Proceedings

of the British Society of Animal Science
in association with the
Agricultural Research Forum

2018

Advances in Animal Biosciences

This book is part of a series which is a companion to the journal ANIMAL

Blood immune transcriptome analysis of artificially fed dairy calves and naturally suckled beef calves from birth to 7 days of age

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Application This study investigated the molecular mechanisms involved in the developing immune response of neonatal beef and dairy calves. Identified genes of interest have potential as biomarkers of immunocompetence in future studies.

Introduction Neonatal calves possess an immature and naïve immune system and are reliant on the intake of maternal colostrum. Variation in colostrum management in beef and dairy calves is thought to affect early immune development. Therefore, the objective of this study was to examine changes in gene expression and investigate molecular pathways and biological functions involved in the immune-competence development of neonatal Holstein dairy calves and naturally suckled beef calves using next generation RNA-sequencing during the first week of life.

Material and methods Jugular whole blood samples were collected into RNA tempus tubes from Holstein dairy calves (n= 8) artificially fed 5% B.W. colostrum, and from naturally suckled Charolais-Limousin (CL; n = 7) and Limousin-Friesian beef calves (LF; n = 7), for subsequent RNA isolation. Blood samples were harvested at 0, 48, 72 and 168 hours (h) post-birth. mRNA was isolated from the whole blood, cDNA libraries prepared from mRNA and subsequently sequenced with single-end reads. RNAseq processing was carried out as described by Johnston *et al*., 2016. Quality assessment of the filtered data was performed by FastQC and reads were UMD3.1 *Bos taurus* genome. Read alignment was performed using STAR. Analysis of the count data was performed using DESeq2, and functional analysis of DEG was carried out using IPA.

Results Dairy calves were first compared to LF beef calves (Figure 1A). At 48h post-birth, comparison of LF to dairy calves highlighted a number of biologically interesting networks as enriched, including haematological system development and function. Top canonical pathways enriched included *IL-1* and *IL-8* signalling were both upregulated in dairy calves. At 72h post-birth, a significant number of pro-inflammatory cytokines, including *IL-2* complex, were upregulated in dairy calves. At 168h post-birth, comparison of dairy to LF calves highlighted enrichment of a number of networks including humoral immunity. A number of upstream regulators such as *TNF* and *LPS* were present at lower levels in LF calves. Dairy calves were also compared to CL beef calves (Figure 1B). Enriched networks included cell mediated immune response. Upstream regulators activated in beef CL calves after 48h included *IG,* with a significant number of pro-inflammatory cytokines upregulated in dairy calves. Comparison of beef CL to dairy at 72h highlighted *IL-6* and *IL-8* signalling as upregulated canonical pathways in dairy calves. At 168h post-birth, comparison of CL to dairy calves resulted in the enrichment of cell maintenance and structure, and also inflammatory response, which were upregulated in dairy calves at 168h. Upstream regulators identified during the comparison included a number of cytokines which were upregulated in dairy calves and immunoglobulin which was upregulated in CL at 168h post-birth.

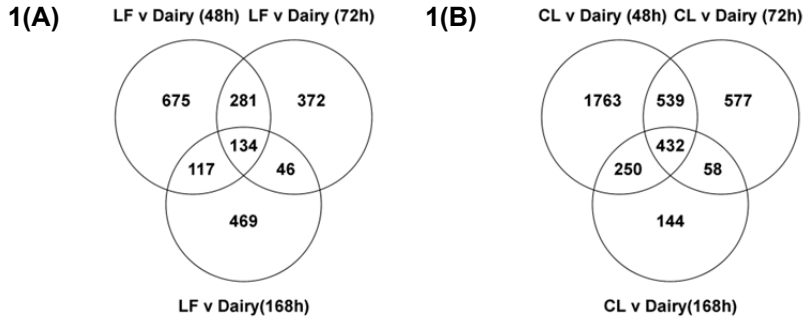

Figure 1 DEG identified in the comparison of LF beef calves (Figure 1A) and CL beef calves (Figure 1B) to dairy calves at 48h, 72h and 168 timepoints.

Pathway and functional analysis were performed using ingenuity pathway analysis (IPA).

Conclusion These data provide a greater understanding of the molecular control of the early development of the neonatal immune system of dairy and beef calves, highlighting some of the molecular mechanisms regulating the immune response, likely due to variations in colostrum ingestion. Dairy calves initially demonstrate a surge in pro-inflammatory cytokines with major differences observed between beef and dairy calves at 168h post-birth including increased abundance of *IG* in beef CL calves.

Acknowledgements The authors gratefully acknowledge Department of Agriculture, Food and the Marine (DAFM) Ireland, research stimulus funding (RSF) (Project 11/S/131).

Reference

Johnston, D., Earley, B., Cormican, P., Kenny, D.A., McCabe, M.S., Kelly, A.K., *et al*., 2016. PLoS ONE11(8).