Application of Metabolomics on Selecting for Litter Size in American Mink (*Neovison vison)*

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Summary

Selection for litter size has the greatest impact over the other economically important traits when increasing the profitability of mink farmers. The blood metabolic fingerprint of 11 mink dams with good reproduction (GR), yielding a high litter size and 10 mink dams with poor reproduction (PR), yielding a low litter size from the Canadian Centre for Fur Animal Research at Dalhousie University was measured to characterize the metabolic phenotype underlying litter size. No clearly distinctive phenotypes could be assigned to the two groups, however, patterns were observed within each group. Five spectral bins showed significant differences between two groups, where a larger NMR signal intensity was seen in the GR group. These patterns of difference will be further investigated in future studies to identify the single metabolites associated with litter size. To the best of our knowledge, the current study is one of the first metabolomics studies in mink and this study confirms the potential of metabolomics technology for detection of biomarkers in mink.

Keywords: metabolomics, American mink, nuclear magnetic resonance (NMR), principal component analysis (PCA), reproductive performance.

Introduction

Improving the reproductive performance is an important aspect of mink farming in North America to meet the export demands. The demand for mink pelts in China is much larger than the current supply (Petry and Liting 2010). Novel technologies such as genomics, transcriptomics and metabolomics may be valuable approaches in detection of biomarkers underlying reproduction traits that can be used as selection tools for farmers. Recent metabolomics studies have been focused on several animal species to predict the levels of numerous economically important traits including body mass (Cerasale & Guglielmo, 2006), growth rates (Hegarty et al., 2006), meat quality (D'Alessandro et al., 2011), as well as being used to discover the biomarkers for diagnosing diseases such as diabetes (Kulkarni, 2012). Proton nuclear magnetic resonance (H NMR) spectroscopy is a one-dimensional spectroscopy method, which is commonly used for metabolomics study (Cox et al., 2014).

Therefore, the objectives of this study are to: a) determine the associations of metabolites with litter size in mink, and b) assess the potential of metabolomics approach in selection for economically important traits in mink.

Materials and methods

Animal Resources and Care

The procedure was approved by the animal care and use committee (ACUC) under special requirements, instructions, and procedures, and mink were cared for in accordance with the Canadian council on animal care (CCAC) guidelines. Twenty one mink from the Canadian Centre for Fur Animal Research (CCFAR) at Dalhousie University were selected based on their previous litter records, in which 11 of them had high litter sizes (average of 9 kits), and 10 of them had low litter sizes (average of 0 kits). The mink dams selected ranged from two to four years old, were given a controlled diet, and were housed in cages 30.5 cm wide, 76.2 cm long, with a 20X20 cm jump up style nest box.

Sample Collection

Blood was taken from the mink by clipping the toenail past the quick. Five capillary tubes (~350μL) of blood was taken from each mink, sealed with critoseal, and placed into labelled containers. The capillary tubes were centrifuged for seven minutes, and plasma samples transferred into cryovials, then sanitized with Virkon, and placed into liquid nitrogen. Samples were kept at -80◦C degrees and shipped to the National Research Council of Canada (NRC) in Halifax, NS, Canada.

Metabolite Assays using NMR

Samples were prepared at the NRC for NMR spectrometry by diluting 50μL of plasma with deuterated water to about 10-20% for an appropriate volume, and then transferred the samples into 1.7mm NMR tubes. The spectra of the samples were acquired on a Bruker Avance-III 700 MHz spectrometer. Data were collected into 32k complex points in the free induction decay (FID). Carr-Purcell-Meiboom-Gill pulse sequence was used with an echo time of 2.4 ms and a total T2 filter of 24 ms. Time domain data were processed by Fourier transform into 32k real points after apodization with an exponential decaying function with a line broadening factor of 0.3 Hz. Spectra were phased to pure absorption, and referenced with trimethylsilylpropionic acid (TMSP) resonance at 0 ppm and baseline corrected using a cubic spline function.

Data Analysis

The spectral data were imported into NMRProcFlow 1.2 (Jacob et al., 2017), in which alignment was done, water signal was removed, and intelligent binning was performed. The binned NMR was further analyzed in MetaboAnalyst 3.0 (Xia & Wishart, 2016), in which principle component analysis (PCA) was used to visualize the impact of reproductive performance on plasma metabolic fingerprints. A two-sample unpaired t-test at a confidence interval of 95% was performed to determine the significance of differences in each spectral bin between the high and low reproductive groups.

Results

The heat map visualization in Figure 1 shows the differences between the metabolic fingerprint of each mink and their reproductive performance. A higher percentage of red, as well as more intense reds, indicative of higher metabolite concentrations, are seen in the GR group,

however there is a strong inter-individual variation and no clear pattern separating the two groups.

A t-test was run on the spectral bins with a significance level of $P<0.05$, revealing the majority of spectral bins not to be significantly different between the GR and PR groups. A significant difference was observed between 5 spectral bins. The GR group displayed significantly greater concentrations in each of these 5 bins (Figure 2).

