Effect of energy restriction during late gestation in the skeletal muscle and blood transcriptome of Angus calves after preconditioning

Leticia P Sanglard[1], Moyses Nascimento[2], Philipe Moriel[3], Jeffrey Sommer[4], Melissa Ashwell[4], Marcio S Duarte[2], Nick VL Serão[1§]

[1]Iowa State University, 50011, Ames, IA, USA
[2]Universidade Federal de Viçosa, 36570-900, Viçosa, Brazil
[3]Range Cattle Research and Education Center, University of Florida, 33865, Ona, FL, USA
[4]North Carolina State University, Raleigh, 27695, NC, USA
[§]serao@iastate.edu

Summary

Maternal nutrition during gestation has long-term effects on skeletal muscle development of the offspring. The objective of this work was to identify differentially expressed genes (DEG) in the blood and muscle tissues of preconditioned beef calves born from cows with and without energy restriction during gestation. Thirty pregnant Angus cows were fed 70% or 100% of their energy requirements during the last third of gestation. The offspring were vaccinated for iBrV and BVDV 8 days post weaning (dpw). Blood samples were collected at 0, 3, 6 and 15 dpw from 12 steers, and muscle samples were collected at 21 dpw from 12 steers and 12 heifers. Total RNA was extracted and sequenced (RNA-seq). For statistical analysis, a negative binomial model including the effects of maternal diet, sex, diet-by-sex interaction, sequencing lane, and RIN score were used for muscle. For blood, sex was substituted for time, and a covariance structure AR(1) was tested. In addition, contrasts (i.e. effect of vaccination and weaning) were analyzed. For the DEG (q-value < 0.05), an enrichment analysis was performed in PANTHER. A total of 521, 1085, and 1317 DEG (q-value < 0.05) were identified in the muscle for the effects of diet, sex, and diet-by-sex interaction, respectively. For blood, 126, 529, and 4 DEG (q-value < 0.05) were identified for effects of diet, time, and diet-by-time interaction, respectively. In the muscle tissue, DEG for diet were enriched for *muscle contraction*, *carbohydrate metabolic process*, *metabolic process*, and *cellular glucose homeostasis*. For the effect of sex, the DEG were enriched for categories related to acid nucleic metabolic process, such as *DNA metabolic process* and *RNA metabolic process*. For blood, for the effect of vaccination and weaning there was an overrepresentation with immune response and stress processes, such as *B cell mediated immunity*, *immune response*, and *response to external stimulus*. In conclusion, enrichment analysis showed that maternal energy restriction affects the expression of genes related most to carbohydrate metabolic process, and to immune response in the blood tissue.

Keywords: Fetal programming, gene expression, maternal nutrition, RNAseq.

Introduction

Maternal nutrition has been discussed as one of the main factors affecting intra-uterine environment (Duarte et al., 2014). Nutritional deficiency may impact the regulation of genes

associated with myogenesis and immune response in the fetus (He et al., 2013). The increase in nutritional requirements by beef cows during late gestation (Larson et al., 2009) can cause lack of nutrients for optimum fetal development, which can affect physiological functions in the animal and, consequently, have an effect on the productive performance (Duarte et al., 2014).

The preconditioning phase is a critical period for beef calves, since animals undergo stressful procedures such as vaccination and weaning (Cooke and Bohnert, 2011). As a response, there is a mobilization of protein from muscle (Jahoor et al., 1999) to increase the synthesis of proteins and cells involved in the immune system (Reeds and Jahoor, 2001). Consequently, growth performance is compromised due limited availability of nutrients to muscle development.

Therefore, the objectives of this work were to identify differentially expressed genes (DEG) in the muscle and blood of beef calves born from cows with and without energy restriction, and to assess the biological relevance of differentially expressed genes (DEG).

Material and methods

Thirty multiparous, nonlactating, pregnant Angus cows were assigned to one of two diets: 100% or 70% of net energy requirement. After calving, cow–calf pairs were transferred to tall fescue pastures. At 8 days post weaning (dpw), calves were vaccinated against infectious bovine rhinotracheitis virus (iBrV) and bovine viral diarrhea virus (BVDV).

