**Gut microbiota structure of growing pigs fed with two physicochemically different dietary fiber diets- alginate and resistant starch diets.** By Ö.C. Onarman Umu, M. Oostindjer, P.B. Pope and D.B. Diep, *Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway*

## Introduction

Gut microbiota consisting hundreds of different species have various physiological effects on host. The impact of gut microbiota depends on its composition that can be influenced by environmental factors such as stress, antibiotics, host immunity and diet. Diet appears to be one of the most important factors; it can act as direct energy source for bacteria in gut being indigestible to digestive enzymes of host (1). Dietary fibers constitute a large group of carbohydrates that are indigestible by host but can be fermented by gut microbiota in colon. Alginate (gel forming dietary fiber) and resistant starch (fermentable dietary fiber) are included in this carbohydrate group contributing to host health (2,3) and affecting host physiology differently due to their different physicochemical properties. The aim of this study was to investigate growing pigs fed with alginate and resistant starch for their gut microbiota activity and structure during 12 weeks and comparing them to the ones of control diet fed growing pig.

## Material and Methods

Each 3 pigs were fed with alginate containing diet (ALG), resistant starch (type 3, retrograded tapioca starch) containing diet (RS) and control (CON) diet having 9 pigs in total. Feeding lasted for 12 weeks and feces were collected from every pig at 7 time points (day 0, day 1, day 3, day 7, week 3, week 7 and week 12). Cell dissociation (4) and phenol-chloroform DNA extraction (5) were performed for each feces and DNA was further processed to amplify 16S V1-V3 regions in PCR using universal bacteria primers 8F and 515R together with 454 Fusion adaptors. Pyrosequencing was performed on the 454 GS FLX sequencer (Roche) at Norwegian Sequencing Center (Oslo, Norway). Quantitative Insights Into Microbial Ecology (QIIME) Pipeline (6) was used to analyze sequencing data. Quality filtering and chimera checking was carried out using USEARCH (7) and Operational Taxanomic Units (OTUs) were picked based on 97% similarities between the sequences. Taxanomy was assigned by RDP Classifier (8) to the aligned sequences. Unweighted UniFrac distance metric (9) was generated from phylogenetic tree that was built by Fast Tree (10). Unweighted UniFrac

distance metric was visualized by Principle Coordinate Analysis (PCoA). ANOVA plot was generated by Calypso Version 3.4 (http://bioinfo.qimr.edu.au/calypso/).

**Results and Discussion**

As a result of pyrosequencing, 232,732 sequence reads with 524 nt of average length were obtained from 61 samples after quality filtering. Diversity was significantly lower in RS samples compared to ALG and CON samples, that is most probably a consequence of selection of specific Firmicutes genera in RS samples. PCoA shows that the bacteria composition of RS samples are significantly different from ALG and CON samples while ALG samples are more similar to CON (Fig.1). Resistant starch as fermentable fiber had a more remarkable impact on gut bacteria structure of growing pigs. The change in composition may affect energy regulation of host as this type of diet improved satiety in adult pigs previously (11). Moreover, the microbiota composition was influenced by time within each diet group. Almost all samples of all pigs at day 0 converged at the bottom of PCoA plot meaning that most of the initial gut bacteria compositions of the pigs were similar as all pigs were fed with same control diet at day 0. By time, other samples diverged following a pattern within each diet group and the higher variation was observed within RS group (Fig. 1). Although there were some inter individual variations, the effect of fibers was remarkable for each individual pig that belongs to the same diet group. The predominant phyla were same for each pig regardless of the diet type. The most predominant phyla were Firmicutes (88.9 ± 1.07 %) and Bacteroidetes (9.5 ± 0.82 %) at all diet groups. Only Tenericutes phylum was affected significantly by diet having the lowest abundance in RS fed pigs. Higher number of variations in abundance was observed in lower taxanomic levels. In family level, Uncultured Bacteroidales family, Prevotellaceae, Lachnospiraceae, Veillonellaceae and Erysipelotrichaceae had significant increase in RS fed pigs while Streptococcaceae, Clostridiaceae and Uncultured RF39 families were reduced by the same type of diet (Fig. 2). Moreover, compared to the previous studies that used type 3 resistant starch as feed component (12,13), more genera had significant change in abundance. Alginate also had impact on the abundances of some bacteria although it is known as gel forming dietary fiber having little fermentability (14). Prevotellaceae, Veillonellaceae and Streptococcaceae became more abundant in ALG fed pigs while abundances of Clostridiaceae, Erysipelotrichaceae and Uncultured RF39 decreased in number (Fig. 2).

To sum up, long term (12 week) effect of alginate and resistant starch containing diet was investigated on growing pigs. RS had the most significant impact on gut microbiota

compositions of growing pigs and the abundances of particular bacteria. ALG fed pigs had more similar gut bacteria composition to CON fed pigs. Although the impact of alginate was not as remarkable as resistant starch, the abundance of some specific bacteria was influenced by ALG diet.

