Measuring skeletal muscle NADH production to assess feed efficiency and milk production

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Introduction

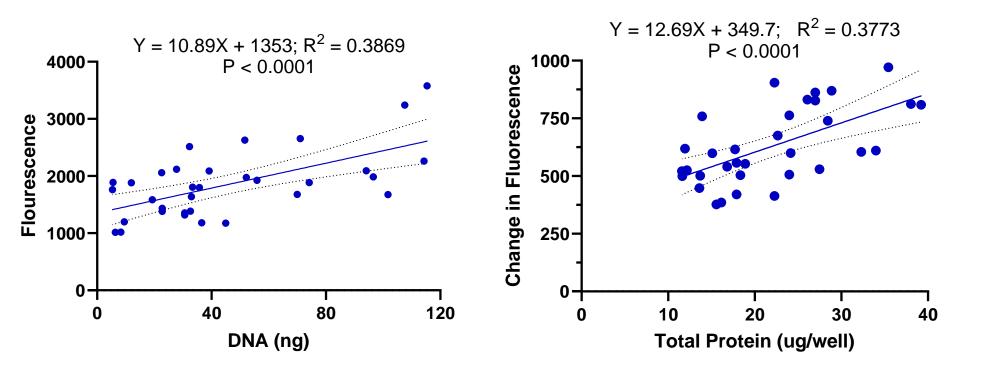
Opportunity:

- Feed is largest single cost in animal production.
- Feed production accounts for 36% of greenhouse gas emissions associated with dairy production.
- Improvements in feed efficiency can:
 - Feed costs & \uparrow producer profits
 - \downarrow Carbon footprint of feed production
- Current technology to improve feed efficiency:

Data Analysis

Larger Biopsies Create More Signal

Biopsy DNA content (May) or protein (October) proxies for biopsy size associated with increased resorufin caused fluorescence.



Results

Maturity & Lactation Status

- Skeletal muscle biopsies from growing heifers expend more energy than those from mature cows (P < 0.05).</p>
- Lactation stage does not affect residual (P > 0.50).

- Residual feed intake
 - Labor intensive/specialized equipment
- Oxygen Consumption
 - Specialized equipment

Objectives:

- 1) Test efficacy of measuring reducing equivalents produced by skeletal muscle biopsy to assess feed efficiency.
- Assess repeatability & ability of assay to distinguish between stage of production.

Hypotheses:

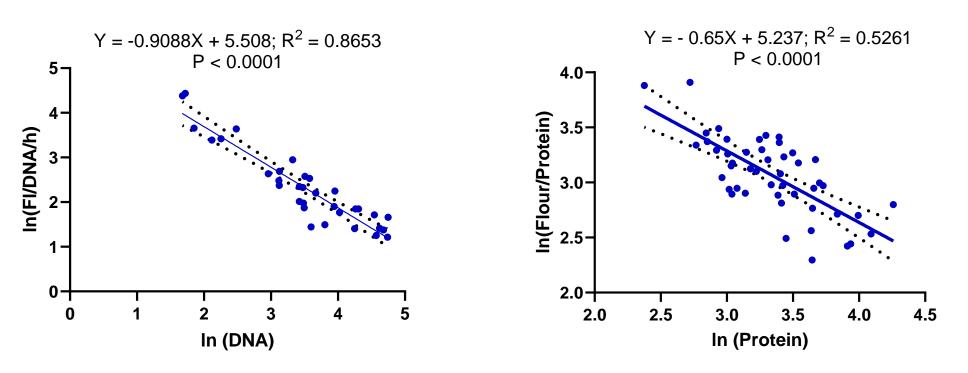
- 1) Skeletal muscle reducing equivalent production is inversely related to feed efficiency.
- 2) Feed efficient cows will produce more milk b/c less dietary energy is going toward maintenance energy requirements.

Principle: Fluorometric Assay of

Reducing Equivalents

Correct For Biopsy Size

In(signal/DNA or Protein) regressed on In(DNA or Protein)



