



Amino acid profiles of calves experiencing bovine respiratory disease

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Introduction

Bovine Respiratory Disease (BRD):

- ❖ (BRD) was first discovered in the 1800s, and has become the most economical impactful problem to the beef industry. (Taylor et al., 2010).
- ❖ BRD, often referred to as shipping fever, is a general term that refers to pneumonia induced by a series of viral and bacterial infections that severely impair the respiratory system.
- ❖ Cattle with BRD present symptoms of respiratory distress such as dyspnea (shortness of breath), fever, and pneumonia. (Sinha and Abinanti, 1962).

Amino Acids:

- Building blocks of proteins
- Required for growth and development
- ❖ Proteins are broken down in a process called catabolism into energy in order to help fuel the immune response.
- ❖ BRD infection proved to result in smaller daily gains and over all reduced thrift in feedlot cattle (reviewed by Gifford et. al., 2012).

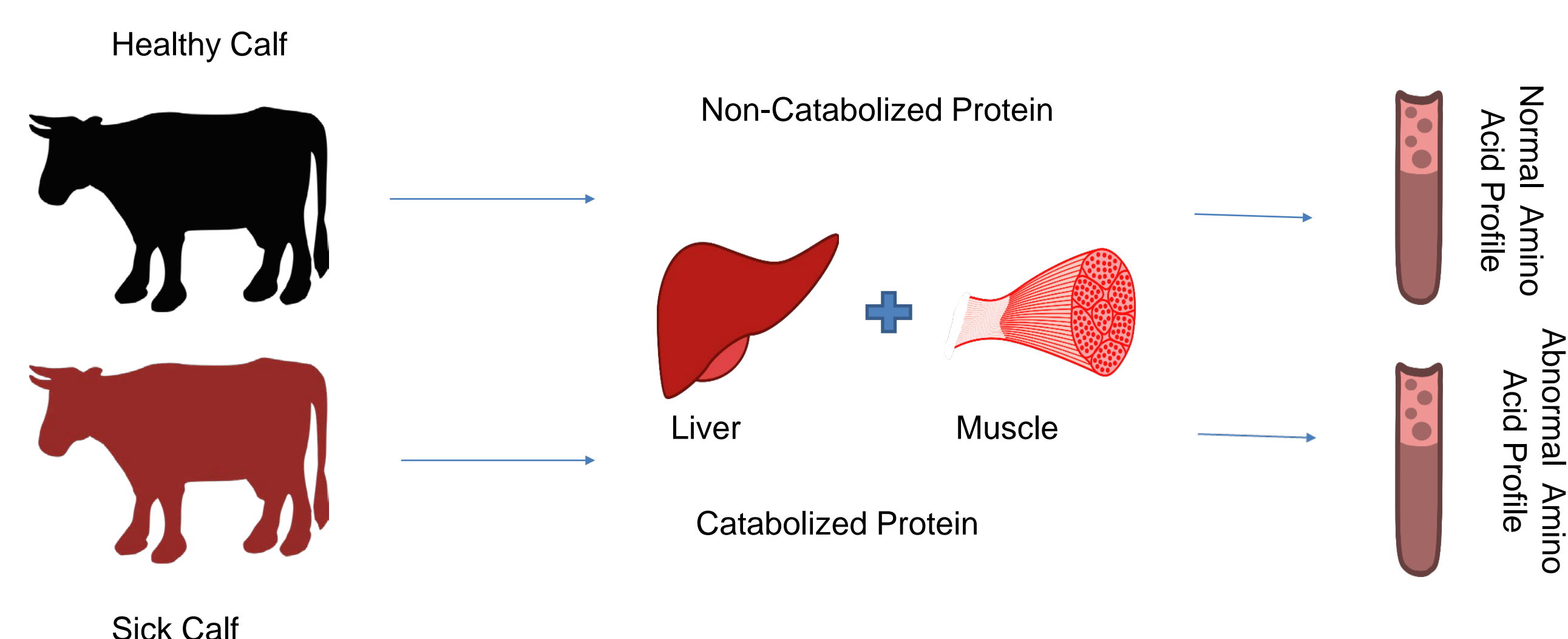
Hypothesis

- ❖ When comparing serum of BRD infected versus healthy calves, serum from BRD infected calves will show altered amino acid components.

Objectives

- ❖ Collect serum from cattle with varying degrees of BRD severity.
- ❖ Develop a method to isolate amino acids in the collected serum.
- ❖ Evaluate the serum using a Gas chromatography–mass spectrometry machine to compare the amino acid profiles.

Amino Acids in Disease



Materials and Methods

Amino acid extraction for profiling by GCMS

Clean up:

1. 2 ml glass GC vial
2. Pipette 100 ul of sample into glass GC vial
3. Pipette 500 ul of 1-N acetic acid and vortex for 15 to 30 seconds.
4. Prepare c18 column. Place 10 ml beaker under column.
5. Slowly pipette sample onto the c18 column.
6. Using the syringe, rubber stopper and needle, slowly push the sample through the column.

Do not force air though the column

7. Rinse column 3 times using a total of 2 ml of ddH2O and push the sample through the column.
8. Replace beaker with 2 ml GC vial under column.
9. Pipette 1 ml methanol onto C18 column.
10. Using the syringe, rubber stopper and needle, slowly push the sample through the column.

