SHORT-TERM IMPLANTING ALTERS THE BEEF TRANSCRIPTOME – EFFECTS ON MICRORNA EXPRESSION

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I. INTRODUCTION

Implanting cattle with growth stimulants is a common practice in the United States, where 91% of steers are implanted at least one time during the finishing phase. Implants increase levels of somatotropin and insulin-like growth-factor leading to an increase of growth hormone and consequently muscle growth. This research was conducted to evaluate the effects of a short term-implant strategy on microRNA (miR) profile of beef. MiRs are small, highly conserved non-coding RNAs, with 20 to 22 nucleotides, that modulate up to 60% of protein-coding genes at the translational level [1]. They are initially transcribed by the RNA polymerase, processed by ribonuclease enzymes and coupled with the AGO2 protein to form the RNA-induced silencing complex (RISC). Once active, the RISC attaches to a complementary RNA sequence inhibiting protein translation and silencing genes. Therefore, if ingested, homologous miRs from beef may have the ability to silence genes of hosts, modulating their homeostasis. Our goal was to evaluate the miR profile of implanted and non-implanted beef from a nanonutrient standpoint, assuming that miRs may play an important role in modulating genes associated with chronic diseases.

II. MATERIALS AND METHODS

Sixteen, twelve-month-old, Angus steers, weighing approximately 1052.8 ± 12.1 lb were randomly assigned to 1 of 2 treatments (implant n=8 and non-implant n=8 animals) Implanted steers received one pellet of Revalor®XS (200 mg of trenbolone acetate and 40 mg 17ß-oestradiol) for 100 days prior to slaughter. All steers were individually fed a diet containing 39.96% alfalfa and 40.24% corn for for the same period of implanting. Animals were harvested at a commercial processing plant and an aliquot weighing approximately 10g was collected from the logissimus dorsi et lumborum muscle 24 h post mortem to evaluate the miR profile in fresh uncooked samples. Subsequently, a 2.54 cm steak was obtained from the left strip loin, aged for 14 d, cooked at 71°C and digested in vitro with pepsin and trypsin to mimic human digestion. RNA was extracted and isolated from all samples via Triazol extraction and quantified via spectrophotometry on the NanoDrop 1000 (Thermo Scientific, USA). Barcoded miRNA-Seq libraries were prepared using the NEXTflex Small RNA Sequencing v3 kit (Bioo Scientific, Austin, Texas) with randomized adapters according to the recommendations of the manufacturer. Libraries were sequenced on one lane of a HiSeq 4000 sequencer (Illumina, San Diego, CA) with single-end 100 bp reads. Small RNA sequencing data were summarized by the SPORTS computational pipeline. The differentially expressed genes and miRNAs were identified by the edgeR tool.

III. RESULTS AND DISCUSSION

Results for fresh and digested samples are presented in Figure 1 and 2, respectively. In fresh beef, only 4 miRs were expressed differently when comparing non-implanted and implanted samples. Bta-miRs-2368, 2887-2, 2887-1 were highly expressed in implanted animals whereas the bta-mir-677 was relatively highly expressed in some non-implanted samples. In digested beef, different expressions of 16 miRs were observed when comparing implanted and non-implanted beef. Non-implanted beef showed greater expressions of bta-miRs 2296, 2341, 2411, 1246, 2389, 2885, 2332, 873, 2447, and

6531. Implanted beef showed greater expressions of bta-miRs 503, 200c, and 2339. According to TargetScan's cumulative weighed context++ score [3,4], upregulated miRs from non-implanted beef most favorably (≤ -0.80) target 41 transcripts, whereas upregulated miRs in implanted beef target 9 transcripts.

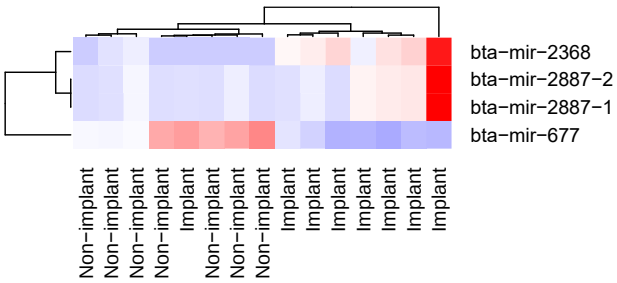

Figure 1. Hierarchical clustering of microRNAs in fresh non-implanted and implanted beef

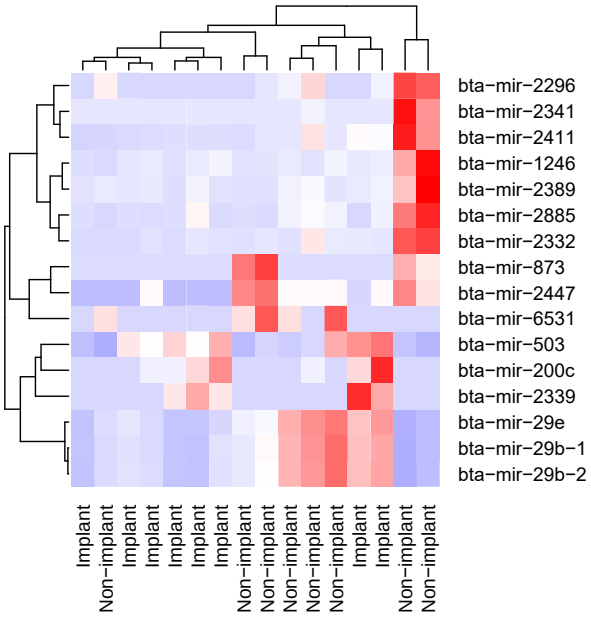

Figure 2. Hierarchical clustering of microRNAs in digested non-implanted and implanted beef

IV. CONCLUSION

Different implant strategies led to different miR profile of beef. If absorbed by hosts, miRs may silence genes altering human homeostasis. In the future, the profile of nanonutrients, such as miRs found in meats, may predict protein synthesis due to their ability of silencing genes. Further research must be conducted to elucidate the role of exogenous food-derived miRs on human health and how production systems modulate the composition of nanonutrients in foods.

ACKNOWLEDGEMENTS

This project was supported by the USDA-NIFA HATCH project # NEV00767. The sequencing was carried out at the DNA Technologies and Expression Analysis Cores at the UC Davis Genome Center, supported by NIH Shared Instrumentation Grant 1S10OD010786-01

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