Calculate Residuals From Graphs Above

Residuals are not biased based on biopsy size

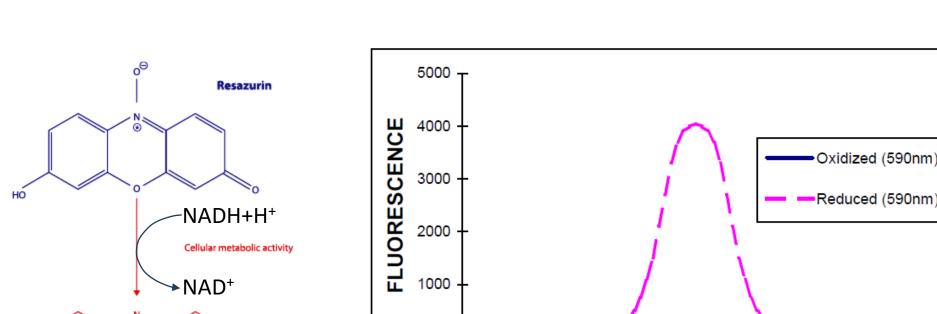
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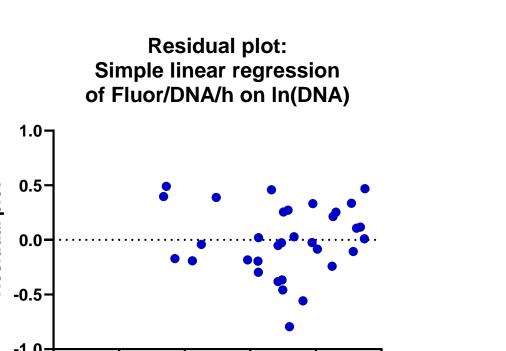
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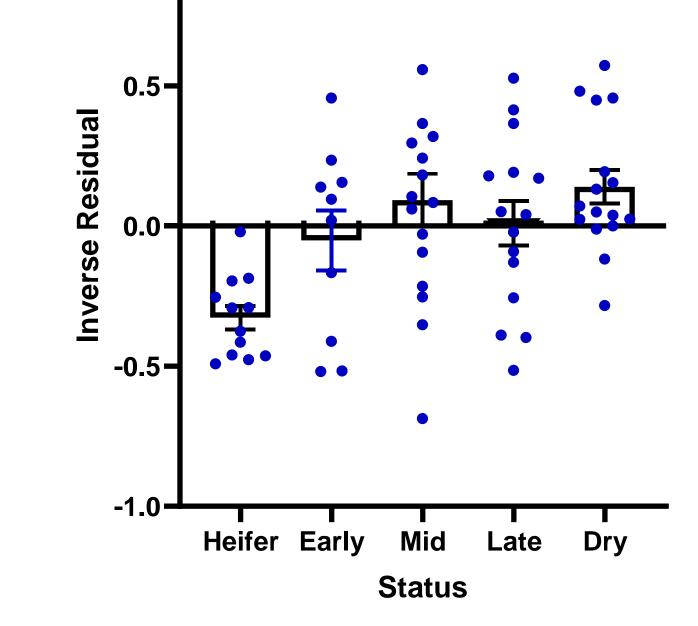
Residual plot:

Simple linear regression

of Fluor/Protein on In(Protein)

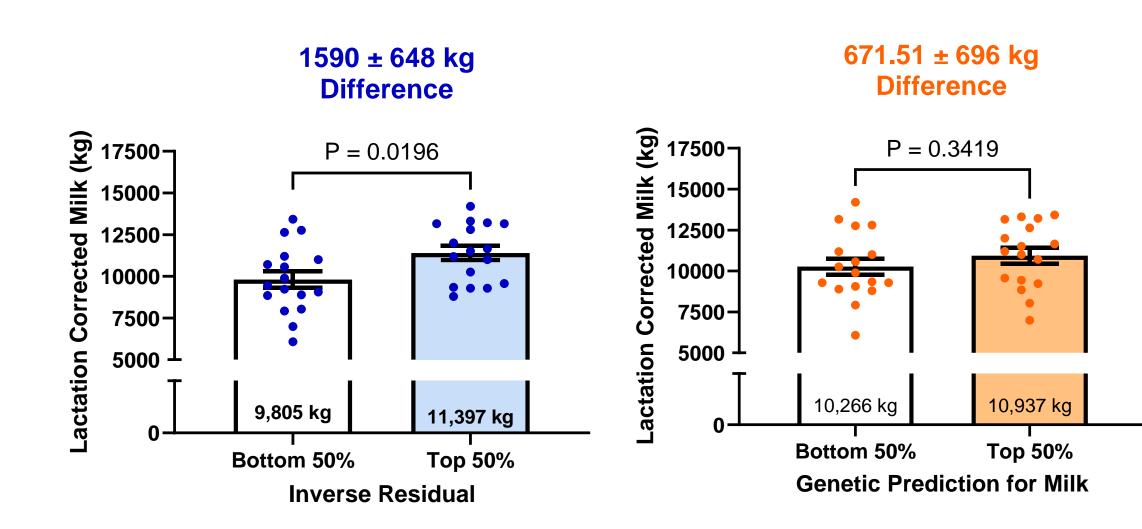


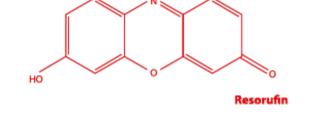


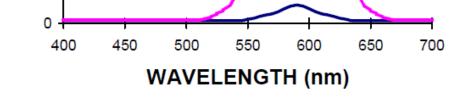


Large variation highlights potential for improvement through selective breeding programs.

Segregating Cows Into High & Low Halves Shows that the Inverse Residual of Skeletal Muscle Metabolic Rate is More Predictive of Milk Production than a Commercially Available Genomic Test for Milk Production.







