March 27, 2013

IMPROVING THE EFFICIENCY OF PORK MEAT PRODUCTION AND DECREASING THE ENVIRONMENTAL BURDEN WITH HYDROGENATION

Ministry of agriculture and forestry
Project Dnro 2056/312/2011

Alimetrics final report, 27.3.2013

Conclusions:

- Hydrogenation showed a positive effect on pig daily weight gain, but at the same time carcass meat percentage decreased. Financial gain was not statistically significantly different from the control group
- Both total microbes and *Streptococci* were inhibited in the faecal samples of the hydrogenation group at the last time point. This result correlates with the improved growth performance results and may therefore be an indicator of improved nutrient absorption in the small intestine
- Hydrogenation decreased the numbers of potentially pathogenic *E.coli* in the faecal samples
- The trials did not confirm the hypothesised decrease in ammonia concentrations
- The mode-of-action of the deodorisation pipe could not be clarified with the *Escherichia coli* spiking trial

1. Background

Ceramic tips made out of titanium oxide have been used to purify water for decades. The process is based on strong redox reactions which filter impurities from water. Hydrogen gas is a by-product of the purification reaction. An example of a purification unit consisting of titanium oxide pellets is illustrated in Figure 1.

Figure 1. Example of a purification unit used in the trial

This invention is based on observations at some pig farms which have installed a deodorisation pipe. Hydrogenation of drinking water has shown positive effects on body weight gain, tail-biting, digestion disorders and manure odour problems. All these observations indicate that the digestion of protein is enhanced by hydrogenated drinking water. Less digestion problems indicate a decreased amount of faulty fermentation which is partly explained by the decreased amount of protein ending up in the lower digestive tract. The decrease in the amount of ammonia and improvement of odour problems in manure indicates strongly that the overall utilisation of feed protein is enhanced. Hydrogenation may additionally protect the unsaturated fatty acids and other nutrients, such as E-vitamin, from the acidic environment in the stomach.

2. Objectives

The objective of the project was to find out how hydrogenation of drinking water affects the digestion of protein and does it benefit animal health. Possible beneficial environmental impacts of hydrogenation were also investigated. One of the assumptions was that hydrogenation improves body weight gain of pigs. Additionally, hydrogenation could benefit to overall animal health and decrease the amount of health problems.

3. Materials and methods

3.1. Trial setup

Growth performance trial was conducted at HK-Ruokatalo contract farm, in Rusko, north of Turku, Finland. The trial began 8.10.2012 and ended 18.1.2013. 48 pigs were included in the trial, of which 24 were female and 24 male. The pigs were divided into two treatment groups which were placed in separate buildings. The deodorisation pipe was installed in one of the buildings. Optimal situation would have been that both groups were in the same building. This was impossible, however, since the installation did not allow seperation of water into two groups inside one building.

24 pigs were placed in each building, half of them female and half male. The pigs included in the trial were further divided into 6 pens with 2 females and 2 males in each pen. The pens used in this trial are illustrated in Figure 2. One group was the control group with untreated drinking water, while the other group's drinking water was hydrogenated. The pigs included in the trial were marked with earmarks in the beginning of the trial.

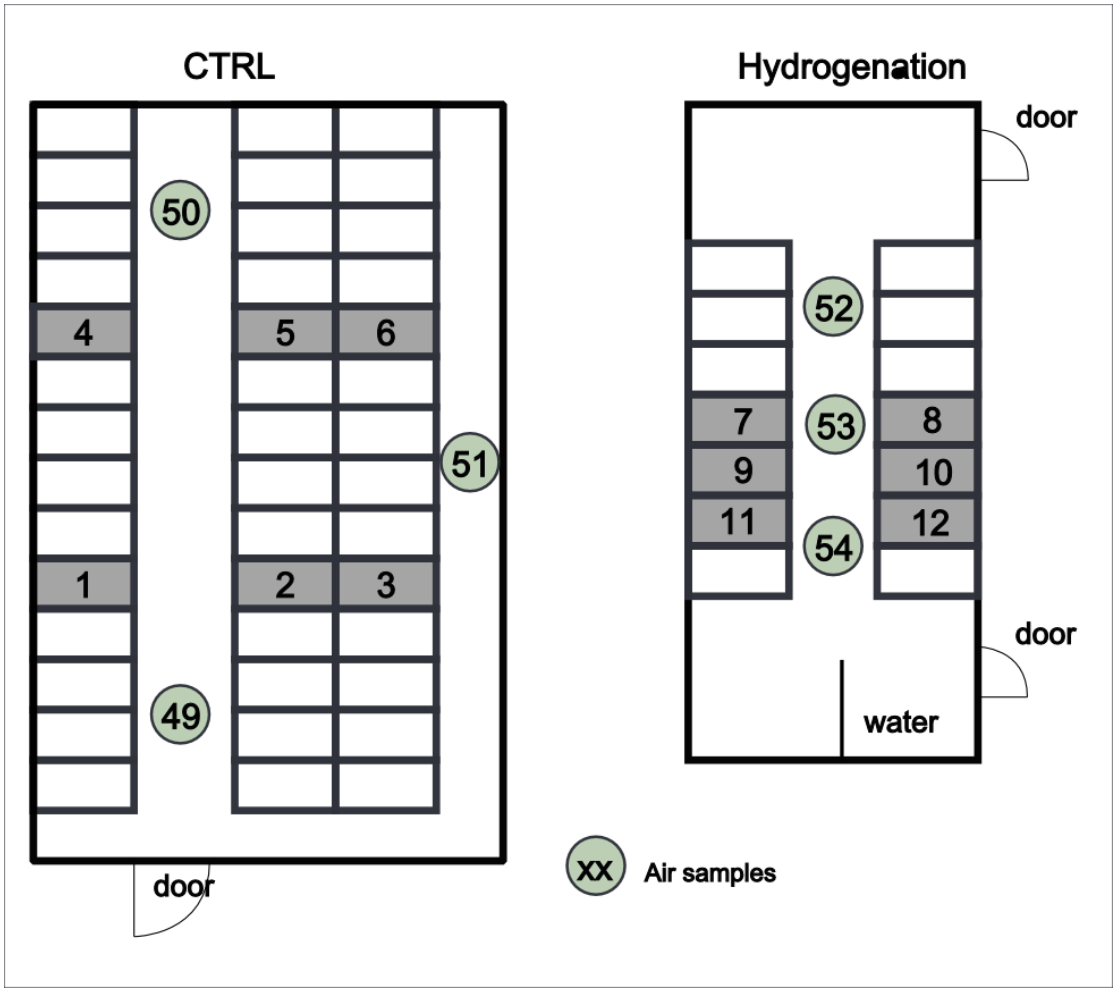

Figure 2. The pens used in the control group (left panel) and in the hydrogenation group (right panel). Green circles indicate the sites for air samples.

3.2. Feeding and sampling schedule

The pigs were fed with two-phased dry feeding. The cereal was grinded with a hammermill and the proportion of cereal and semiconcentrate was adjusted with frequency modulation. The composition of the feed was identical in both test groups. In the first feeding period feed was given *ad libitum,* while in the second feeding period the amount of feeding times was limited. In the first feeding period 42% of the feed was semiconcentrate (Rehuraisio rape seed semiconcentrate 1) and 58% was barley. In the second feeding period 40% of the feed was semiconcentrate (Rehuraisio rape seed concentrate 2) and 60% was barley. Both test groups had unlimited amount of drinking water.

Test pigs were weighed at the beginning of the trial and before second feeding period. The carcass weight and meat percentage at the end of the trial were provided by HK Ruokatalo. Faecal samples were collected at three time points; in the beginning of the trial, after first feeding period and after second feeding period. Air samples were collected at the same time points. The air samples were collected with a 100 ml syringe, and stored in a vacuumed glass container. The air samples were collected from 3 sites in both buildings, as seen in Figure 2.

Manure samples were collected before the growth performance trial, at the end of the previous batch. At the end of the trial, manure samples were collected from all pens included in the trial. The composition of manure samples was mainly dried faeces, sawdust and feed. The sampling time was right before slaughter, which means the samples taken at the end of the previous batch and the trial batch are comparable.

3.3. Sample analysis

3.3.1 Volatile fatty acids, lactic acid, ammonia and qPCR-analysis

Faecal samples were analysed for volatile fatty acids (VFAs) and lactic acid with gas chromatography using a packed column. Ammonia was analysed from faecal samples, air samples and manure samples using a colorimetric method, in which the amount of light emitted by the reagent is proportional to the amount of free ammonia in the sample. The intensity of the emission was measured with a colorimeter and converted to millimoles per litre using a standard curve.

Additionally, faecal samples were analysed for microbial diversity. Initially, bacteria in the samples were washed and separated by differential centrifugation. Subsequently, bacterial cell walls were disrupted using both enzymatic and mechanical lysis procedure and the chromosomal DNA was quantitatively purified using the Alimetrics DNA extraction protocol, which is optimised and validated for the bacterial DNA isolation. Consequently, all individual DNA samples were subjected to quantitative real-time PCR analyses. Ten different bacterial groups including total microbes were analysed.

3.4. Echerichia coli -spiking

The effect of hydrogenation to drinking water quality was studied *in vitro* by spiking the water with *E.coli.* Two doses of initial *E.coli* concentration was used in the trial ($4.8*10^3$ ja $4.8*10^6$ cfu/ml). These concentrations were diluted from freshly grown culture of *E.coli*. The test was conducted with the deodorisation pipe provided by Johematic oy. The water flow speed was set to a constant 500 ml/h. The water used in the trial was taken from the same farm where the growth performance trial was performed. The sampling scheme is illustrated in Figure 3. One sample was taken before the treatment (sample 1), two samples when the machine was on (samples 3 and 5) and two samples when the machine was off (samples 2 and 4). The test was carried out separately for the *E.coli* doses, first with the lower concentration and then with the higher concentration. The machine was on/off 15 minutes before sampling, in order to make sure the water from which the sample was taken from was representative. The concentration of *E.coli* in each sample was analysed with MPN-method (Thomas, 1942). The analysis was done with 4 replicate sterile microtiter plates with TSB as growth media. The samples were incubated for 24 hours at 37°C.

