral microbiome and calf health implications.

Results

Table 1: Quantification of bacterial content by colostrum source

	CQ Value	SE	P-value
Dam (n=27)	23.54	0.87	<0.0001
Fridge (n=13)	19.46		

Fig. 1: α-diversity by colostrum source

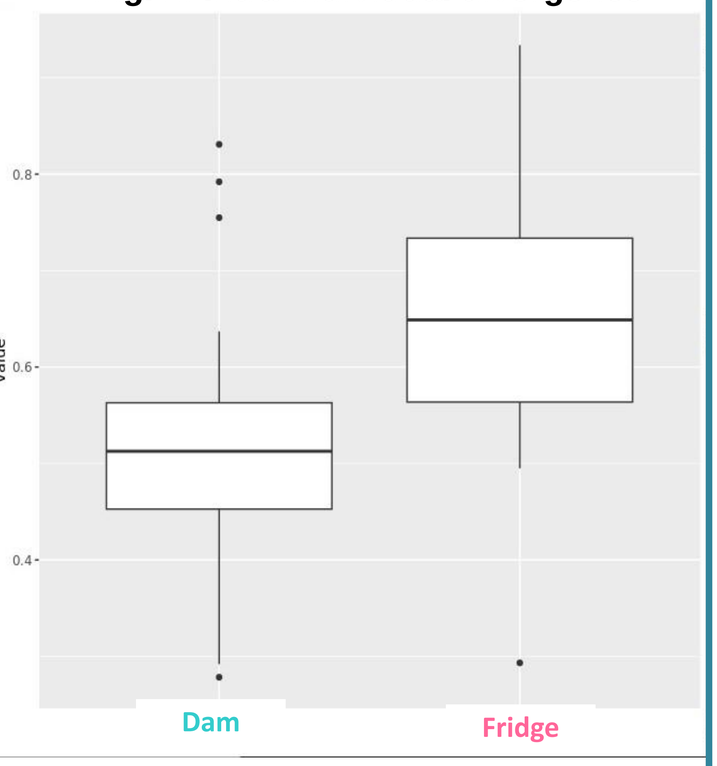


Dam colostrum had later CQ values and therefore lesser bacterial loads than **fridge** colostrum (Table 1).

Regarding microbial diversity and composition, there was no effect of breed or parity, only colostrum source (Table 2).

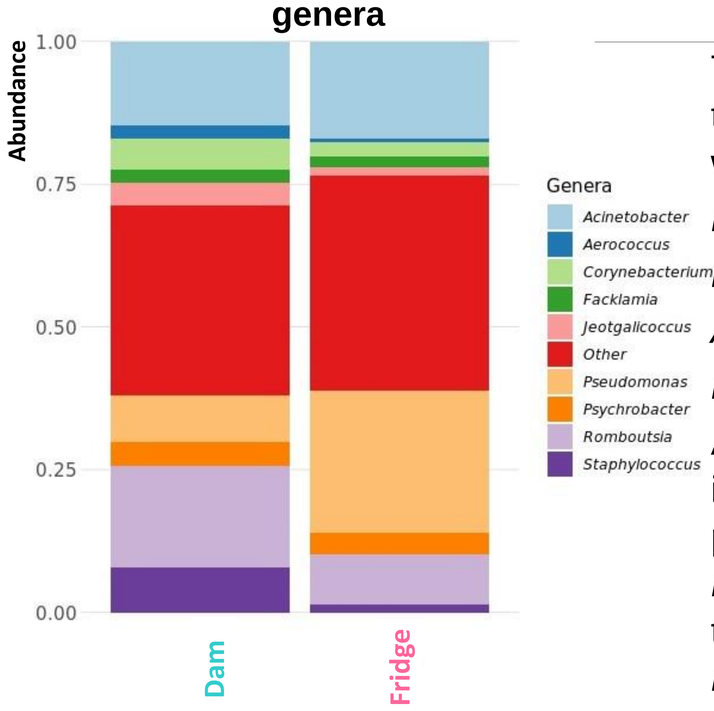
Table 2: Effect on Microbiota	p-Value
Colostrum source (Dam v. Fridge)	0.0002
Cow breed (Ho v. Je)	0.17
Cow parity (Primi- v. Multiparous)	0.11

Fig. 2: Intra-individual divergence



Dam colostrum was observed to have a diverse (Fig.1) and homogenous microbial community (Fig.2), indicating that there is a wide variety of bacteria present that appeared across all samples within the **Dam** subset. **Fridge** colostrum was observed to have reduced α-diversity (Fig. 1) alongside heterogeneous community membership (Fig. 2), indicating that there were few bacteria present, and microbial composition was varied between the **Fridge** subset.

Fig. 3: Relative abundance of top genera



The bacterial phyla identified with the highest relative abundances were

Bacillota	42.5%
Pseudomonadota	34.2%
Actinomycetota	11.2%
Bacteroidota	7.6%.

A variety of bacterial genera were identified (Fig. 3), including several pathogens such as *Yersinia* and *Klebsiella*. The archaeal genus with the highest relative abundance was *Methanobrevibacter* (85.5%).

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