THE EFFECTS OF EXERCISE AND ESTROGEN ON GUT MICROBIOTA IN FEMALE MICE

By

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Dr. Sara Campbell

And approved by

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ABSTRACT OF THE THESIS

The Effects of Exercise and Estrogen on Gut Microbiota in Female Mice

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Thesis Director:

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The gut microbiota has recently been acknowledged as having an impact on overall systemic health and obesity. To date, research has primarily focused on male mice due to the unknown effects of the menstrual cycle in females. Exercise is a known mediator of obesity related diseases, and the literature demonstrates an effect on the microbiome in males thus far. As post-menopausal obesity continues to rise, there is a need to explore the relationship between estrogen and the microbiome, with exercise as a possible moderator. In this study, female mice either had an ovariectomy, to simulate estrogen deficiency, or a sham procedure. Mice were placed into either a continuous exercise group, high intensity, or sedentary control group for six weeks. Microbial analysis was completed to view differences between groups. The estrogen deficient group had higher body weight and body fat percentages, regardless of exercise. Microbial analysis indicated a decrease in diversity in the estrogen deficient group, as well as a higher Firmicutes/Bacteriodetes ratio. These results are similar to obesity studies, suggesting that changes in the microbiome may be one mechanism that promotes obesity

in the estrogen deficient state. Exercise interventions increased microbial diversity, with a higher percent of Bacteroidetes compared to Firmicutes.

Keywords: *estrogen, female, microbiome, exercise, gut,*

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To Dr. Lee Kerkhof and Lora McGuinness, for your patience and assistance in microbial analysis. Thank you for teaching me different techniques and allowing me to work in your lab.

Dedication:

I would like to dedicate this thesis to my loving parents, Rod and Laurie Melvin. Thank you for always pushing me to do my best and encouraging me to pursue all of my dreams, no matter how big. Thank you for always feeding my constant need to learn.

Mom, thank you for being my person to talk to, for sending care packages, and pushing me to always look to the stars. Dad, thank you for teaching me that the world is full of adventures, and I should see as many as I can. Because of you both, I will be successfully moving on to my next adventure soon.

And to Austin, for keeping me sane during the duration of my Master's program, thank you. You will always be my rock.

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Introduction

Obesity was recognized as a disease in 2013 and affects more than two thirds of United States adults.[1,2] Widely considered a preventable cause of death, obesity related medical treatments resulted in an excess of $147 billion in health care expenditure.[1] Proposed mechanisms include energy imbalance, adipose tissue dysregulation, homeostatic imbalance, and hormonal imbalances, including estrogen.[2] Recent evidence suggests that the gut microbiome plays an integral role in the obesity relationship, with studies reporting a higher Firmicutes to Bacteriodetes ratio. [3,4,5] Additionally, evidence suggests that gut microbiota changes occur in response to diet, exercise, stress, and possibly estrogen levels.[3,4,5,6,7] To date, diet, exercise and stress have begun to be studied extensively, however, estrogen deficiency is a relatively unexplored area. Estrogen deficiency is a condition that occurs in postmenopausal women whom have stopped having a menstrual cycle. Menopause can occur in women between the ages of 45-58 and due to medical advancements, the average US woman is expected to live until the age of 81. This means women could spend approximately 30 years in an estrogen deficient state.[8] Exercise is a known mediator of obesity-related cardiometabolic disorders, and was found to mitigate increases in visceral adipose tissue and lower serum estrogens in postmenopausal women.[9] It is plausible to suggest then that exercise could help weight maintenance in the postmenopausal state. Due to lack of female populations within previous studies, microbiome gender differences remain unknown. The purpose of this study is to examine the relationship between estrogen levels, exercise interventions, and microbiome shifts in female mice.