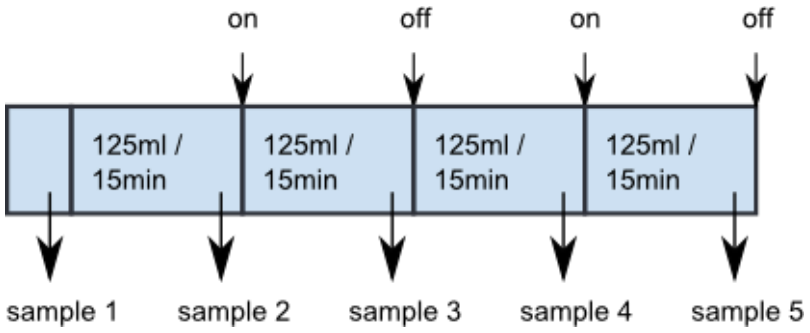

Figure 3. Sampling scheme in the *E.coli* spiking trial.

3.5. Statistical analyses

Statistical analysis consisted of analysis of variance (ANOVA), and Tukey's post-hoc test. In ANOVA the effect of each variable is measured by the amount of variance it explains. In this trial the test treatment (CTRL, Hydrogenation), sex (female/male) and time was taken into account.

The Tukey letters divide the treatments into groups, starting with letter A given to largest values. Treatments which have the same letter in their labels are not statistically significantly different from each other. For example treatment with label B differs from treatment with label CD, but C and CD do not differ statistically significantly.

The carcass value was evaluated by HK-Ruokatalo. The base of the calculation is the carcass weight. Meat price for carcasses between 75 and 100 kg was 1.5€/kg. For carcasses below 75kg and over 100kg the price was slightly lower. Meat percentage has an cumulative effect of 0.02 €/%, i.e. decrease of one percentage unit in the meat percentage decreases the meat price for the carcass by 0.02€/kg. The start value for meat percentage was 59.5 – 60.5%.

4. Results

4.1. Escherichia coli -spiking

The results of the *in vitro* spiking trial confirmed the validity of the test setup. The measured *E.coli* levels from the intake were at the desired level. Additionally, the two doses could reliably be distinguished from each other which means the scaling of the trial was appropriate. Replicate MPN measurements showed approx. 0.5 log variation, which means differences of approx. 1 log could have been distinguished.

The hydrogenation of water did not show a statistically significant effect to the microbial concentration (Figure 4). This indicates that the mode of action is probably not based on inhibition of (pathogenic) bacteria. The temperature in the machine provided by Johematic increased over 30°C. This probably does not happen in the machine used in growth performance trial.

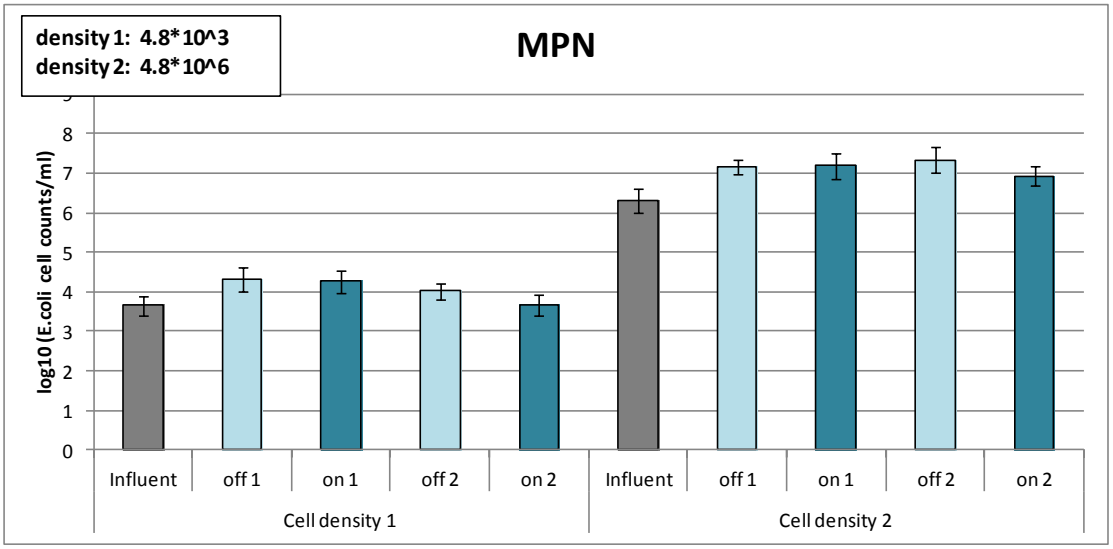

Figure 4. Results of *Escherichia coli* -spiking. Unit is the number of *E. coli* cells/ml water. NOTE: logarithmic scale.

4.2. Manure and air samples

The composition of manure samples varied greatly. The amount of moisture in the samples affects the results. Therefore, the results should assessed with reservation. Hydrogenation decreased the concentration of ammonia to half what it was at the end of the previous batch (Table 1). At the same time, the concentration of ammonia increased in the control group. This indicates that sampling time and other variables have significant effect in the composition of the sample and thereby to ammonia concentration. The concentration of nitrate/nitrite was inverse to the concentration of ammonia; high concentration of ammonia means low concentration of nitrate/nitrite and vice versa. This phenomenon is related to nitrogen cycle, which is the process where nitrification bacteria transform ammonia to nitrate/nitrite at the presence of oxygen.

Table 1. Ammonia concentration in the manure samples.

	Previous batch	Trial batch
CTRL	2.73	11.97
Hydrogenation	15.73	5.90
	mM	mM

Good air quality in production facilities is one of the basic prerequisites of production animal welfare. Constantly changing weather conditions present a challenge in Finland. The ventilation systems in the buildings were different from each other. Especially in the end of the growth performance trial the building with hydrogenation had higher interior temperature and air moisture content compared to the control building. Higher interior temperature increases the emission of ammonia from the faeces on the floor. This partly explains the increased concentration of ammonia in the hydrogenation building. The concentration of ammonia in the air samples of both building is presented in Figure 5. The variance in the results is explained by local weather conditions at sampling time, different building properties and the indoor temperature.

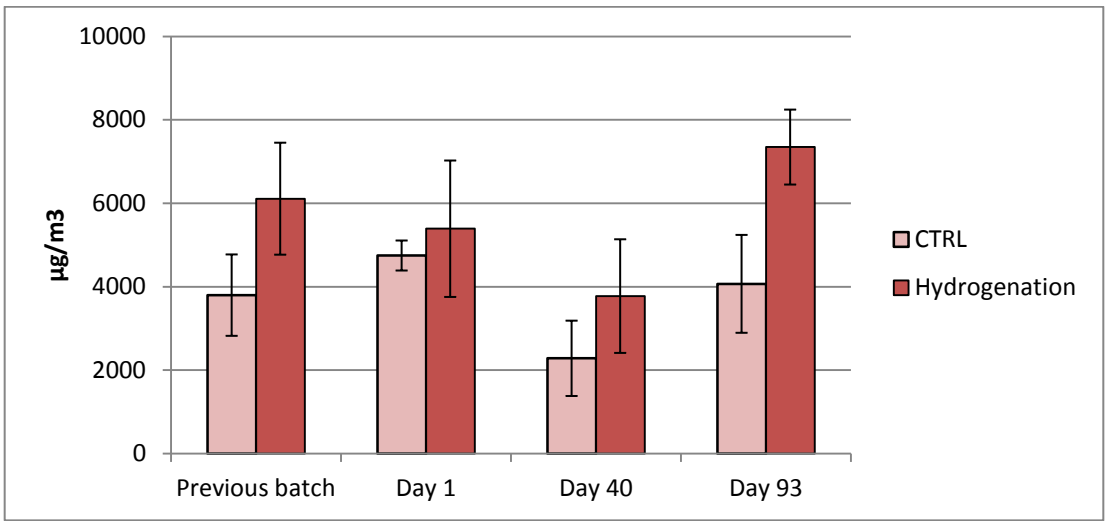

Figure 5. Ammonia in the indoor air samples. The error bars indicate standard error between three replicate samples.

4.3. Pig performance results

The body weight of the pigs was measured for the duration of the trial (Figure 6). The results showed that sex had significant effect on the body weight gain (BWG). Female pigs had statistically significantly larger BWG in the hydrogenation group when compared to the control group. The differences in BWG with male pigs were not statistically significant, but on average the hydrogenation group showed increased BWG when compared to the control group.