Blood samples were collected from 12 steers via jugular venipuncture at 0, 3, 6, and 15 dpw. At 21 dpw, a biopsy of the muscle *Longissimus dorsi* was collected in 12 steers and 12 heifers. Total RNA was extracted by Tempus™ RNA isolation kit. RNA quantity and quality were determined by Agilent 2100 Bioanalyzer. Sequencing was performed in an Illumina NextSeq 500, generating 75 bp single-reads and 150 bp paired-end reads for muscle and blood, respectively.

Raw reads were evaluated for quality with FastQC and then mapped to *Bos taurus* UMD3.1 using Bowtie2. The number of counts for each sample was obtained from SourceForge. A total of 2,076,680,240 and 1,353,110,288 reads were generated for muscle and blood samples.

For statistical analysis, a negative binomial model with the fixed effects of maternal diet, sex, diet-by-sex interaction, sequencing lane, and RIN score were used for muscle. For blood, sex was substituted for time, and a covariance structure autoregressive order 1 (AR(1)) was tested; the final model was chosen based on Akaike Information Criteria (AIC). All data were analyzed using GLIMMIX procedure of SAS 9.4. The enrichment of Gene Ontology (GO) terms associated with DEG was analyzed using PANTHER Enrichment Analysis.

Results and discussion

In the muscle tissue, 429 of the 521 DEG (q-value < 0.05) were overexpressed in the control group, suggesting that limiting energy availability during late gestation limits the expression of genes in the muscle (Fig 1; A). On the other hand, in the blood, there was a clearly overexpression (q-value < 0.05) of genes in the energy-restricted group (118 out of 126), compared to the control diet (Fig 1; B).

The enrichment analysis in the muscle for DEG for diet showed an overrepresentation (P-value < 0.05) of general categories, such as *transport* (GO:0006810), *catabolic process* (GO:0009056), and *cell cycle* (GO:0007049), but we also found relevant categories related to carbohydrate and protein metabolism such as *protein transport* (GO:0015031), *carbohydrate*

metabolic process (GO:0005975), and *muscle contraction* (GO:0006936). For the effect of sex, DEG were enriched for very general biological processes most of them associated with acid nucleic metabolism (e.g. *DNA recombination* (GO:0006310), *RNA metabolic process* (GO:0016070), and *DNA metabolic process* (GO:0006259). These functional terms are related to chromatin biology and epigenetic activities, suggesting that these changes are responsible for the alterations in gene expression observed in fetal tissues.

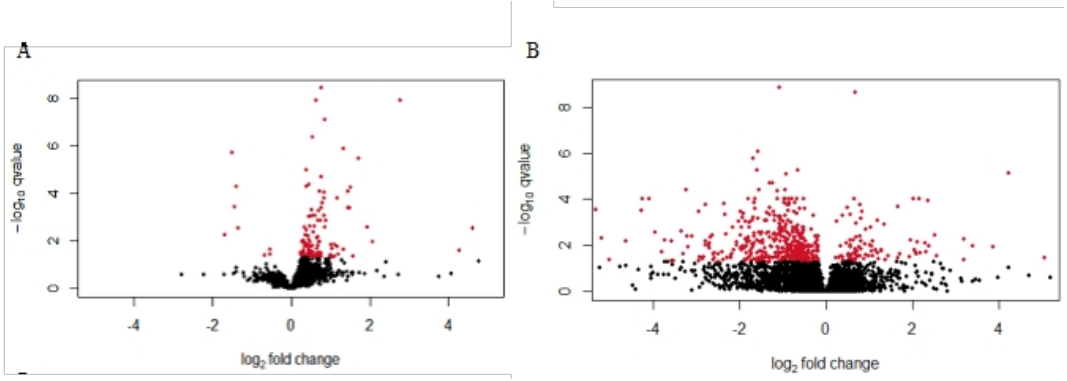

Fig. 1: Volcano plot for the effect of diet in analyses of muscle (A) and blood (B). Y-axis represents the –log10 *q*-values for the effect of diet, whereas the x-axis represents the $\log_2$ fold change, with negative and positive values representing the overexpression of the control and restricted diets, respectively. Significant genes (*q*-value < 0.05) are highlighted in red.

From the top 10 most significant genes overexpressed in the control group, we identified 5 associated with muscle metabolism and development as well as energy metabolism: ISM1, TMTC1, ZNF174, IGF2BP2, and ATP5S. These findings associated with the relevant functional terms in the enrichment analysis may indicate a change in the energy metabolism (from oxidative to glycolytic pathway) in the restricted group. In contrast, the energy-restricted group showed a higher expression of genes related to oxidative stress, such as CYP4B1, and DAPK2. This effect suggests that a restricted diet may require energy expenditure in the muscle tissue to neutralize or excrete greater quantities of toxic products and metabolites (Sreekumar et al., 2002) being a possible negative effect in feed efficiency.