Microbiome

The gut microbiome consists of the collective beneficial and pathogenic bacterial species in the digestive tract. These gut microbes are responsible for nutrient harvesting, immune modulating, and accessing nutrients that the body cannot normally harvest.[14] Currently, there is no consensus on a "normal healthy" gut microbiome profile, however, the most common conclusion is a balance between two major phyla, the Firmicutes and Bacteroidetes. [4,10,11] A higher F/B ratio has been positively correlated with obesity, while lower ratios correlate to healthier, leaner mice [4,10,12] To date, no clear cause and effect have been linked to obesity and microbiome. Some evidence suggests that high fat diets alter the F/B ratio and this may predispose animals to obesity phenotypes. [3,4] While others suggest that lysing of gram negative bacteria produce lipopolycharride, a pro-inflammatory molecule, and that this disrupts the intestinal barrier promoting systemic inflammation predisposing the animal to obesity.[11]

While majority of gut bacterial species have not been elucidated, research has uncovered a variety of physiological and environmental triggers which influence alterations in gut microbe abundances. Some of these include diet, exercise, environment, and hormonal influence. Evidence demonstrates a correlation between poor dietary pattern and decreased microbial ecology, found to be reversible and altered by caloric restriction or reduced fat diet.[13,14] Exercise has a protective role on cardiovascular function, weight maintenance, cognitive health, and microbial diversity, however mechanisms of this still remain unclear. Mice permitted voluntary exercise showed an increase in microbial diversity, compared to their sedentary counterparts.[6,7] Mice with exercise wheel access had lower body mass, lower blood glucose, reduced intestinal

inflammation, and increased microbial diversity. [6,7] Voluntary exercise has also been found to alter the intestinal short chain fatty acid profile, particularly butyrate, in rats.[15] Exercise may protect the integrity of the microbiome, even in the presence of a high fat diet or environmental toxins, such as PCBs. [6,7,42]

A large majority of studies to date exploring microbial composition, changes, and effectors have been conducted in male mice. Abundant differences in gender exist across all scientific literature, and we believe the microbiome is no exception. Conflicting evidence suggests that genders have no more than random expected difference in microbiome, while others suggest sex hormones and genetics greatly affect microbiota composition, whereas females have demonstrated species that were absent in males, including Roseburia, Blautia, Coprococcus 1, Parabacteroides, and Bilophilia [16,17] The effects seen in male mice cannot be directly applied to females at this time because of the difference in hormones and physiological pathways.

Estrogen Deficiency

Estrogen is a major regulator of bodily function, including development and maintenance of the reproductive system, adipose metabolism, and cardiovascular health. Menopause is the cessation of menses, defined by absence of a menstrual period for at least 12 months. This conclusion of menstruation results in less estrogen production, as ovaries become inactive, and the primary source of estrogen secretion becomes aromatization of androgens in adipose tissue.[18] Estrogen deficiency is involved in a variety of health concerns such as decreased collagen synthesis, endothelial dysfunction, decline in bone mineral content, increased blood pressure, loss of lean body mass, reduced glucose tolerance, and increase in both total and abdominal mass.[19,20] The shift in

body composition can lead to further health complications including higher risk for cancers, cardiovascular disorders, and joint issues. Excess adipose tissue is proposed to synthesize estrogen, through aromatization of androgens, although the physiological pathway is not fully understood.[21] In addition, obese women have been found to have higher levels of both estrogen and follicular stimulating hormone (FSH) than their lean counterparts.[18] These increased hormonal levels may in part be a result of excess adipose with androgens for aromatization.[18]

At this point, it is unclear why the lower circulating estrogen causes weight gain, as observed after menopause or with ovariectomy in animal models. Further research is needed to explore menopause linked weight gain, while proposed mechanisms include chronic inflammation, hormonal changes, and gut microbiota disruption. Systemic low grade inflammation is found to be associated with increased levels of circulating C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor (TNF) α, and leptin.[22] In obese women, the relationship between elevated levels of inflammation was correlated with fat amount.[22] Estrogen deficiency was found to cause insensitivity to central leptin administration, leading to increased food intake and suppressed spontaneous physical activity in female rats.[23] Another study found that leptin levels were correlated with body mass index in women of all ages, with women having higher leptin concentrations than men.[24] Obese females supplemented with estradiol benzoate had a significant reduction in obesity, hyperglycemia, and spontaneous food intake, suggesting protective effects of estrogen.[25]