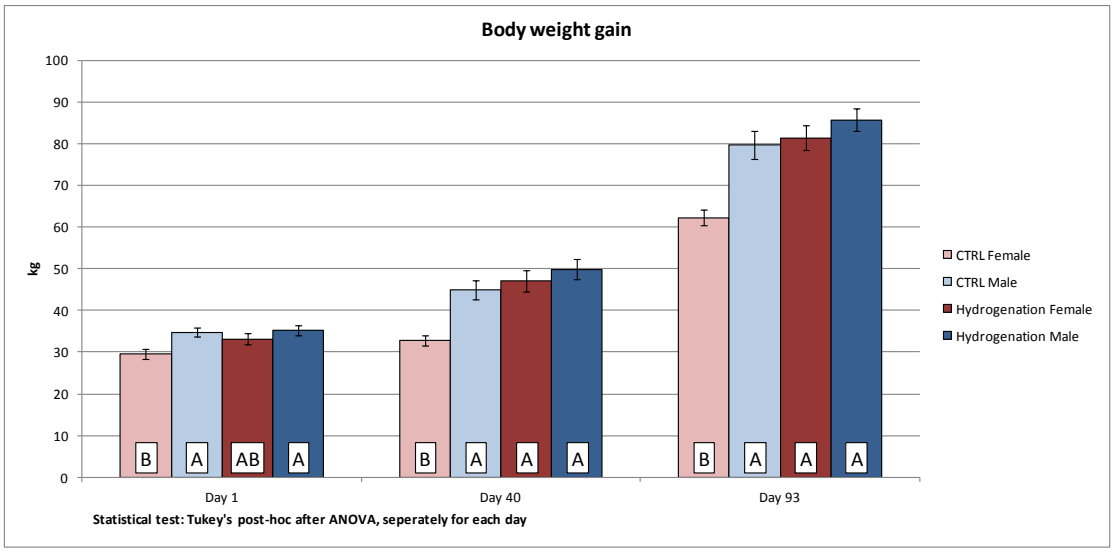

Figure 6. Body weight gain, grouped by treatment and sex. On the left: BWG from day 1 to day 40, in the middle: BWG from day 40 to day 93 and on the right: BWG from day 1 to day 93. Statistical analysis with Tukey's post-hoc test after ANOVA.

The slaughter weight at the hydrogenation group was significantly larger than in the control group (Figure 7 upper panel). The difference was statistically significant with female pigs. The sex has also an influence to the slaughter weight since the slaughter weights of female pigs were smaller in both buildings when compared to male pigs. The composition of the pig carcass shifts when the total weight increases. The amount of fat in the carcass increases and thereby decreases the meat percentage.

As seen in the lower panel of Figure 7, increased slaughter weight decreased the meat percentage also in this trial. Females have higher meat percentage than males. Meat percentage in the hydrogenated groups was lower than in the control group. Statistical comparison by pens is shown in Table 2. Calculating the values from pen averages shows slightly different results, but they are irrelevant statistically speaking. The amount of produced meat shows no statistically significant difference between test groups.

HK provided carcass weight and meat percentage also from pigs outside this trial. These pigs were treated with the same drinking water as the trial pigs, but no faecal samples were collected from them. Figure 8 shows the slaughter weight and meat percentage comparison between trial pigs and the pigs outside the trial. Sex was not reported in the data set and therefore average values are presented. The results confirm the previous observation, that hydrogenation increases the slaughter weight of pigs. The data also supports the conclusion that a larger number of trial animals would provide more accurate results. In the control building the average slaughter weight of trial pigs has bias when compared to the average slaughter weight of other pigs. Additionally, more trial animals would yield smaller variance in the data.

In the hydrogenation building two pigs died of leg weakness. Additionally, a third pig was not sent to slaughter also because of leg problems. The mortality was not caused by the treatment but it has to be taken into account in the statistical analyses.

The value of the carcasses was analysed by HK. Without mortality, the value of the carcass in control group was on average 109.42€ and in the hydrogenation group 123.04€. The difference is statistically significant (Student p-value 0.02). In case the mortality is taken into account, the financial benefit between test groups is not statistically significant, because the carcass value is 107.66€ for the hydrogenation group.

Table 2. Pig performance trial results. Statistical analysis made from pen average values.

	Carcass weight, kg	Meat-%	Meat amount*	BWG, kg			BWG, g/pv
				1-40	40-93	1-93	
CTRL	77.92	60.05	46.62	32.15	38.88	71.02	0.76
Hydrogenation	88.65	58.40	45.22	34.25	48.03	83.17	0.89
p-value (Student)	< 0.001	0.088	0.162	0.077	0.005	< 0.001	< 0.001

*The amount of meat is calculated by (slaughter weight x meat-%). Mortality was taken into account.

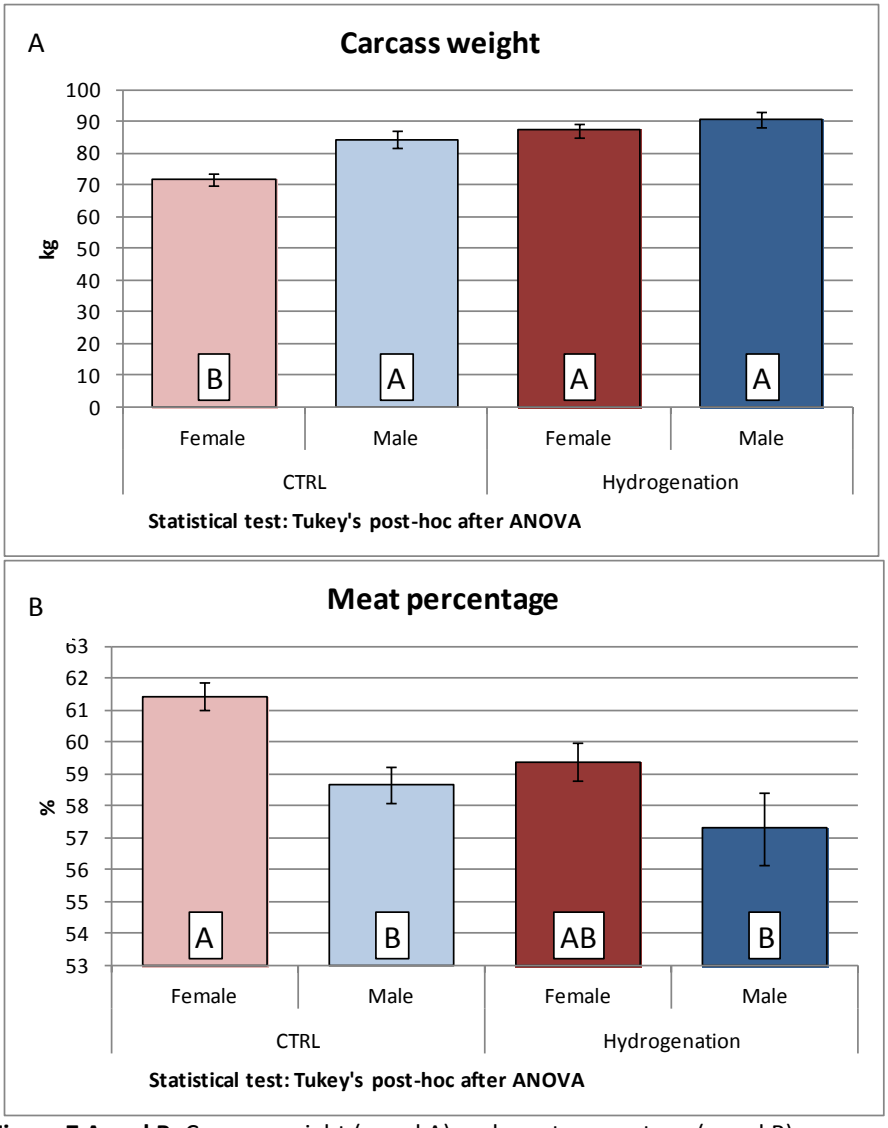

Figure 7 A and B. Carcass weight (panel A) and meat percentage (panel B), grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

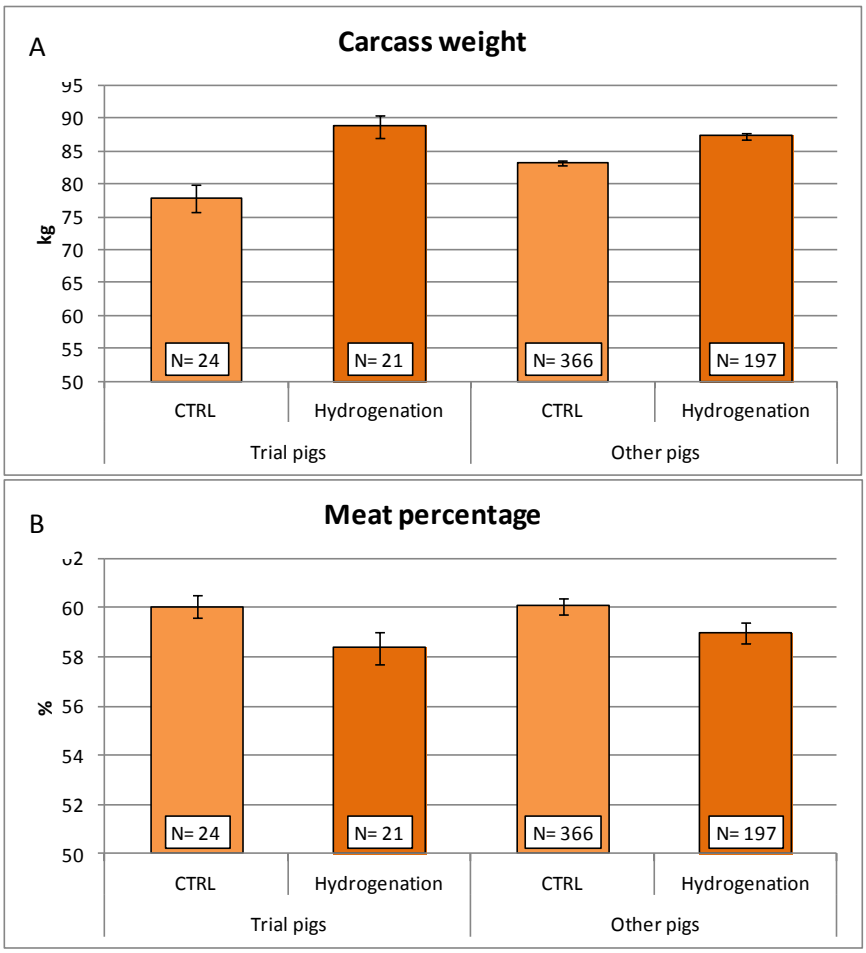

Figure 8 A and B. Carcass weight (panel A) and meat percentage (panel B) separately for trial pigs and other pigs in the buildings. Note scale.

