ALASKA DEPARTMENT OF FISH AND GAME
DIVISION OF WILDLIFE CONSERVATION
PO Box 115526
Juneau, AK 99811-5526

Alaska Department of Fish and Game
Wildlife Restoration Grant

GRANT NUMBER: AKW-23

PROJECT: P1.68

PROJECT TITLE: Moose Diet FY2018: Factors affecting moose forage quality and subsequent reproductive success

PROJECT DURATION: 1 July 2016 to 30 June 2020

REPORT DUE DATE: 1 September 2018

PRINCIPAL INVESTIGATOR: William B. Collins

COOPERATORS: Don Spalinger, University of Alaska Anchorage; Lindsey Solden, and Kelly Wrighton, The Ohio State University

WORK LOCATION: Matanuska Research Farm, Togiak Valley, Colville River, Nelchina Basin, Game Management Units 15, 17

I. PROGRESS ON PROJECT OBJECTIVES DURING LAST SEGMENT

OBJECTIVE 1: Availability of digestible nitrogen to moose across a spectrum of marginally to highly productive moose ranges in Alaska.

We have completed analysis of our Nelchina, Placer, and Denali samples, and most of our Colville samples. We are in the process of completing our Togiak and Goodnews samples. Until most recently, we have been encumbered by a much larger number of samples than we originally intended to collect. To remedy this problem, we have been setting up a laboratory and program to specifically support our analytical requirements. Most of this laboratory has now been assembled, and the remainder should be completely up and running by 1 October 2018.

Our ultimate goal is to compare the quality of diets across a broad spectrum of ranges to better explain variations in forage quality and related herd productivities. Our intermediate goal is to have completed analyses of all the samples we currently have by the end of 2018.

The following paper was finalized and published during this segment:

Carnahan, A., D. Spalinger, and W. Collins. 2017. Alkane and long-chain alcohol recovery in moose (*Alces alces*), a browsing herbivore. Can. J. Zoology.

OBJECTIVE 2: Effects of climate and utilization on browse quality.

No further work was done regarding this objective, as most of the Salix pulchra plants in our transplant garden died back during winter 2015/2016. Replacement time to propagate new plants from cuttings will go beyond the duration of this study.

OBJECTIVE 3: Microbiological link between diet quality and reproductive performance of moose.

We completed this objective.

Ruminants and their gastrointestinal (GI) microbial communities have co-evolved, with the latter providing host energy by digesting plant materials in the rumen. Our objective is to define microbial contributions to forage digestion in moose in attempt to understand factors potentially limiting to their condition and reproductive potential. Assessment of rumen flora responses to variations in diet quality has required use of rumen fistulated moose. Thus, our initial approach has been to focus on microbial responses to variations in quality of diets selected by individuals by season, rather than to assess response differences across geographic gradients. Microbial communities of digestive tracts respond to specific variations in diet quality similarly, whether they are of seasonal or geographic origin. Identification of GI microorganisms and their function in the various aspects of forage digestion, and comparisons of those organisms across moose populations will help us will help us better understand the linkage between forage quality and animal performance.

To uncover lignocellulose degrading taxa and establish an ecosystems biological ruminant model, we sampled rumen fluid from moose consuming native Alaskan plants in winter when lignin and cellulose were 2.5X higher than herbaceous diets in spring. Metagenomic reconstruction yielded 207 genomes, 82 of which were unique and complete to near complete. Thirty-three of the unique genomes provided first genomic representatives for a novel TM7 class, two orders previously identified in rumen (RF9 and RFP12 in the Tenericutes and Lentisphaera, respectively), and prevalent rumen Bacteroidetes families (BS11 gut group) and genera (RC9). In contrast to other studies that suggested Firmicutes cellulosomes mediate rumen carbon cycling, our metaproteomics data detected 91 polysaccharide utilization loci (PUL) predicted to degrade starch, pectin, cellulose, hemicellulose, and mucin substrates. These represent new metabolic activities for uncultivated Bacteroidetes.

Our study provides the first 6 genomes from the RC9 genus revealing extensive complex carbon degradation capacities, including up to 20 PUL systems per genome and pathways for lignin degradation. We also sampled the first four genomes from the BS11 family within Bacteroidetes, which were inferred via proteomics to be involved in hemicellulosic monomer fermentation. Additionally, we identified 1,497 viral contigs,

which represented an estimated 110 new viral genera and included 93 closed genomes and 457 prophages. Of the non-prophages, 48 were active as inferred from representation in metaproteomes and 69 were linked to microbial hosts, including active carbon degraders, suggesting viral predation is active and of paramount ecosystem importance in the rumen. These findings illuminate the cross-kingdom taxonomic and metabolic novelty and activity in the rumen ecosystem, functional roles and metabolic handoffs in the rumen ecosystem.

The following two papers relating to this work were written/published or submitted during this segment:

Solden, L. M., A. E. Naas, S. Roux, R. A. Daly, W. B. Collins, C. D. Nicora, S. O. Purvine, D. W. Hoyt, J. Schuckel, B. Jorgensen, W. Willats, D. E. Spalinger, J. L. Firkins, M. S. Lipton, M. B. Sullivan, P. B. Pope, K. C. Wrighton. In Press. Interspecies cross-feedings orchestrate carbon degradation in the rumen ecosystem. Nature Microbiology.