Methods

Animals

- 36 Holstein cows (BW ± SEM; 662 ± 25 kg)
 - Lactation 1-4
 - Dry, Early, Mid and Late lactation
 - All previous lactation data available
- 12 growing heifers before production (BW ± SEM; 417 ± 8 kg)

Triceps Brachii Biopsy

- Cows sedated with intravenous injection of xylazine HCL
- 35 ug/kg of BW
- Subcutaneous line block with lidocaine HCL (1 mL).
- 14G hypodermic needle inserted & used to guide Cook[®] biopsy needle (18G).
- <100 mg of tissue collected from depth of ~4 cm.</p>

Metabolic Activity of Skeletal Muscle



Residuals = Relative Metabolic Rate

Negative Residuals (Predicted – Actual) = animals that expended less energy than expected

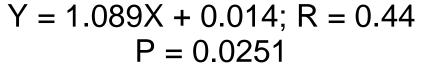
Positive Residuals (Predicted – Actual) = animals that expend more energy than expected

To Ensure that a Positive Efficiency Number is Indicative of a Positive Trait, All Data is Presented as the Inverse Residual

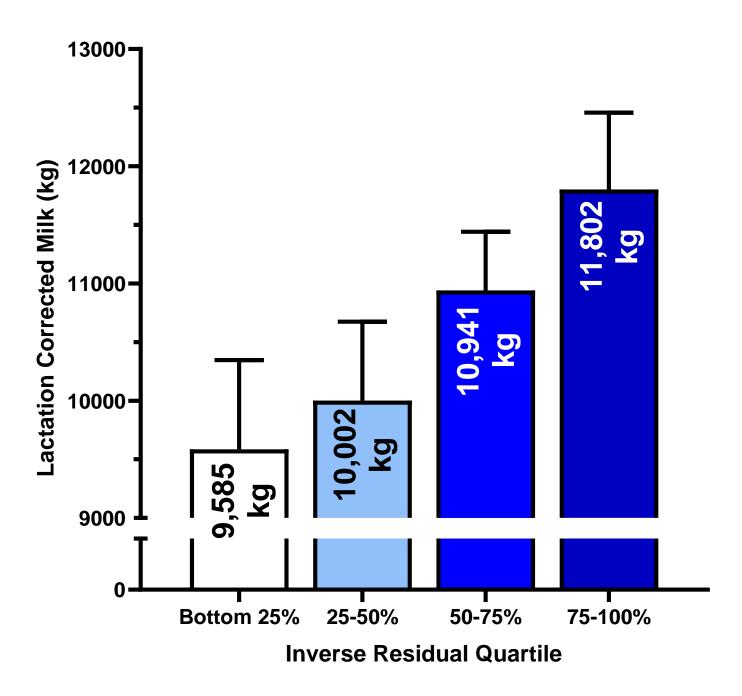
Results

Measurements Taken 7 Months Apart, in the Same Animal, are Correlated Despite

Differences in Lactation Status $X = 1.089X \pm 0.014$ R = 0.44



Milk Production Increased with Quartile of Inverse Residual of Skeletal Muscle Metabolic Rate

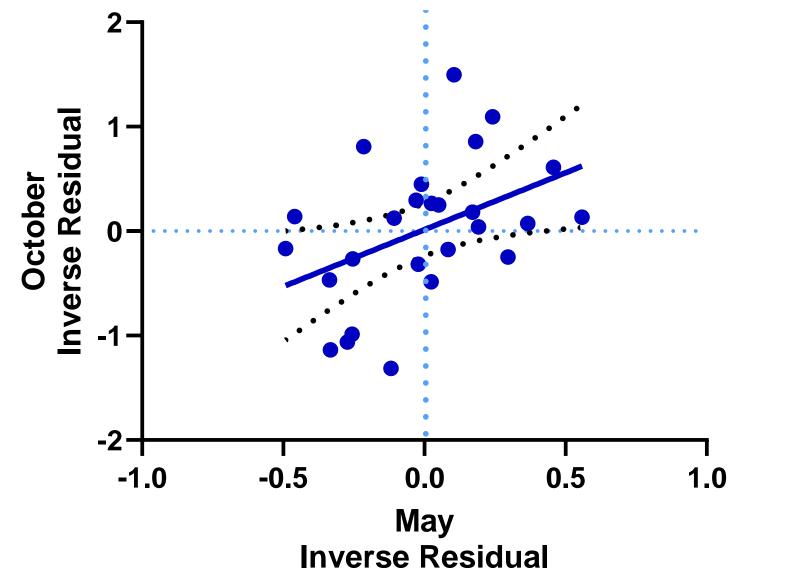


Conclusions

1) Skeletal muscle metabolic rate consistent within animal.



- Biopsies incubated in 96- well plate with sterile media (DMEM/F12 with 1% pen/strep); kept on ice.
- Transferred to test media (DMEM/F12 with 1% pen/strep and 0.01 mg/ml resazurin) within 1h of collection & incubated at 37°C.
- Resazurin based fluorescence (excitation 530 nm; emission 590 nm) measured at 0 and 4h.
- Biopsies digested & corrected for size, based on biopsy DNA (May) or protein (December) content:
- PicoGreen assay kit (Invitrogen[™])
- BCA assay (ThermoFisher Scientific)



2) Growing animals have higher skeletal muscle metabolic rate than mature because they are expending energy for growth & maintenance.

3) Lactation stage does not largely affect skeletal muscle metabolic rate.

4) Cows with a greater inverse residual for skeletal muscle metabolic rate, produce more milk than cows with lower.