4.4. Ammonia, VFA and microbial analyses from faecal samples

4.4.1 Ammonia

Ammonia is generated in the lower gastrointestinal tract during protein fermentation during deamination. The production of ammonia is prevented in case the intestinal microbes are provided with carbohydrate-based energy source.

The faecal samples showed no statistically significant in ammonia concentration (Figure 9). Variation in the results was smaller than in the air samples (Figure 5), partly caused by a larger sample size. The results showed no sign of decreased ammonia concentration by hydrogenation. Temporal effects were not statistically significant, nor did sex have an effect on the results.

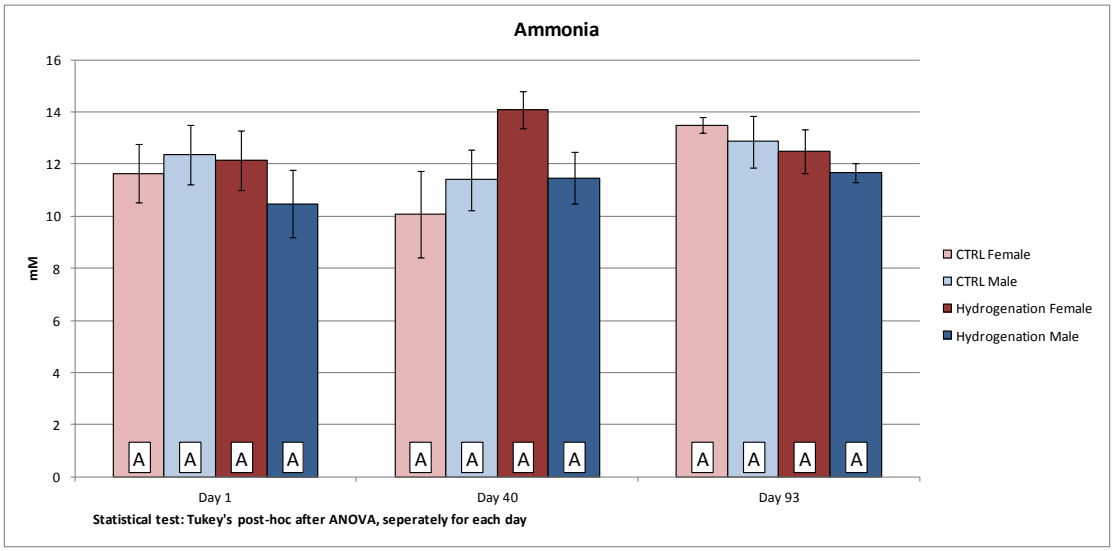

Figure 9. Concentration of ammonia in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

4.4.2 Short-chain fatty acids

The most important end products of the microbial fermentation in the gut are short-chain fatty acids (SCFAs). Most important SCFAs are acetic, butyric, propionic and lactic acids. Up to 95% of the SCFAs produced in carbohydrate fermentation are used by the host animal. Cells are specialised to use SCFAs as an energy source; acetic and propionic acid for brain, muscles and heart while butyric acid is the preferred energy source of the intestinal epithelium cells.

Figure 10 shows the average SCFA concentration in all samples. Acetic acid was the most frequent (60mM), while propionic acid (30 mM) and butyric acid (20 mM) were the next. The concentration of lactic, valeric acid and branched VFAs were below 10mM.

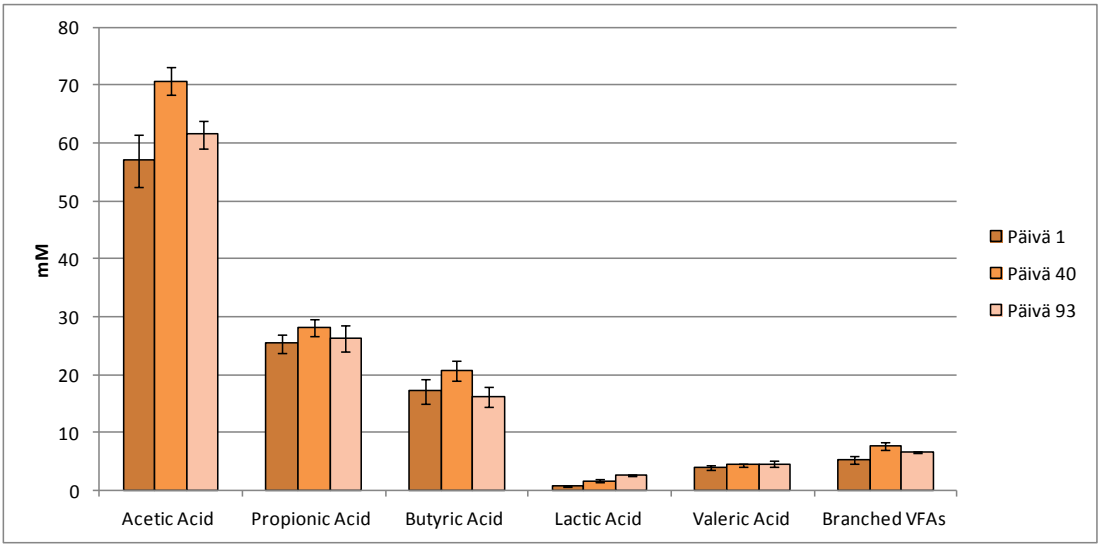

Figure 10. Concentration of short-chain fatty acids at the three sampling dates. (mM)

Hydrogenation showed no statistically significant effect on the total SCFA concentration (Figure 11). Additionally, neither sex nor sampling date showed a statistically significant effect.

As seen in Figure 12, hydrogenation did not affect the percentage of acetic acid in the faecal samples. According to ANOVA, male pigs had statistically significantly larger acetic acid percentage than females (see Appendix 1).

The percentage of propionic acid in the faecal samples was decreased in the hydrogenation group when compared to control group (Figure 13, Appendix 1). Especially male pigs at the end of the trial showed a decreased propionic acid percentage.

The percentage of butyric acid was lower with male pigs at the two last time points (Figure 14). Additionally, at the last time point, hydrogenation showed an increased butyric acid percentage when compared to the control group.

Branched VFAs had no clear relationship to treatment, but the faecal samples from the second time point with hydrogenation showed a decreased percentage of branched VFAs (Figure 15). Branched VFAs is found to correlate with odour problems (Miller & Varel, 2003). In this trial the amount of branched VFAs showed no statistically significant correlation with ammonia concentrations.

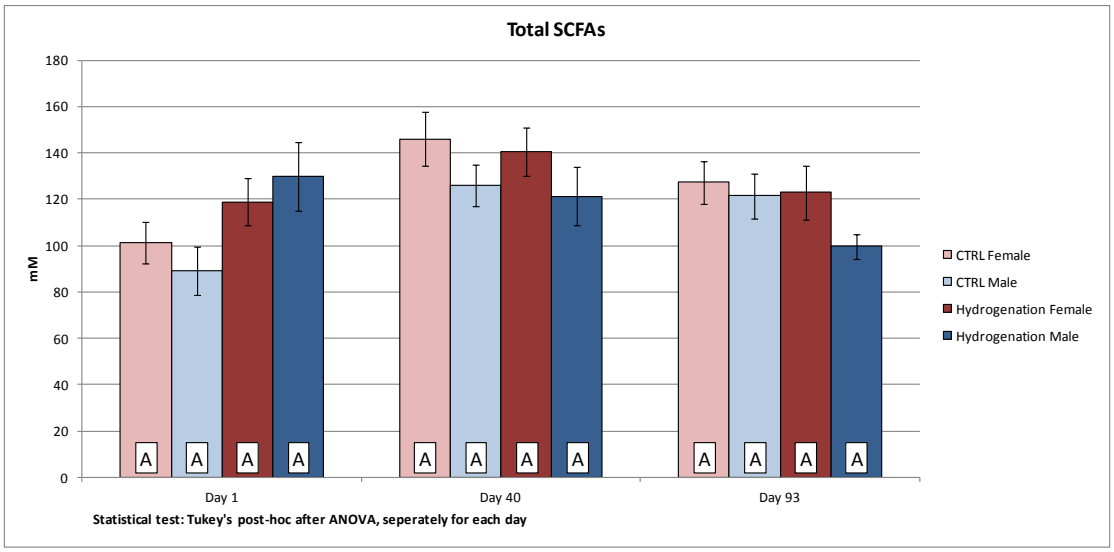

Figure 11. Total short-chain fatty acids (mM), grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