Solden, L. M., D. W. Hoyt, W. B. Collins, J. E. Plank, and R. A. Daly E. Hildebrand, T. J. Beavers, R. Wolfe, C. D. Nicora, S. O. Purvine, M. Carstensen, M. A. Lipton, D. E. Spalinger, J. L. Firkins, B. A. and Wolfe, K. C. Wrighton. 2018. First genomic insight into the enigmatic BS11 gut group reveals roles in hemicellulose degradation. Nature Communications.

OBJECTIVE 4: Hormonal link between diet quality and reproductive performance of moose.

We completed this objective, publishing a paper in Alces detailing the results.

We examined patterns of nutritional hormones (leptin, ghrelin, Insulin-like growth factor-1) in captive, non-pregnant female moose fed a maintenance diet from November to August. Over the 10-month period, plasma concentrations for leptin, ghrelin, and IGF-1 averaged 1.36 ± 0.81 ng/ml, 0.229 ± 0.110 ng/ml, and 114.0 ± 30.5 ng/ml, respectively. Leptin and IGF-1 did not respond to changes in season while ghrelin did. Plasma ghrelin concentrations were significantly elevated during the winter months ($P = 0.009$), suggesting that ghrelin may be sensitive to changes in season and maybe indicative of the animal's nutritional status.

II. SUMMARY OF WORK COMPLETED ON JOBS IDENTIFIED IN ANNUAL PLAN THIS PERIOD

Job/Activity 1-a: Forage N and Protein-binding Capacities of Forages across a Spectrum of Ranges.

Accomplishments: We analyzed moose forages from Colville, Togiak, Good News and Nushagak drainages, determining digestible nitrogen and fiber characteristics.

Job/Activity 1-b: Forage Selection and Food Habits of moose in Colville Range and Habitats Adjacent to Togiak Valley Based on Alkane Analysis

Accomplishments: We analyzed winter and summer diets of moose in the Colville River drainage and in habitats in and adjacent to the Togiak drainage.

Job/Activity 2: Effects of Climate and Utilization on Digestible N, Tannins, Digestibility, and Palatability in Potted Willows

Accomplishments: No work was accomplished on this job, due to dieback of potted plants during the previous winter.

Job/Activity 3: Moose Microbiome responses to diet quality

Accomplishments: This job has been completed. The final manuscript has been accepted in Nature Microbiology. Previous results were reported in Microbial Ecology Journal, Nature Communications, and American Society for Microbiology

Job/Activity 4: Hormonal Link between Diet Quality and Reproduction

Accomplishments: This job is completed, with results published in Alces.

III. SIGNIFICANT DEVIATIONS AND/OR ADDITIONAL FEDERAL AID-FUNDED WORK NOT DESCRIBED ABOVE THAT WAS ACCOMPLISHED ON THIS PROJECT DURING THIS SEGMENT PERIOD

There was no significant deviations during this reporting period.

IV. PUBLICATIONS

Solden, L. M., A. E. Naas, S. Roux, R. A. Daly, W. B. Collins, C. D. Nicora, S. O. Purvine, D. W. Hoyt, J. Schuckel, B. Jorgensen, W. Willats, D. E. Spalinger, J. L. Firkins, M. S. Lipton, M. B. Sullivan, P. B. Pope, K. C. Wrighton. In Press. Interspecies cross-feedings orchestrate carbon degradation in the rumen ecosystem. Nature Microbiology.

Solden, L. M., D. W. Hoyt, W. B. Collins, J. E. Plank, and R. A. Daly E. Hildebrand, T. J. Beavers, R. Wolfe, C. D. Nicora, S. O. Purvine, M. Carstensen, M. A. Lipton, D. E. Spalinger, J. L. Firkins, B. A. and Wolfe, K. C. Wrighton. 2018. First genomic insight into the enigmatic BS11 gut group reveals roles in hemicellulose degradation. Nature Communications.

Carnahan, A., D. Spalinger, and W. Collins. 2017. Alkane and long-chain alcohol recovery in moose (*Alces alces*), a browsing herbivore. Can. J. Zoology.

Stantorf, C. J., L. C. Buck, D. H Keisler, W. B. Collins, and D. E. Spalinger. 2017. Seasonal variation of nutritional hormones in captive female moose. Alces.

Solden, L. M., D. W. Hoyt, W. B. Collins, J. E. Plank, R. A. Daly, E. Hildebrand, T. J. Beavers, R. Wolfe, C. D. Nicora, S. O Purvine, M. Carstensen, M. A. Lipton, D. E. Spalinger, J. L Firkins, B. A. Wolfe, and K. C. Wrighton. 2016. New roles in rumen hemicellosic sugar fermentation for the uncultivated Bacteroidetes family BS11. Int. Soc. Microbial Ecology Journal 1-13.

Solden, L. M., A. Naas, S. Roux, R. A. Daly, W. B. Collins, R. A. Wolfe, C. D. Nicora, S. O. Purvine, D. E. Spalinger, M. S Lipton, M. S. Sullivan, P. Pope, and K. C. Wrighton. 2017. Microbial dark matter in the rumen ecosystem: Assigning the function of uncultivated Bacteroidetes and novel viruses in anoxic carbon transformations. Abstract. American Society for Microbiology.

V. RECOMMENDATIONS FOR THIS PROJECT

Job 2 should be discontinued due to damaged growth of plants which were propagated in advance for use in this experiment. Jobs 3 and 4 should be discontinued because they have been completed.

Prepared by: William B. Collins

Date: September 1, 2018