Sex hormones have been found to influence microbial diversity, which inversely has an effect on metabolism and systemic hormone levels.[16,26,27] A possible mechanism

linking gut bacteria with estrogen status involves the estrogen receptor mediation, specifically estrogen receptor β (ER-B), of gut microbial composition.[28] A recent study found that gut ecology, as well as short chain fatty acid production, may mediate the protective effects of aerobic capacity on cardiometabolic risk in female rats.[29] It can be proposed that gut microbiota alterations mediated by estrogen causes phenotypic changes in postmenopausal females. While this preliminary research is promising, further research needs to be conducted to examine the receptor-microbiota connection.

Systemic health has an extremely complex nature and an almost limitless number of factors. As demonstrated, microbiome, diet, exercise, and environmental factors all influence obesity but exact mechanisms are yet to be understood. The majority of studies performed previously examined male mice or rats, and these studies cannot be directly translated to females or humans at this point. Due to the lack of female microbiome and exercise studies in the literature, there is a clear need for further preliminary research prior to human application. The purpose of this study is to explore the relationship between estrogen status, different exercise intensities, and microbiome shifts in female mice. Our hypotheses are that: (1) estrogen deficient groups will have decreased microbial diversity and (2) exercise will promote enhanced microbial diversity despite estrogen deficiency.

Methods

Animals, Diets, and Exercise

Female C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were exercised in a six week protocol previously described.[30] In summary, mice were assigned to either ovariectomy (OVX) to induce estrogen deficiency or sham-operation (SHAM) and placed

into sedentary, continuous moderate intensity, or high intensity interval exercise groups (Exer3/6, Columbus Instruments, Columbus, OH). The high intensity exercise group performed 30 minutes bouts of 30 seconds sprints followed by 60 seconds walking. The continuous exercise group performed 30 minutes bouts of distance and duration matched exercise, at 13.8 m/min. Body weight, spontaneous physical activity, and food intake were measured weekly. Mice had ad libitum access to water and a Labdiet 5K52 diet (Purina Mills, Richmond, IN). All mice were sacrificed after the completion of six weeks of exercise, during the diestrus phase, confirmed by vaginal smears.

Gut Microbial Community Analysis

Feces were extracted from the cage of each animal, immediately snap frozen, and stored at -80 degrees until analysis. Fecal analysis was completed using an aquaRNA protocol (AquaRNA^{TM} Kit)**.** One million cells were harvested and centrifuged at 2000xg for 2 minutes. Excess supernatant was aspirated and discharged, leaving the cell pellet. Two hundred µL AquaGenomic Solution was added to a 1.5µL tube which was vortexed vigorously for 30-60 seconds, then incubated at room temperature for four minutes. Sample was again vortexed vigorously for 30-60 seconds and centrifuged at 21,000xg for two minutes to pellet the debris. 200µL isopropanol was added and contents were centrifuged for 15 minutes at 21,000xg in -4 degrees to pellet the DNA. Supernatant was transferred to a new 0.5mL tube and 70% ethanol wash was performed as the tube was centrifuged for 5 minutes at 21,000xg. All liquid was removed and DNA pellet was resuspended in 50µL PCR H_2O. Presence of DNA was verified using photo imaging of agarose gel, post ethidium bromide bath, containing 5 µL DNA and lambda for DNA

reference. PCR amplification was completed using GoTach at 25 cycles and verified again by agarose gel photo imager.