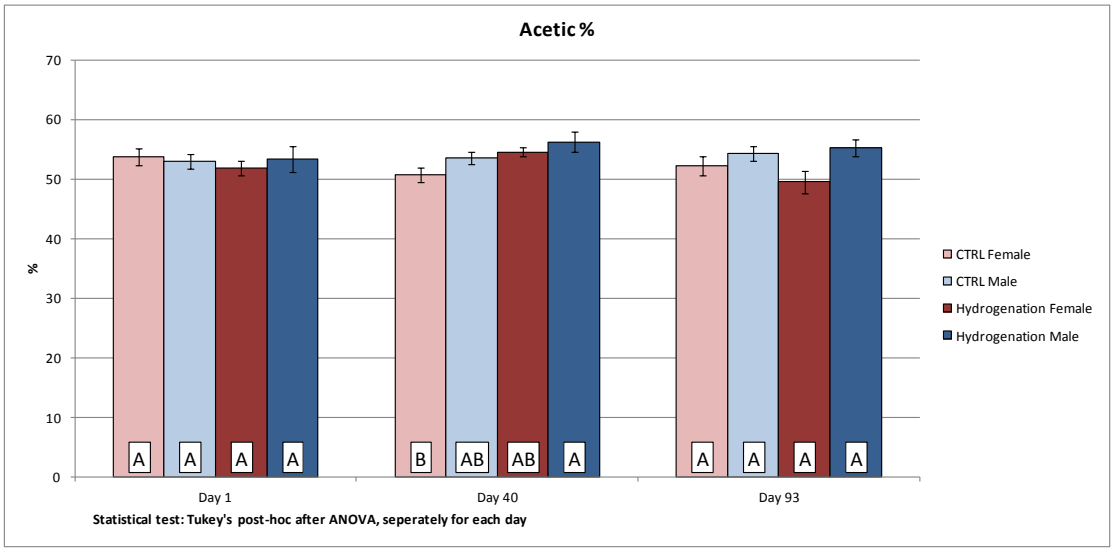

Figure 12. Percentage of acetic acid (%), grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

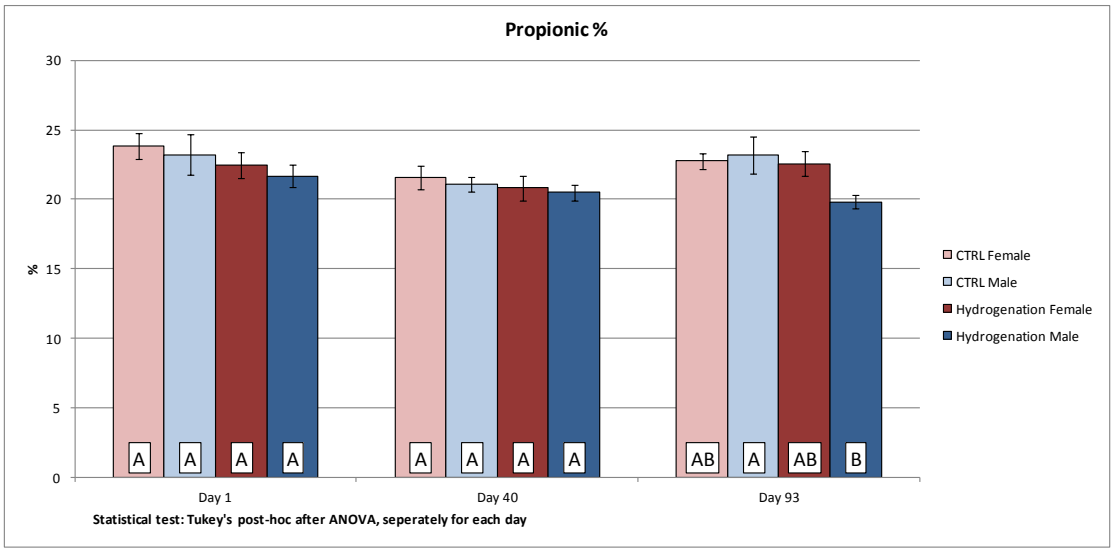

Figure 13. Percentage of propionic acid (%), grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

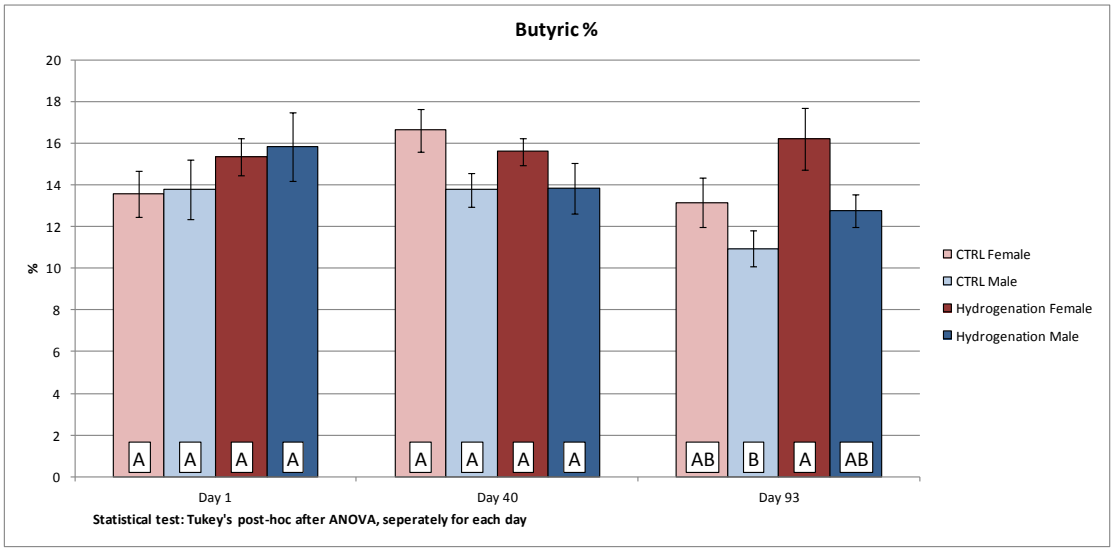

Figure 14. Percentage of burytic acid (%), grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

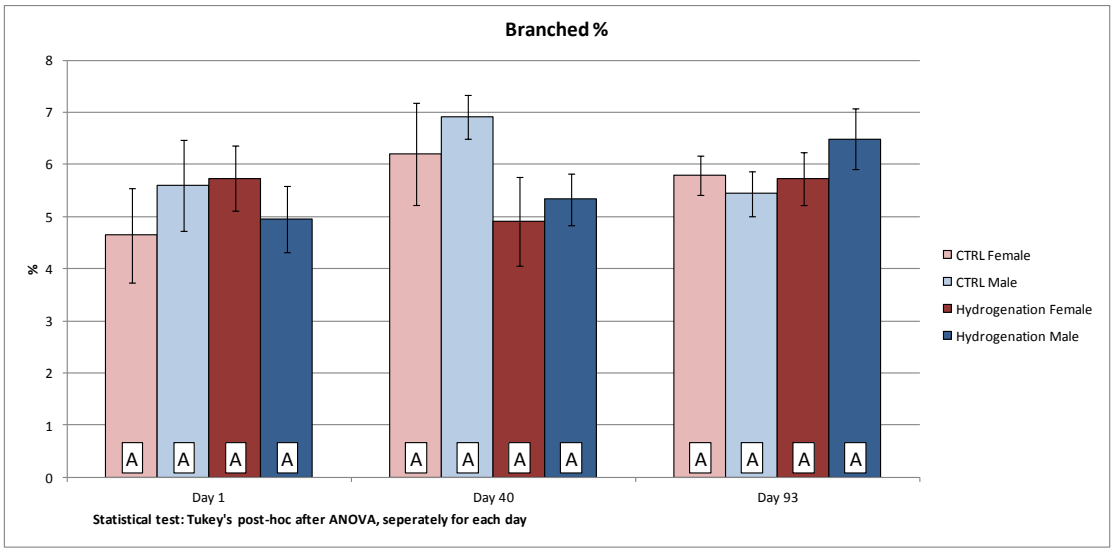

Figure 15. Percentage of branched VFAs (%), grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

4.4.3 Microbial analyses (qPCR)

The gut microbiota is generally very stabile in healthy animals indicating that certain mechanisms promote the vitality of desirable bacteria in the gut. Disrupting the equilibrium of the gut microbiota has been shown to cause many kinds of health problems. The quantity of microbes in the gut is a good indicator for possible disorders and health problems.

The quantity of microbes in the faecal samples was very stabile during this trial (Figure 16). Microbial counts in the last time point are higher than in the previous time points with almost all measured microbes. The quantity of *E.coli* bacteria is increased more in proportion to the other microbial groups. This may indicate a decrease in hygiene levels towards the end of the trial.

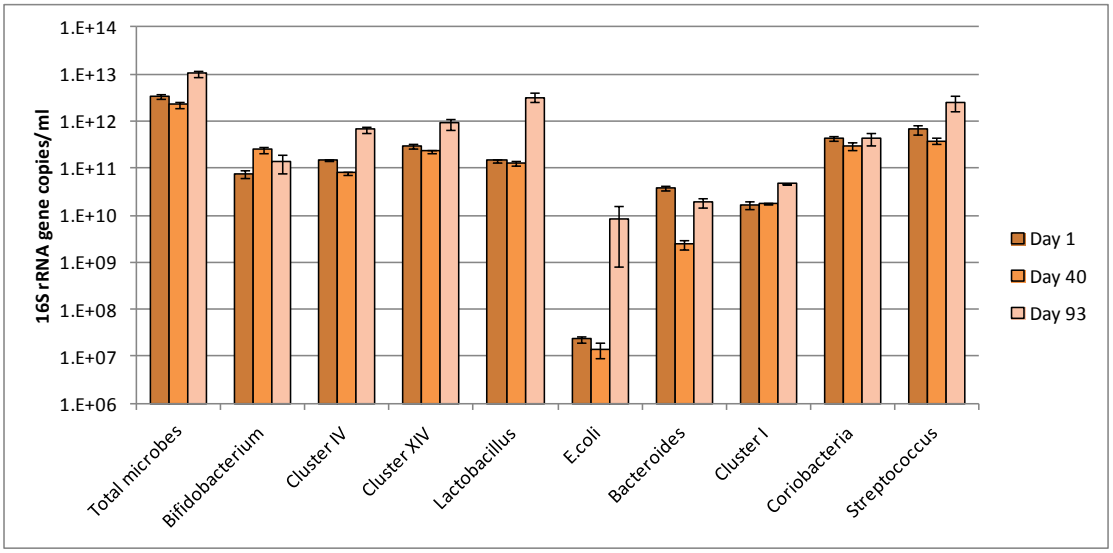

Figure 16. Average microbial numbers in the faecal samples (16S rRNA gene copies/ml).