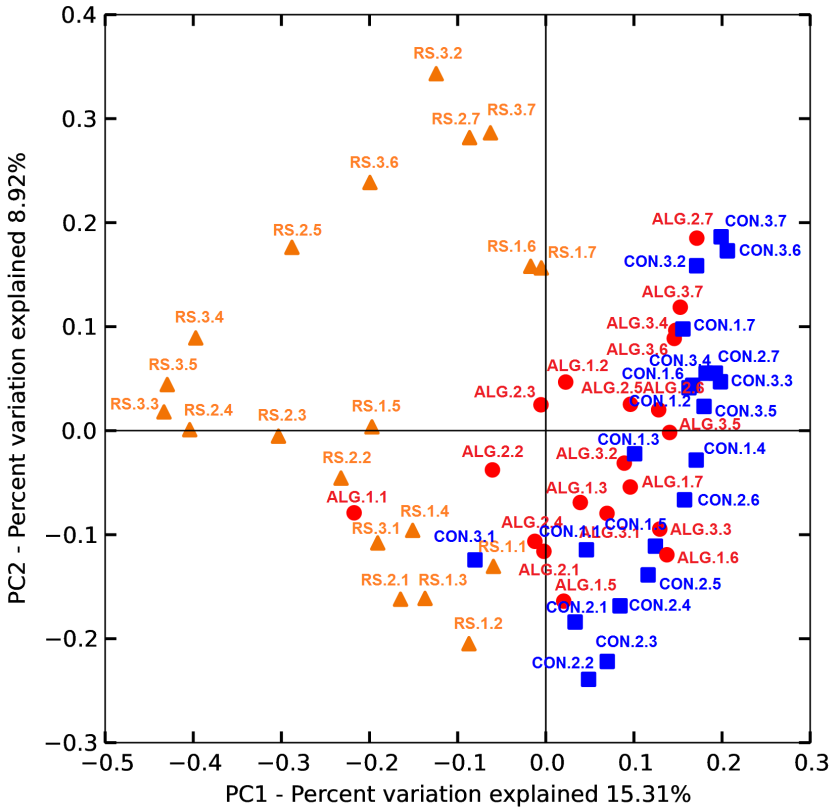


Fig. 1. 2D PCoA plot showing the differences in composition of microbial communities between each samples. RS (▲): resistant starch fed pig sample, ALG (●): alginate fed pig sample and CON (■): control diet fed pig sample. Firs number after diet name represents pig number and second number represents time points from day 0 to week 12.

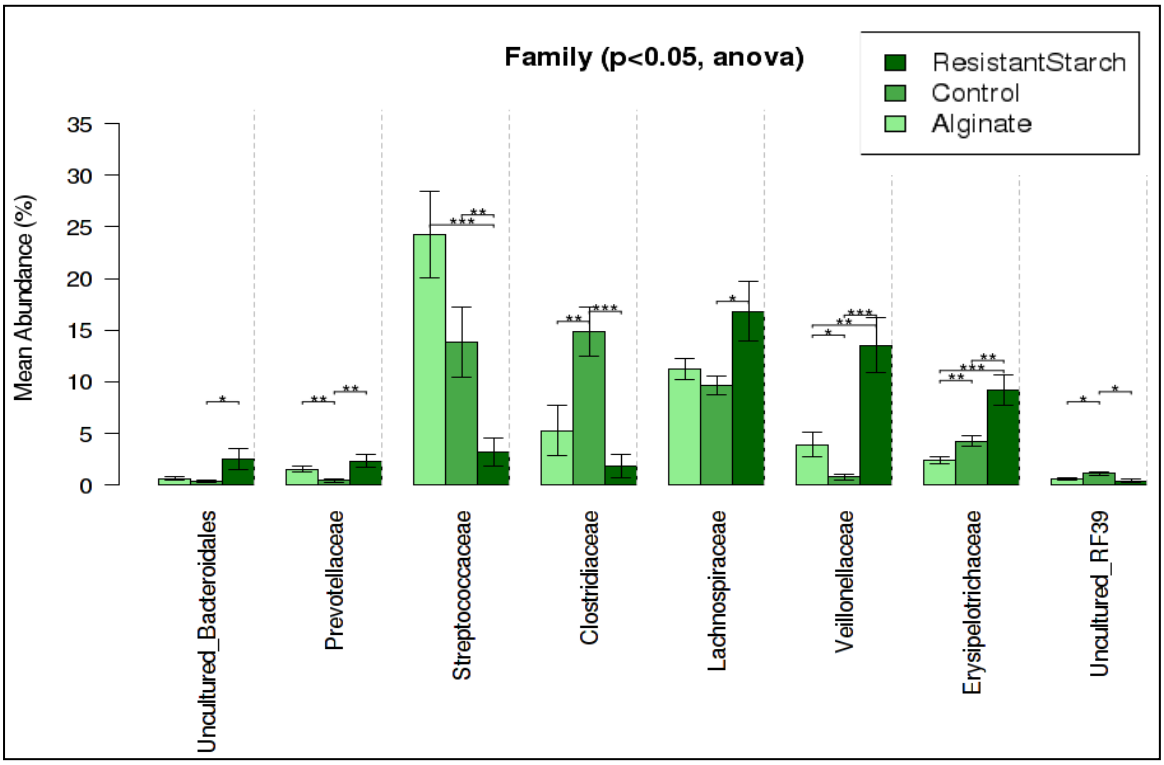


Fig. 2. ANOVA bar graph showing the significantly changed bacteria families in abundance after feeding growing pigs with alginate and resistant starch containing diets.

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