TRFLP fingerprints were generated using a protocol previously described.[31] In summary equal amounts of PCR product (20ng) were digested with *Mnl* I for 6 hours at 37˚C degrees. For analysis, digests were precipitated and re-suspended with 0.3µl of ROX 500 Standard in 19.7 µl deionized formamide. Samples were then analyzed on an ABI 310 Genetic analyzer using Genescan software for peak detection and quantification (Applied Biosystems, Foster City, CA).[31]

Genomic DNA was pooled from sample extracts by group (n=1/group) and sent for 454' pyrosequencing of bacterial community to Mr. DNA (16S rRNA gene sequencing; approx. 3,000 reads/sample, MR. DNA Labs, Shallowater, TX). Barcoded amplican sequencing process (bTEFAP©) was performed by a method previously described.[32] In summary, subunit rRNA sequences were generated using a Roche 454 titanium instrument according to manufacturer's guidelines. Chimeras, barcodes, and primers were removed, data was denoised, and sequences >200bp were removed. OTUs were clustered and defined with a set parameter of 97% similarity (3% divergence). OTUs were taxonomically classified against a GreenGenes, RDPII and NCBI database by BLASTn, compiled by both counts and percentages.[33,34,35]

Results

Uterine Weights

OVX vs SHAM uterine weights were dramatically reduced (20 vs 80 mg, main effect of surgery, $p<0.0001$) indicating success of the surgery and estrogen deficiency.

Body composition and food intake

Body weight was significantly higher in OVX compared to SHAM overall, and at each week 0-6 throughout the training ($p<0.001$) (Figure 1). Body fat percentage was elevated in OVX overall, and significantly at weeks 4-6 ($p<0.01$) (Figure 2). Weekly food intake was lower in OVX vs SHAM when normalized to bodyweight and FFM, but no differences were expressed in overall intake (Figure 3). Results from analysis of food intake indicated that there were no main effects of training and no interactions between factors (Data not shown).

Fecal Analysis

Fecal analysis via TRFLP showed minimal differences between conditions (Data not shown). When comparing merely SHAM vs OVX, levels showed differences in peak OTUs between the two estrogen statuses. Heat maps of TRFLP data reveal differences in peak presence (Data not shown). Bray Curtis and Sorenson similarity indexes, at exclusion cut offs of .2% and 1.0%, show grouping between mice who underwent sham versus those who underwent ovariectomy (Figure 4,5). Sorenson index uses a simple presence or absence comparison, while Bray Curtis similarity index identifies the quantitative compositional differences between populations.[36,37] This evidence suggested justification for deep sequencing as further analysis.

Upon pooling samples per group, 454' pyrosequencing identified bacterial phyla and species. At the phyla level, the OVX group showed high percentages of Firmicutes, with reduced Bacteriodetes, and minimal levels of other phyla including candidatus saccharibacteria, actinobacteria, verricomicrobia, proteobacteria and tenericutes. The OVX CE group specifically had higher presence of Tenericutes and Proteobacteria, 3.63% and 4.26%, respectively. SHAM groups collectively had a reduction of Firmicutes,

comparatively, and increased Bacteriodetes, with all other phyla comprising <2% of the community, including Tenericutes, Cyanobacteria, Candidatus Saccharibacteria, Verrucomicrobia, and Actinobacteria. Species level analysis was also completed and heat map shows distinct differences between each group (Figure 13). Attached figures show the bacterial composition of each group (Figures 7-12). *Barnesiella spp*. was found in all groups, at very different proportions. In OVX CON and HI samples, *Barnesiella spp.* comprised approximately 35% of the community. The OVX CE group had extremely decreased presence at 3.81%. All SHAM groups had high proportions of *Barnesiella spp.* ranging between 55-67%. *Helicobacter hepaticus*, comprised all 4.26% of the OVX CE group's Proteobacteria. *Akkermansia muciniphilia* was found in only the SHAM CON group. *Clostridium spp*. was present in highest quantities in the OVX CON, CE, and HI groups making up 32, 28, and 38% of the communities respectively. Clostridium spp. was present in all SHAM groups, creating between 11-13% of the microbial community. found in each group, but in less quantities in all SHAM groups and the OVX CE group. *Eubacterium spp.* was present in each group, highest concentration in OVX CE of 13%, and consistent concentrations in all SHAM groups(1-3%). Various strains of *prevotella* and *alistipes* were found throughout all six groups.