High microbial numbers in the lower intestine indicate that some feed components have not been used by the host animal in the upper intestine, thus providing energy for the bacteria in the lower intestine. The microbes in the lower intestine convert some feed to SCFAs that the host animal is able to use. However, the absorption rate of SCFAs is much slower than in the upper intestine.

The total number of microbes is presented in Figure 17. In the control group the levels in the last time point are approx. 1 log larger than in the previous time points. Corresponding increase in microbial numbers was 0.5 log in the hydrogenation group. ANOVA reveals the difference between the two groups to be statistically significant (see Appendix 1). The decrease of total microbes in the hydrogenation group may indicate, that the microbes in the lower intestine have less available compared to the control group. This correlates with the growth performance, since enhanced digestion in the upper intestine leaves less energy for the microbes in the lower intestine.

Bifidobacteria are saccharolytic bacteria which are believed to promote health both in humans and animals. Probiotics, which increase the amount of *Bifidobacteria* in the gut, are much discussed in the literature. They are known to show positive effects, especially with piglets. (Abe, et al., 1995). The numbers of *Bifidobacteria* in the faecal samples varied greatly and no clear conclusions could be made (Figure 18).

This Clostridial cluster together with the cluster IV is the most dominant bacterial group in the caecum of broiler chickens. It contains many bacterial species the exact identities of which are poorly known. The common feature appears to be the production of butyric acid and positive correlation with animal health and performance. Butyrate is known to improve overall gut health and provide energy to the epithelial cells. As seen in Figure 19, the Cluster XIVa numbers correlate with total microbial numbers. Hydrogenation group showed a statistically significantly smaller increase in the last time point when compared to the control group. Strong correlation between Cluster XIVa numbers and the butyric acid concentration was not observed.

Clostridial cluster IV is taxonomically distant from the cluster XIVab, but like XIVab accommodates producers of butyric acid. It contains bacterial species such as *Faecalibacterium prausnitzii*, *Cl. leptum* and *Ruminococcus spp*. all of which are common intestinal bacteria in warm blooded animals. In addition to butyric acid production, members of this cluster are known produce formic acid, which is known to inhibit pathogenic bacteria in the gut. Additionally, *F.prausnitzii* has been observed to protect against inflammation both *in vitro* and *in vivo.* Figure 20 illustrates the Cluster IV numbers in the faecal samples. The total numbers are slightly lower than with Cluster XIVa, but in general the treatment effects are very similar.

Like B*ifidobacteria*, also *Lactobacilli* are saccharolytic. Typically they lower the gut pH and thereby have a protective role against pathogenic bacteria, such as *E.coli* or *Salmonella*. *Lactobacilli* numbers is not known to have any effect on the feed conversion ratio. The numbers of *Lactobacilli* in trial (Figure 21) was similar to the previous microbial results discussed.

Characteristics of the bacterial species belonging to the genus *Bacteroides* vary. Some bacteria belonging to this group are known to be putrefactive. Additionally, some members of the *Bacteroides* group are known to be opportunistic pathogens that disrupt the epithelial cells. In this study, the *Bacteroides* numbers was the smallest at the second time point (Figure 22). Additionally, hydrogenation group showed inhibition (Appendix 1).

Escherichia coli belongs to normal intestinal microbiota in all warm blooded animals. However, there are many virulent strains of the bacterium that seriously risk the health of animals. Since the conditions favouring the growth of the harmless *E. coli* hardly differ from those of the virulent ones, it is justified to use the total numbers of *E. coli* as a risk indicator. In this trial the numbers of *E.coli* were the largest at the end of the trial (Figure 23, Appendix 1). In the control group the increase was over 2 logs, while in the hydrogenation group the increase was only approx. 1 log.

Mostly harmful bacteria belong to the Clostridium cluster I. In this trial it was observed that sex or hydrogenation did not have an effect on the Cluster I numbers. At the end of the trial the numbers were higher than in the previous time points (Figure 24, Appendix 1). The

inhibition observed in total microbial numbers with the hydrogenation group indicating that the proportional share of Cluster I bacteria increased in the hydrogenation group. However, negative effects were not observed in growth performance or other parameters.

Coriobacteriaceae belong to the *Actinobacteria* phylum, which is known to harbour strictly anaerobic Gram-positive bacteria. *Eggerthella lenta* and *Collinsella aerofaciens* are the most well known members of this group in mammals. The health benefits of *Coriobacteriaceae* are not widely studied, but some positive indications of health benefits exist. Hydrogenation showed inhibition of *Coriobacteriaceae* numbers (Figure 25, Appendix 1). This inhibition is similar to the inhibition of total microbes which means the percentage of *Coriobacteriaceae* in the faecal samples remains the same.

Streptococcus, such as *Lactobacilli* are Gram-positive lactic acid producers. They dominate the upper digestive tract, but in certain cases they are found in large numbers in the lower digestive tract. The increased amount of lactic acid producing bacteria may be caused by decreased absorption in the upper digestive tract. In this trial the *Streptococci* numbers were decreased at the last time point in the hydrogenation group when compared to the control group (Figure 26). This result correlates with the better growth performance and may be an indicator for improved absorption efficiency in the upper digestive tract.

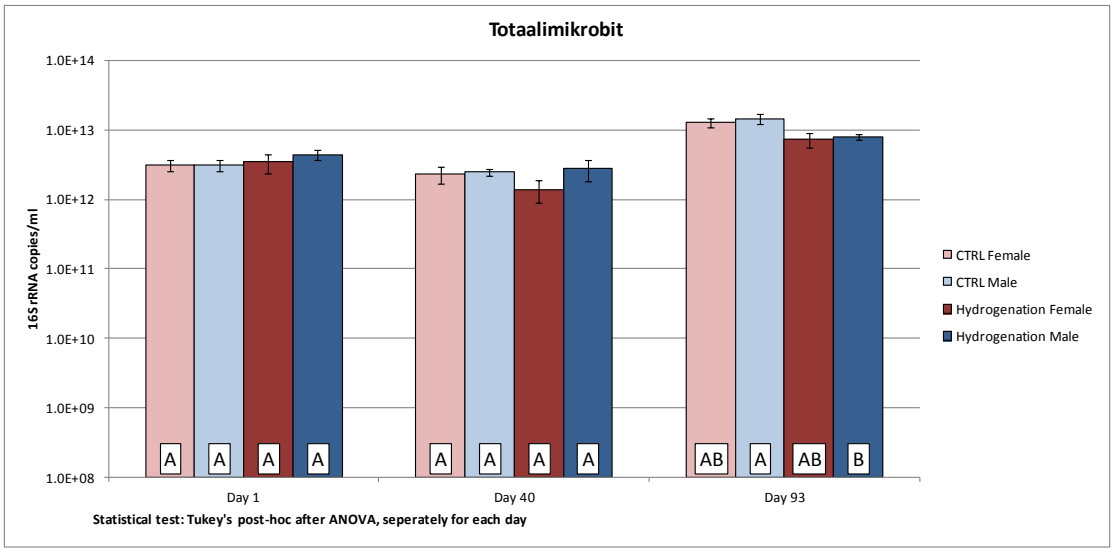

Figure 17. Total microbial numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

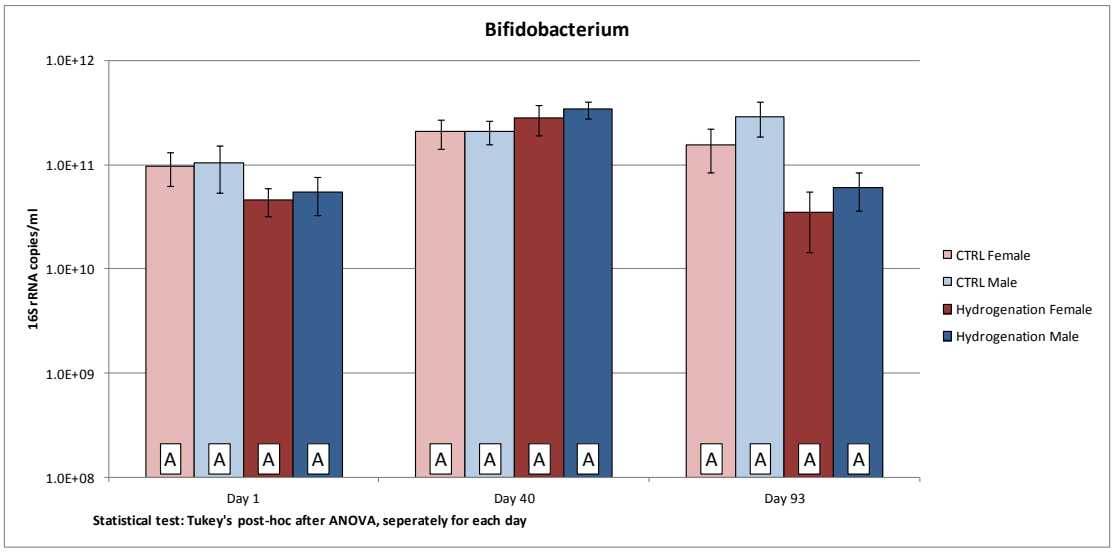

Figure 18. *Bifidobacteria* numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