Push air though column

11. Dry under nitrogen until the volume is 100 to 200 ul.

Do not let all of the sample evaporate

Derivation:

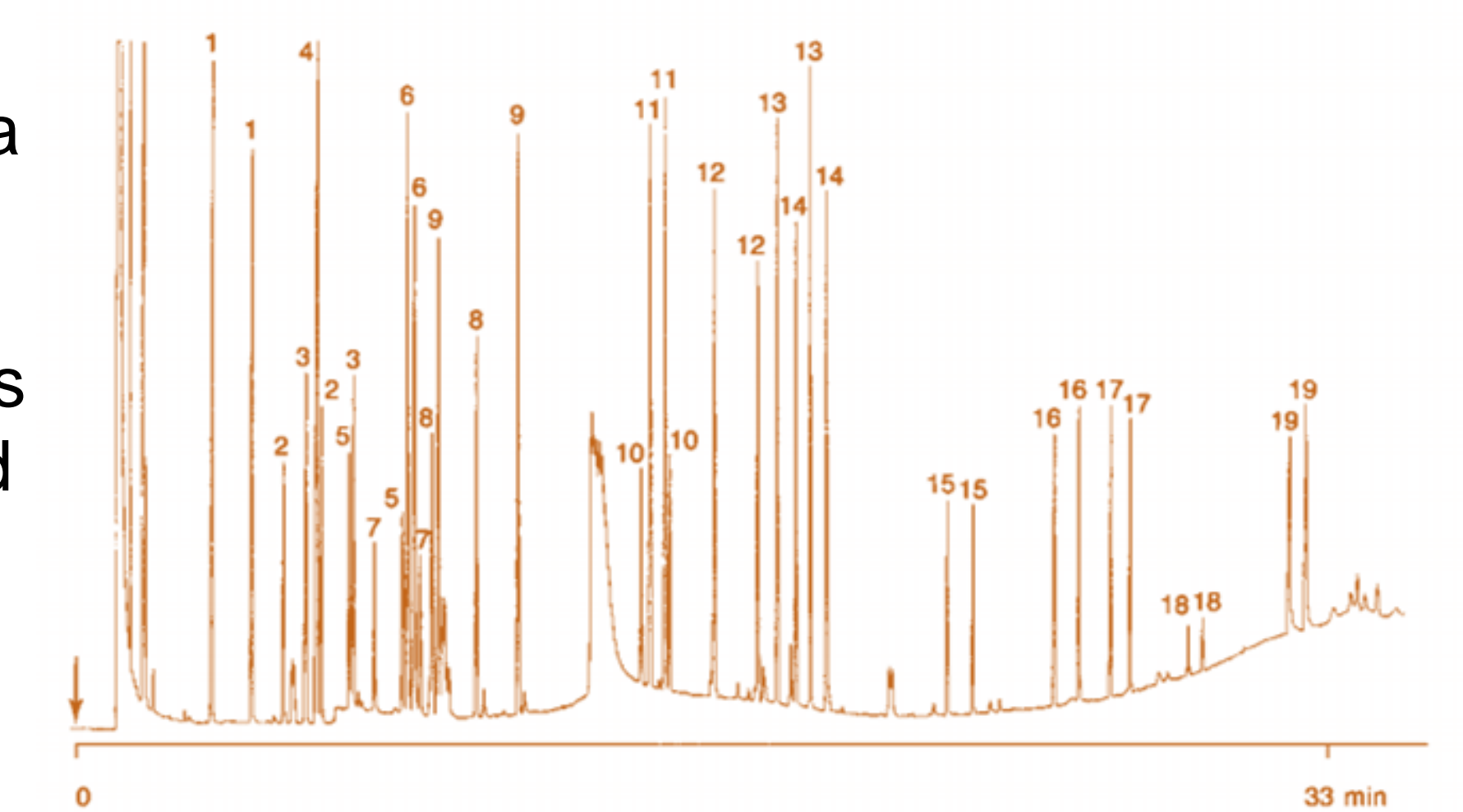
1. Add 100uL of ethoxyamine HCl and allow 90 minutes of incubation at 40° C.
2. Add 200 ul of methyl-N-trifluoroacetamide (MSTFA) and incubate for 50 minutes at 40° C.
3. Dry under nitrogen
4. Add 500 ul of methanol and dry under nitrogen. Repeat 1 additional time.
5. Add ISTD (DI-noravline, 60 mol/L) and QS to 1 ml with methanol.
6. Inject on GCMS

Importance of Research

- ❖ The current economical damage caused by this disease warrants continued research on methods to detect, prevent, and treat BRD.
- ❖ Continuation of this project can lead to a better understating of the effects of stress on the proteins within the serum of cattle.
- ❖ Additional screening of the serum samples collected may lead to identification of particular amino acids broken down during BRD.
- ❖ The connection between inflammation in cattle with BRD and the catabolism of amino acids provides possibilities for amino acid supplementation to aid the immune system or growth.

Technology

- ❖ On the right is an example of what data peaks we will be evaluating.
- ❖ Each peak represents a different amino acid present in the sample.



- ❖ On the left is a GCMS machine.
- ❖ It can determine the presence of amino acids.

Summary

- ❖ When cattle develop Bovine Respiratory Disease, the associated symptoms cause stress induced catabolism of amino acids. By analyzing the change of amino acids found in serum of bull and steer calves with or without BRD, the results may yield insight to the affect that BRD has in calves that have varying degrees of infection.

Conclusions and Future Directions

- ❖ Further testing of the collected samples is needed for actual results of this experiment.
- ❖ Results from this experiment can lead to future development on the connection between the respiratory stress of BRD and changes in amino acids.

Acknowledgements

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