Determining potential for on-farm fecal collection for DNA extraction

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Introduction

- Grazing cattle diet composition determination methods include: field observations, ruminal or esphogial contents, fecal microhistological analysis, and "Markers" (saturated hydrocarbons, alkanes) but can be labor intensive, costly, and under/over predicts some plants in the diet (summarized by Ho et al., 2010)
- Wildlife researchers have used fecal samples and DNA to determine mammalian intake of feeds, but quantification of exact diet composition was not determined (Jarman et al., 2002; Deagle et al., 2005; Deagle and Tollit, 2007)
- Ruminant plant fecal identification has been completed with moderate success, but diet quantification was not attempted (Pegard et al., 2009; Joo et al., 2014; Mohammadzadeh et al., 2014)
- (DeMay et al., 2013), but Fecal DNA quantity can be reduced by temperature and UV radiation in rabbit fecal DNA analysis has not been completed

Objective

The intention of this project is to determine DNA concentration differences based on fecal physical composition, time of collection, and animal classification.

Experimental Procedures

- Holsteins (n=4), heifer calves (n=3), and dry, pregnant beef cows (n=4) were fed a diet at 1.68% of BW for 21-d
  - 20% alfalfa, 50% fescue, 20% corn, and 10% soybean meal (DM basis)
- On d 22-24 at 7 AM and 3 PM individual fecal samples were collected and on d 25 at 7 AM
  - FRESH: sample collected directly from cow or from ground when observing defecation
  - DRY: dry sample collected from pen. Samples were ~90% dry in the fecal pile
- Samples frozen immediately at -20°C
- DNA was extracted following kit protocols (Quiagen Quick Spin Fecal DNA kit, illustrated in Figure 1)
- Purity and quantity were assessed through a spectrophotometer.
- Samples with a low 280/260 ratio underwent a DNA contamination clean-up (Zymo DNA PCR Clean-up & Concentration Kit)

Statistics

- The study is a completely randomized block design with repeated measures
- Quality (280/260 ratio) and quantity (ug/mL) were analyzed using appropriate t-tests and generating 90 and 95% confidence intervals for:
  - Freshness – FRESH vs DRY
  - Time of day for collection – AM vs PM
  - Animal classification – Cow, Holstein, or Heifer
  - Interactions

References:

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Ho, K. W., G. L. Krebs, P. McCafferty, S. P. van Wyngaarden, and J. Addison. 2010. Using faecal DNA to determine consumption by kangaroos of plants considered palatable to sheep. Animal. 4:282-288.

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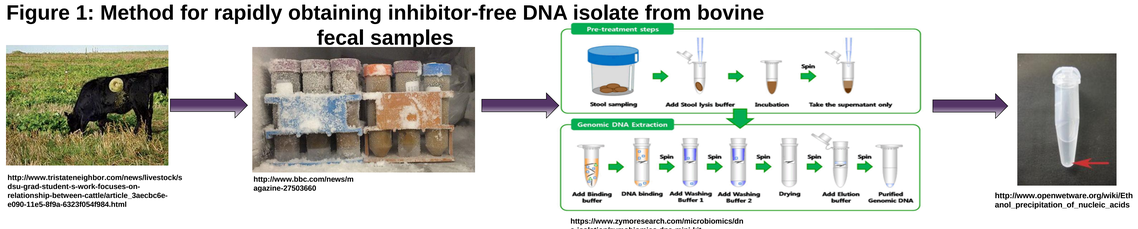
Joo, S., D. Han, E. J. Lee, and S. Park. 2014. Use of length heterogeneity polymerase chain reaction (LH-PCR) as non-invasive approach for dietary analysis of Svalbard reindeer, Rangifer tarandus platyrhynchus. PLos One. 11:e91552

Mohammadzadeh H., D. R. Yanez-Ruiz, G. Martinez-Fernandez, and L. Abecia. 2014. Molecular comparative assessment of the microbial ecosystem in rumen and faeces of goats fed alfalfa hay alone or combined with oats. Anaerobe. 29:52-58.

Pegard, A., C. Miquel, A. Valentini, E. Coissac, F. Bouvier, D. Francois, P. Taberlet, E. Engel, and F. Pompanon. 2009. Universal DNA-based methods for assessing the diet of grazing livestock and wildlife from feces. J. Agric. Food Chem. 57:5700-5706.

Experimental Results and Methods

Figure 1: Method for rapidly obtaining inhibitor-free DNA isolate from bovine fecal samples



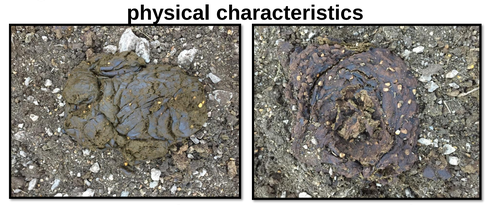
http://www.tristateneighbor.com/news/livestock/sdsu-grad-student-s-work-focuses-on-relationship-between-cattle/article_3aecbc6e-e090-11e5-8f9a-6322f054f984.html

http://www.bbc.com/news/magazine-27503660

https://www.zymoresearch.com/microbiomics/dna-isolation/zymobiomics-dna-mini-kit

http://www.openwetware.org/wiki/Ethanol_precipitation_of_nucleic_acids

Figure 2: Photos illustrating examples of fecal physical characteristics



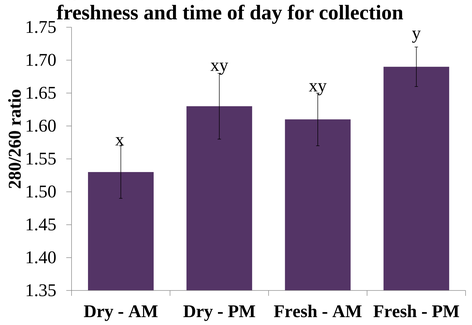
FRESH sample DRY sample

Table 1: Quality (280/260) comparisons of fecal type, time of day for fecal collection, and animal classification.