For the analysis of DEG in the blood, we focused on the effect of vaccination and weaning (Table 1), and it was found biological processes related to immune response activities and response to stress, respectively (i.e. *B cell mediated immunity* (GO:0019724), *immune response* (GO:0006955), and *response to external stimulus* (GO:0009605)). These findings suggest that the nutritional status of the animal influenced the response to the immune challenge after weaning and vaccination.

Table 1: Enrichment analysis showing overrepresented[1] Biological Process for Differentially Expressed Genes[2] in blood tissue.

Term name	# genes	FE	P-value
DEG for effect of vaccination			
Immune response (GO:0006955)	10	2.24	1.49E-02
Localization (GO:0051179)	25	1.57	1.57E-02

B cell mediated immunity (GO:0019724)	4	4.03	1.81E-02
Transport (GO:0006810)	23	1.58	1.94E-02
Response to external stimulus (GO:0009605)	6	2.56	3.15E-02
DEG for effect of interaction vaccination-by-diet			
Immune response (GO:0006955)	5	5.81	1.54E-03
Localization (GO:0051179)	8	2.61	9.10E-03
Intracellular signal transduction (GO:0035556)	5	3.33	1.59E-02

[1]*P*-value < 0.05; [2]*q*-value < 0.05; FE = fold enrichment

For the effect of weaning, among the top 10 major DEG we found genes related to response to stress, such as PYCR1 and MPV17L. These genes were overexpressed in the energy-restricted group after weaning. Similar to the results in the muscle tissue for the effect of energy-restricted diet, we hypothesized that the restricted animals triggered a more pronounced stress process than the control group during the acute response phase. When we looked at the effect of interaction diet-by-vaccination, we found genes involved in the immune response being overexpressed in the control group after vaccination, such as CCL4, KLRF, CCL5, and KIR3DS1. These findings suggest that the control group showed a greater response to the immune challenge.

In conclusion, enrichment analysis showed that maternal energy restriction during pregnancy affects the expression of genes related to carbohydrate metabolic process and muscle contraction in the muscle, and immune response and response to stress in blood. Therefore, alterations on the intra-uterine environment can modify prenatal development, with lasting consequences in the adult life.

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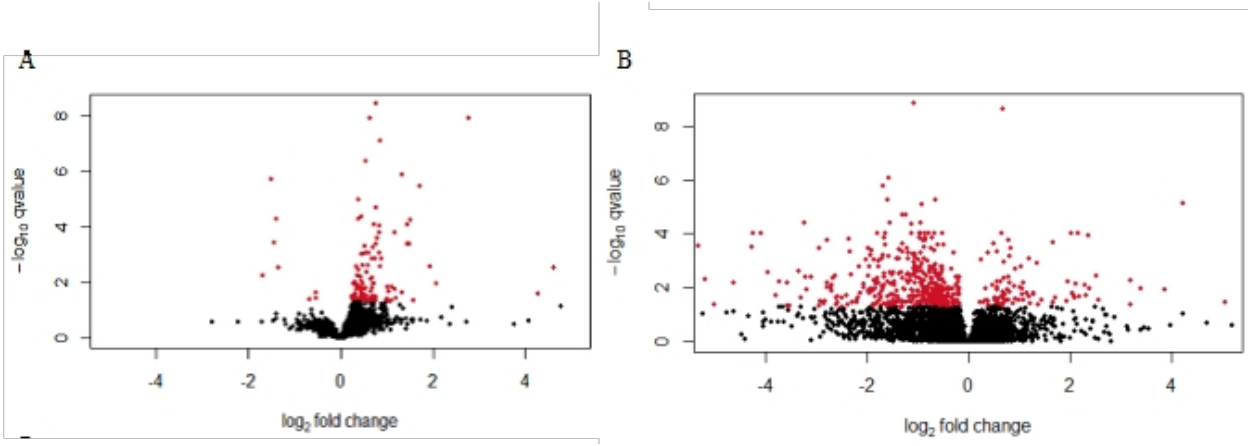
Figure



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