**Master work plan: Anna Hanauska (June 2020)**

**Rusitec Experiment**

**6 Treatments:**

* 5 Seaweeds, incubated together with TMR, inclusion level 5 %
  + 2 *Ascophyllum nodosum*
  + 2 *Fucus vesiculosus*
  + 1 *Asparagopsis taxiformis*
* Seaweed-free Control (Basal diet) -> high protein TMR, also used for extended HGT
* 15 g total weight

Treatments

Treatment 1: 100 % TMR

Treatment 2: 2.5 % Ascophyllum nodosum from Iceland and 95 % TMR

Treatment 3: 2.5 % Ascophyllum nodosum from Scotland and 95 % TMR

Treatment 4: 2.5 % Asparagopsis taxiformis from the Azores and 95 % TMR

Treatment 5: 2.5 % Fucus vesiculosus from Iceland and 95 % TMR

Treatment 6: 2.5 % Fucus vesiculosus from Scotland and 95 % TMR

**2 Experimental runs:**

* 14 days each
* 1 day for the start of the system (d0);
* 6 days for adaptation (d1-6);
* 7 days for sampling (d7-14)

**Data recording & pooling**

* **Adaptation phase (day 1 – 6):**
  + pH (fermenter liquid)
  + Redox potential (fermenter liquid)
  + Amount of gas and methane concentration of produced gas
  + Amount of effluent
* **Sampling phase (day 7 – 14)**
  + pH (fermenter liquid)
  + Redox potential (fermenter liquid)
  + Amount of gas and methane concentration of produced gas
  + Amount of effluent
  + Effluent liquid (70 ml/day 🡪 pool samples)
  + Bag residues (weight independently; day 7-12 🡪 pool samples)

**Samples taken for microbiota analyses**

* Rumen solid phase day 0 from each cow (target amplicon sequencing (TAS), shotgun metagenomics (SM))
* Rumen fluid day 0 from each cow – TAS, SM
* Rumen fluid mixed with buffer (cow 1-3 mixed) – TAS, SM
* Feed residues day 13 from 24 h bag each fermenter – TAS
* Feed residues day 13 from 48 h bag each fermenter – TAS, SM
* Fermenter liquid at d 7 each fermenter – TAS
* Fermenter liquid at d 13 each fermenter – TAS, SM
* Effluent at d 7 each fermenter – TAS
* Effluent at d 13 each fermenter – TAS

**Sample processing for microbial community analysis (TAS and SM)**

* DNA extraction - FastDNA Spin Kit for soil - MP Biomedicals – 158 samples
* DNA quantification – NanoDrop (all samples); Qubit (samples for shotgun metagenomics)
* Amplicon library preparation – Bacteria and Archaea
* Sample preparation for shotgun metagenomics

**Bioinformatic and statistical analysis (TAS)**

* Bioinformatics – MOTHUR pipeline
* Statistics – R packages, PRIMER6, JMP
* Sequencing library preparation
* Sample preparation for shotgun metagenomics