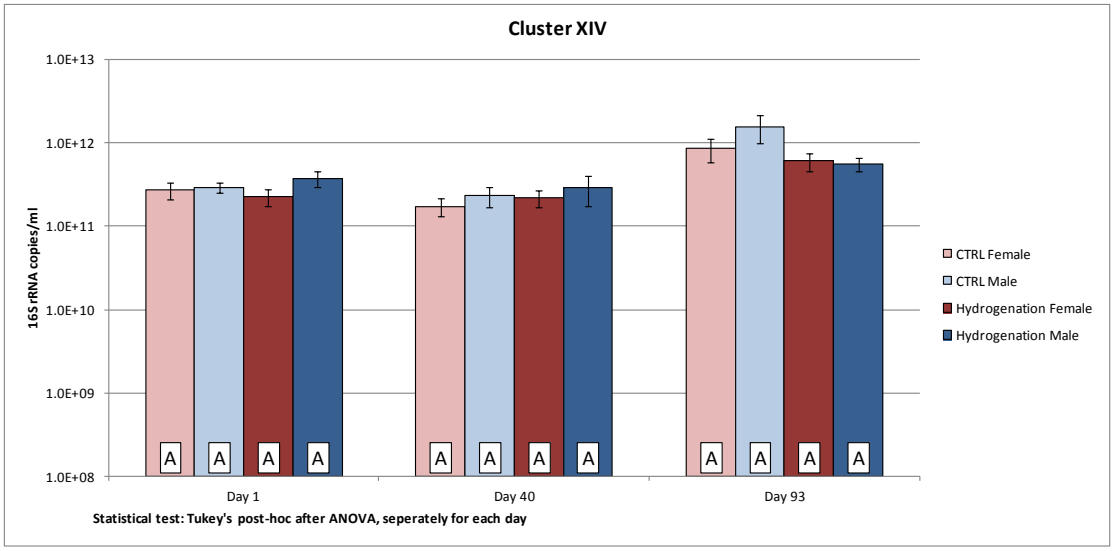

Figure 19. Clostridium cluster XIVa numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

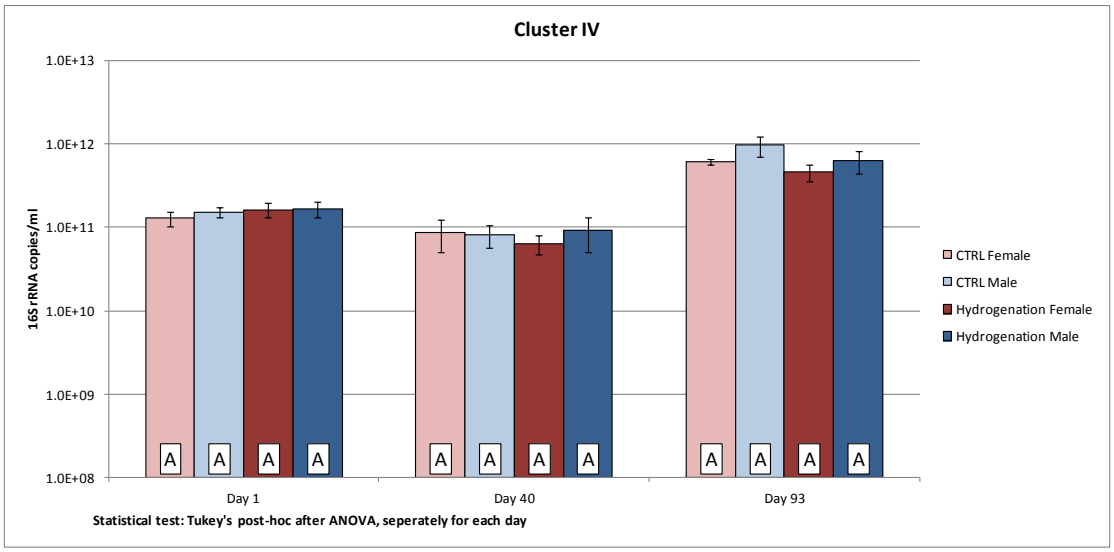

Figure 20. Clostridium cluster IV numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

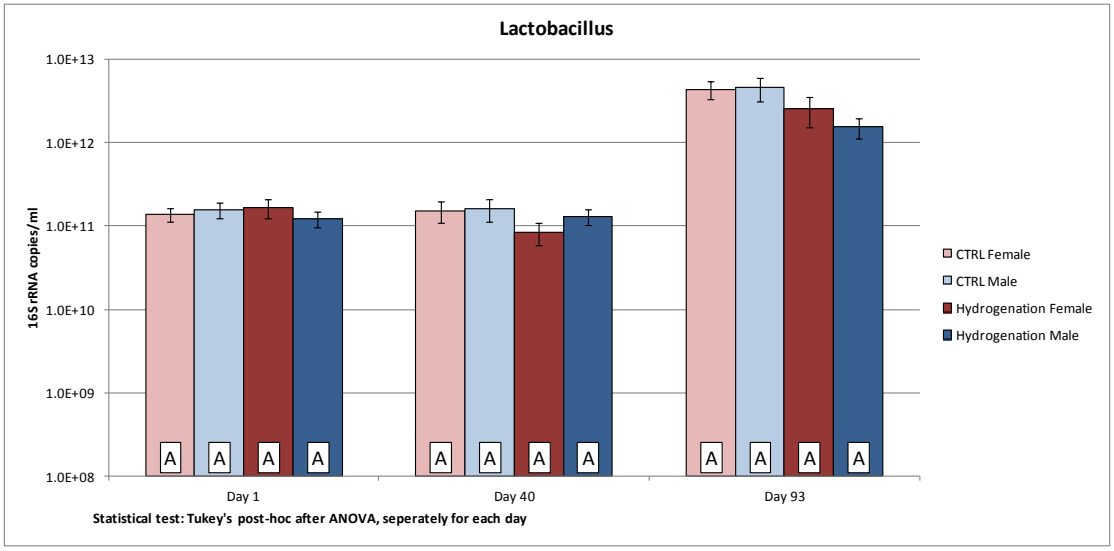

Figure 21. *Lactobacilli* numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

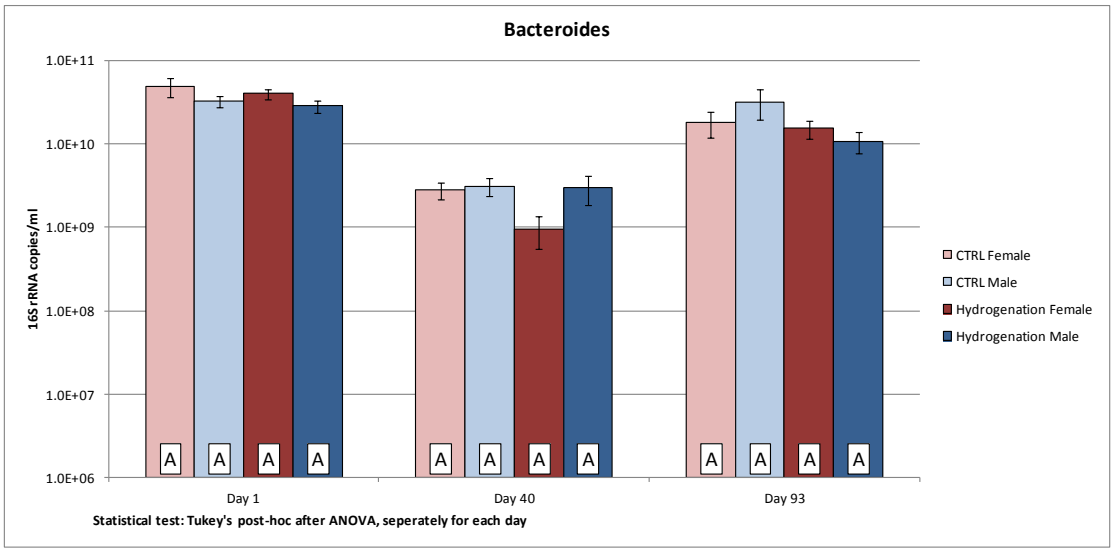

Figure 22. *Bacteroides* numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