Principle component (PC) 1 and PC2 accounted for 49.4% of the total variation between the two groups (Figure 3). The results showed a pattern of difference between the two groups of GR and PR. Less variation was observed within the PR group, but a greater variation was observed within the metabolic fingerprints of the GR group.

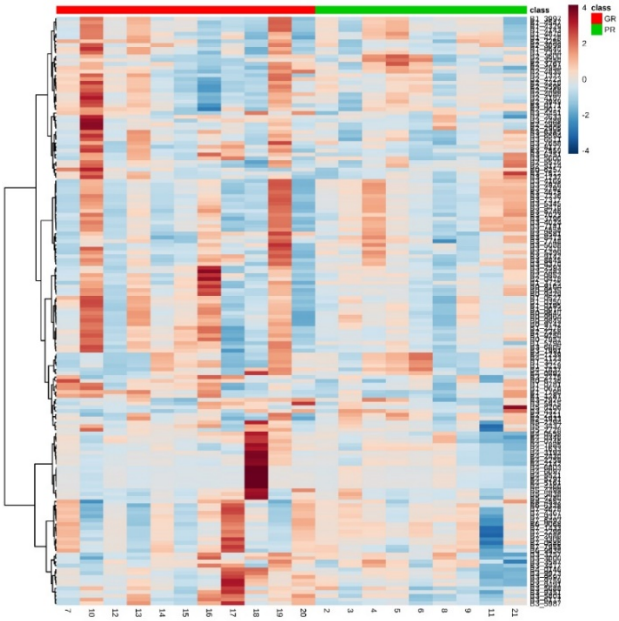

Figure 1. Heat map[1] *visualizing the difference of several spectral bins between high (GR), and low (PR) litter size mink.*

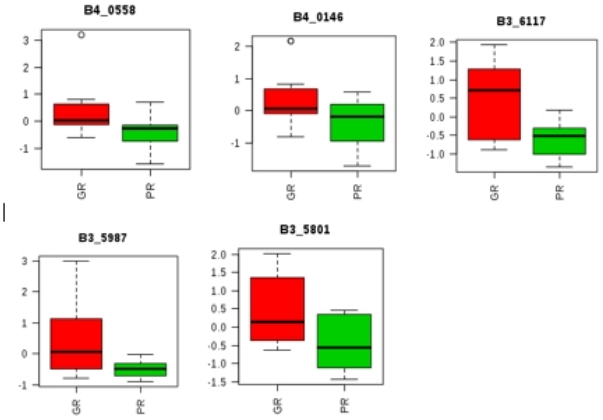

Figure 2. Unpaired t-test box plots comparing the significantly different NMR spectral bins of the metabolome between the GR (red), and PR group (green).

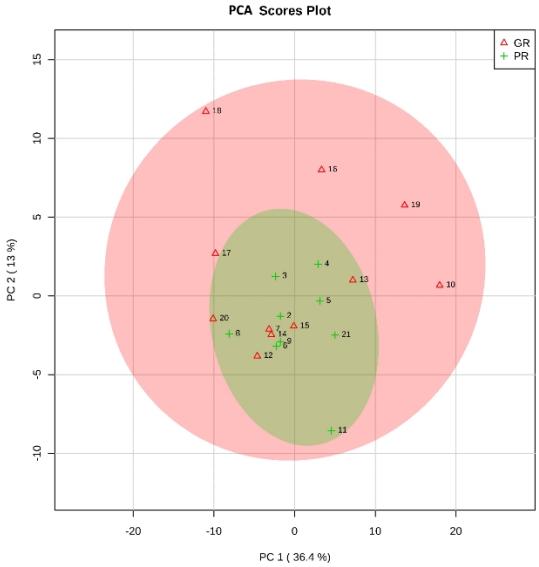

Figure 3. Principal component analysis on the metabolic fingerprints of high litter size (GR, in red, n=11) and low litter size (PR, in green, n=10) mink.

Discussion

Although no clearly distinctive phenotypes could be assigned based on the metabolic fingerprints of the GR and PR mink dams, the results may suggest the unique patterns of differences between high and low litter size mink. The results suggested that these patterns of differences may have possibly been more pronounced with a larger sample size.

The results determined that five spectral bins were statistically different in the GR and

PR groups. In the future studies, these unknown metabolites will be identified and quantified and their physiological role and associations with litter size will be further investigated. It is recognized that although the results showed the effect of reproductive status on the metabolic fingerprint, there are several other factors that account for variations in litter size that cannot be accounted for, such as the reproductive status of male, stress levels, etc. Also, the large variation between individuals indicates that metabolic fingerprints are end products of the complex interactions between genome and environment, so reproductive traits are just one of a myriad of factors that all together determine metabolic phenotypes.

The implications of this research were to assess the potential of metabolomics in selecting for economically important traits in mink, which has been studied very little. This study was a pilot study, and suggested potential for future applications of metabolomics in understanding and selecting for key traits including feed efficiency, fur quality and disease resistant traits in mink.

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