Treatment	Mean	SE[1]	Lower[2]	Upper[3]	*P* - value[4]
Comparison of FRESH fecal samples to DRY pen-grabbed fecal samples					
DRY	1.57	0.23	1.51	1.63	NS
FRESH	1.64	0.17	1.60	1.69	NS
Comparison of time of day for fecal collections					
AM	1.53	0.22	1.52	1.63	NS
PM	1.66	0.17	1.60	1.71	NS
Comparison of fecal samples collected based on animal classification					
Cow	1.66	0.20	1.60	1.73	NS
Heifer	1.59	0.18	1.52	1.65	NS
Holstein	1.57	0.22	0.149	1.64	NS

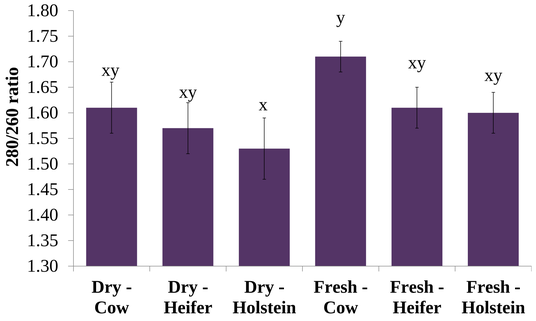
[1] SE: standard error
[2] Lower limit of confidence interval
[3] Upper limit of confidence interval
[4] *P*-value of NS means non-significant with $P > 0.10$
* If confidence intervals are overlapping then treatments are the same with $P > 0.10$

Figure: 3: Quality of DNA based on fecal freshness and time of day for collection



[xy] Different letters are different at $0.05 < P < 0.10$
* Classifications are sample freshness (DRY or FRESH) then time of day for collection (AM or PM)

Figure 4: Quality of DNA based on fecal freshness and animal classification



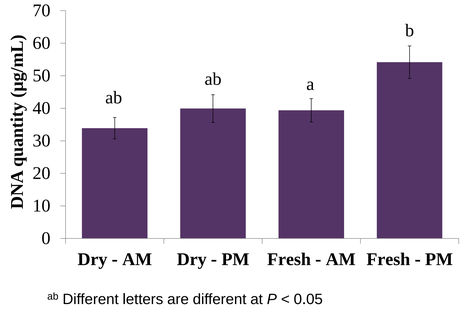
[xy] Different letters are different at $0.05 < P < 0.10$
* Classifications are sample freshness (DRY or FRESH) then animal classification (Cow, Heifer, or Holstein)

Table 2: Quantity (µg/mL) comparisons of fecal type, time of day for fecal collection, and animal classification.

Treatment	Mean	SE[1]	Lower[2]	Upper[3]	P - value[4]
Comparison of FRESH fecal samples to DRY pen-grabbed fecal samples					
DRY	36.21	18.81	30.96	41.46	NS
FRESH	45.51	22.42	39.31	51.72	NS
Comparison of time of day for fecal collections					
AM	36.59	19.28	31.73	41.45	NS
PM	47.39	22.35	40.41	54.37	NS
Comparison of fecal samples collected based on animal classification					
Cow	40.10	21.92	32.87	47.33	NS
Heifer	47.47	22.53	39.03	55.90	NS
Holstein	36.43	18.20	30.45	42.50	NS

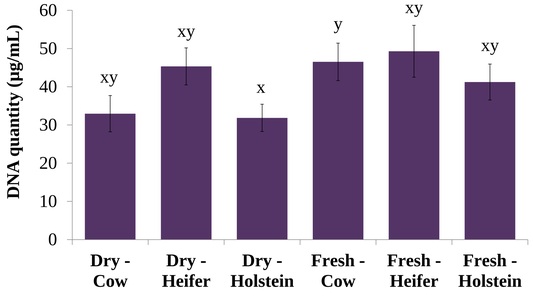
[1] SE: standard error
[2] Lower limit of confidence interval
[3] Upper limit of confidence interval
[4] *P*-value of NS means non-significant with $P > 0.10$
* If confidence intervals are overlapping then treatments are the same with $P > 0.10$

Figure 5: Quantity of DNA based on fecal freshness and time of day for collection



[ab] Different letters are different at $P < 0.05$
* Classifications are sample freshness (DRY or FRESH) then time of day for collection (AM or PM)

Figure 6: Quantity of DNA based on fecal freshness and animal classification



[xy] Different letters are different at $0.05 < P < 0.10$
* Classifications are sample freshness (DRY or FRESH) then animal classification (Cow, Heifer, or Holstein)

Conclusions and Future Work

- Of the 105 samples analyzed, 89 had to complete a secondary clean-up to increase the 280/260 spectral reading to be >1.4.
- There was no difference in the quantity of DNA extracted from samples that were collected FRESH or DRY; in the AM or PM; nor differences based on cattle classification.
- There is evidence that a fresh sample collected in the afternoon would net the highest quality and yields of DNA to be used for downstream use in qRT-PCR.
- Possible explanation is that afternoon samples occurred 7 hr after daily feeding which might correspond in this diet to the time of maximal ruminal digestion and undigested turnover
- Overall, as with other species the fresher the sample collected the higher quality and quantity of DNA. However, for on-farm projects samples that have some moisture will still generate potentially usable DNA.