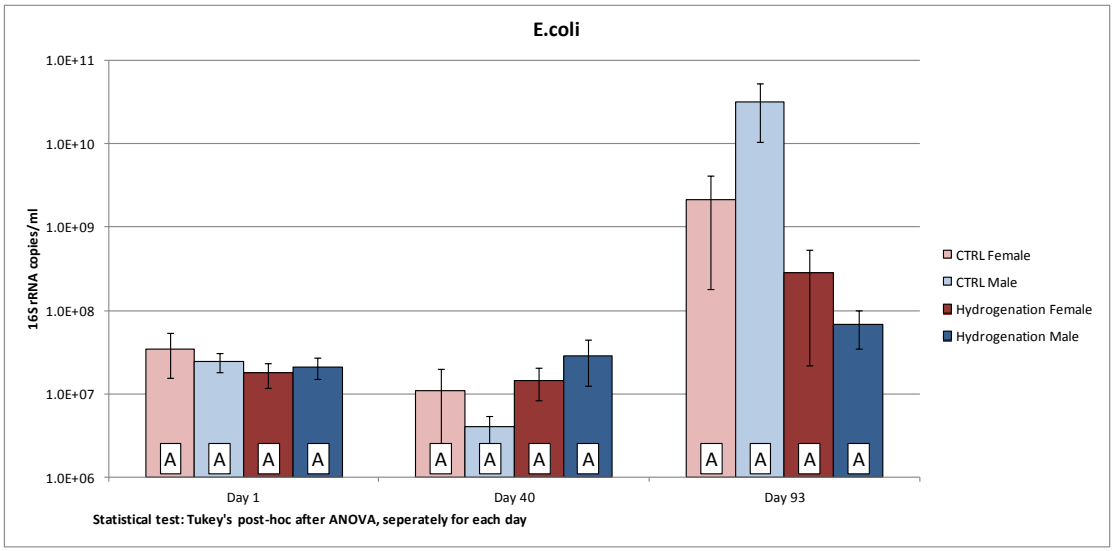

Figure 23. *E. coli* numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

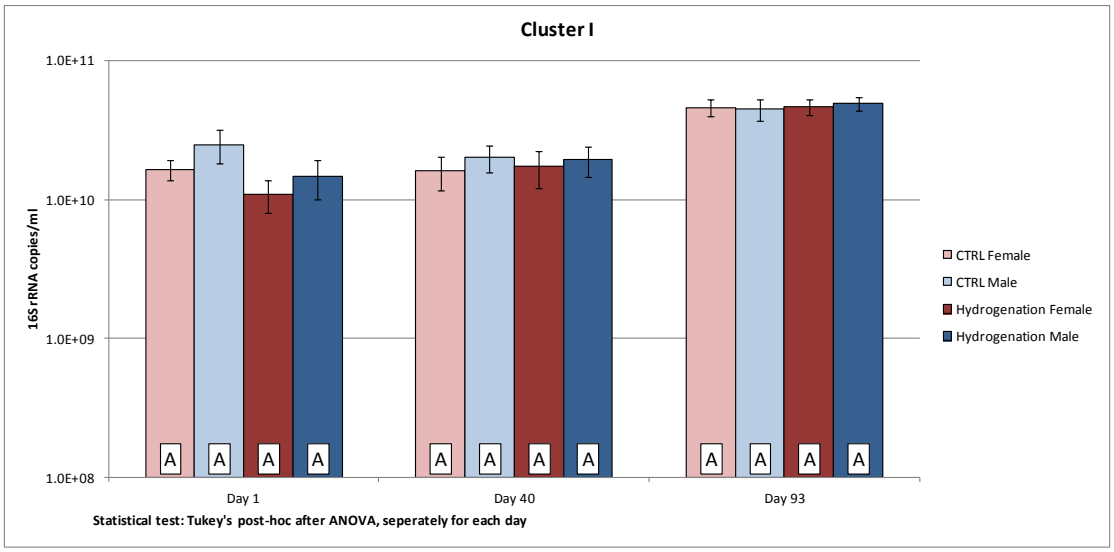

Figure 24. Clostridium cluster I numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

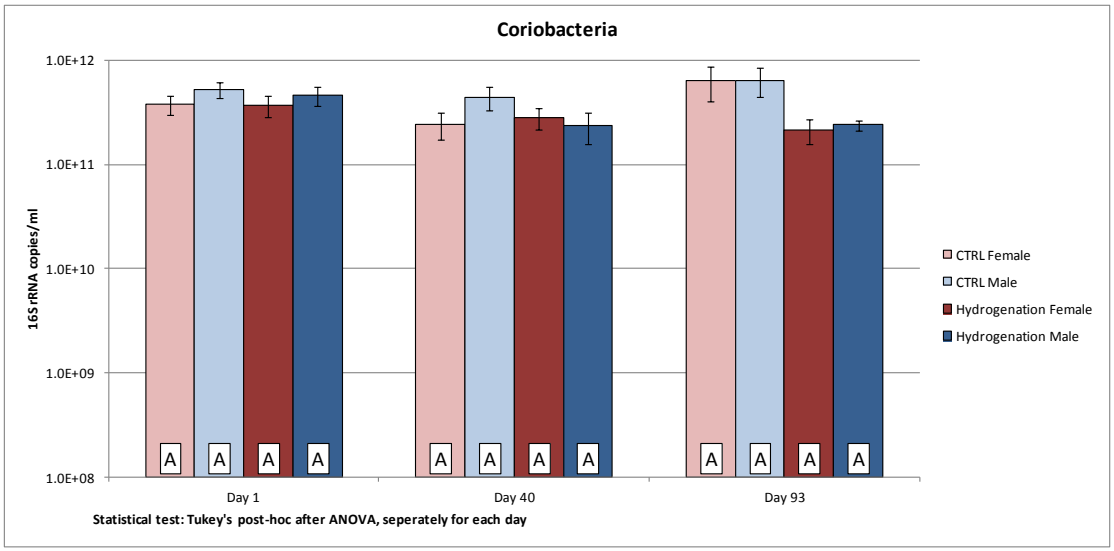

Figure 25. *Coriobacteriaceae* numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

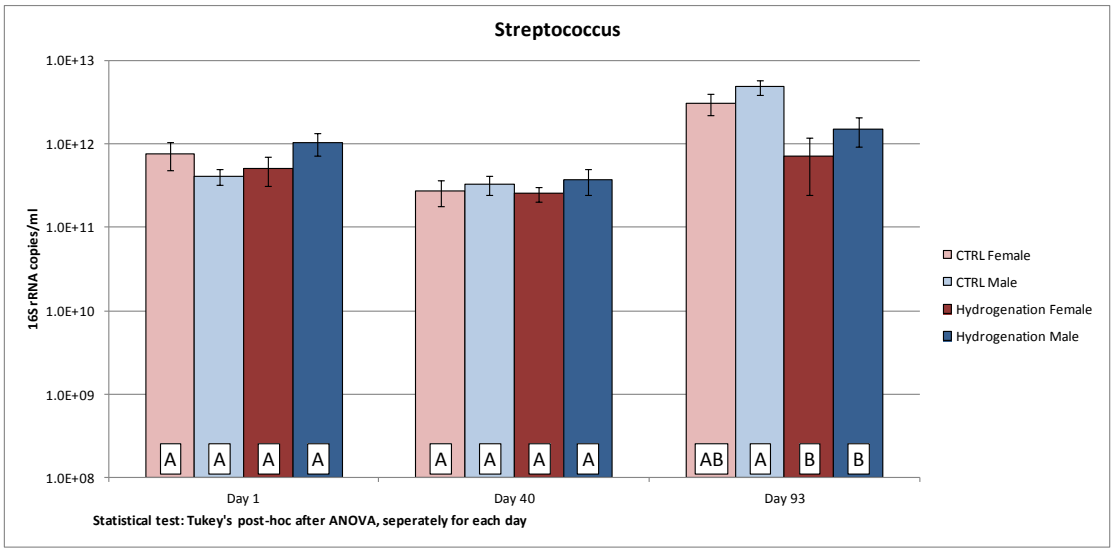

Figure 26. *Streptococcus* numbers in the faecal samples, grouped by treatment and sex. Statistical analysis: Tukey's post-hoc test after ANOVA.

5. Conclusions

Hydrogenation did not show a statistically significant effect on the concentration of ammonia in the faecal samples. However, a small difference in the concentration could have been covered by the large variation caused by other factors. E.g. the concentration of ammonia in the urine was not measured in this trial. Analysing the urine for ammonia would be recommended, since as much as 80% of ammonia is excreted through urine (Leek, et al., 2005). The hypothesis, that hydrogenation reduces the environmental burden, could therefore not be confirmed.

Hydrogenation group showed a statistically significant increase in the body weight gain; during the whole trial the daily weight gain was 130g/day larger in the hydrogenation group when compared to the control group. The average carcass weight in the hydrogenation group was 10 kg larger than in the control group (N=45). The average carcass weight of other pigs in the hydrogenation building was 5 kg larger than the carcass weight of pigs in the control building (N=563). At the same time, however, meat percentage of the carcass decreased. Additionally, mortality in the hydrogenation group was larger than in the control group or other pigs outside this trial.

Financial gain of hydrogenation is difficult to calculate due to the facts mentioned previously. Even though the carcass weight was significantly larger, half of the gain is lost because the meat percentage decreased. Additionally, when mortality is taken into account in the calculations, there was no statistically significant difference in the financial gain between hydrogenation and control groups. The effects to feed conversion ratio could not be assessed because the feed consumption was not measured during the trial.

In vitro spiking trial showed no effect of hydrogenation to the *E.coli* numbers. Therefore, the mode-of-action of hydrogenation is most likely not based on the inhibition of bacteria.

The big picture was that the deodorisation pipe (hydrogenation) showed positive effects on growth performance and gut microbiota of pigs (e.g. decrease in *Streptococci* numbers). However, definite proof supporting the function of the deodorisation pipe could not be stated based on this trial. The operation of the deodorisation pipe should be observed with test groups living in the same building, thus minimizing the effect of nuisance parameters. Young piglets, which are known to show quick response in performance parameters, would be the most prominent target group.

6. Bibliography

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Appendix 1.

- ANOVA tables

	Hydrogenation	*Sex*	*Date*
Ammonia	0.836	0.3263	0.1571
Total SCFAs	0.766	0.1529	0.3137
Acetic-%	0.2075	0.0234	0.6666
Propionic-%	0.0091	0.1881	0.8578
Butyric-%	0.1334	0.0424	0.0136
Branched-%	0.6895	0.4874	0.1745
Acetic:propionic	0.0142	0.0168	0.7146
Total microbes	< 0.0001	0.3995	< 0.0001
Bifidobacterium spp.	0.7442	0.2129	0.152
Clostridial Cluster IV	0.0077	0.2311	< 0.0001
Clostridial Cluster XIV	0.0099	0.2737	< 0.0001
Lactobacillus spp.	0.0003	0.608	< 0.0001
Echerichia coli	0.0523	0.2159	0.0127
Bacteroides spp.	0.0061	0.4507	0.1198
Clostridial Cluster I	0.4832	0.3521	<0.0001
Coriobacteriaceae	0.0008	0.3544	0.093
Streptococcus spp.	< 0.0001	0.1466	< 0.0001

ANOVA p-value with the assumption that hydrogenation has no effect at day 1. Hydrogenation and sex defined as categorical variables and time as a continuous variable.