Discussion/Further Research

The purpose of our study was to explore the relationship between estrogen deficiency, exercise, and microbial alterations in the gut of female mice. Upon completion of ovariectomy or sham procedure, uterine weight differences showed the efficacy of the surgical procedure. Following the six week protocol, body weights and body fat indicate that OVX mice stored more fat than their SHAM counterparts. (Figure

1, 2) These findings support the literature that suggests estrogen deficiency is related to excess adipose tissue, contributing to obesity.[25] Microbial composition in the gut strongly demonstrated the differences in the OVX vs SHAM group. The relative shift of higher Firmicutes/Bacteriodetes ratio in estrogen deficient mice with higher body fat is consistent with the body of literature,suggesting that microbial shifts may influence the phenotypic shifts in postmenopausal women. .[3,10,12]

Proteobacteria were only found in the OVX CE group, due to one specific bacteria. The presence of *helicobacter hepaticus*, known to be related to liver and colon cancer in mice, in the OVX CE may suggest that one or more of the mice in the group was sick, seeing as it was found at 0% in all other groups.[38] It is also plausible that exercise was not beneficial to this mouse, or group of mice, which resulted in growth of this species. Being the samples were pooled, there is no way to see which mouse was sick, however, nothing in the literature suggests *H. hepaticus* could be brought on by the exercise condition or treatment group. Even with this harmful bacteria present, the CE group showed increased diversity and had a slight protective effect on F/B ratio. It is unclear at this time what effect the presence of *Helicobacter hepaticus* had on the rest of the community.

Tenericutes were found in higher proportions in the OVX CE, SHAM CE, and SHAM HI, suggesting an effect of exercise, especially in the continuous exercise groups. Verrucomicrobioais present in more significant amounts only the SHAM CON and HI groups, specific species being *akkermansia muciniphilia*. Minimal amounts were seen in OVX CON, OVX HI, and SHAM CE. A. muciniphilia is a known mucin degrader in the colon and it has recently been identified to be correlated with positive systemic

health.[39,40] Higher presence in the SHAM groups suggests a protective effect of estrogen, and deficiency is correlated with minimal amounts.

The two most significant species across all groups include Barnesiella spp and Clostridium sp. Changes in Barnesiella spp. are notable between groups. SHAM groups contain more than 50% Barnesiella spp, which is suggested to be a beneficial bacteria as recently, it has been found to suppress antibiotic-resistant bacteria in the human gut.[41]. Our findings suggest that estrogen deficiency reduced population of this helpful bacteria. In addition, Clostridium spp. was a dominant species found in all groups. The species comprised approximately 1/3 of the community of the OVX CON and OVX HI groups while this was reduced by 22% in the OVX CE group. All SHAM groups had reduced concentration, between 10-13% of the community, demonstrating an effect of estrogen. Clostridium spp. is a gram-positive bacteria, responsible for a large portion of the Firmicutes major phyla changes in these mice, possibly implicated with negative health implications in higher amounts. [3,10,12]

These distinct microbial shifts suggest the gut microbiota acts as a mediator to a phenotypic shift in post-menopausal women. Exercise did not have as profound of an effect as previously seen in the literature, however, it did increase microbial diversity correlated with positive systemic health. Fecal samples were collected from the cages of each animal, depending on the time in between collection some species may have had the opportunity to grow or die. A more accurate collection method may be collecting feces during sacrifice straight out of the colon, giving us a better look at what exists inside of the intestines. In the literature, there is still no standard of healthy microbiota and it is possible that a healthy microbiota is dependent on individual factors. Various strains of

different bacteria may have a different effect on immunity, health, and body composition and this should be explored in future research. While this preliminary research demonstrates an influence of both exercise and estrogen, this complex relationship needs to be further explored in hopes for postmenopausal human application.

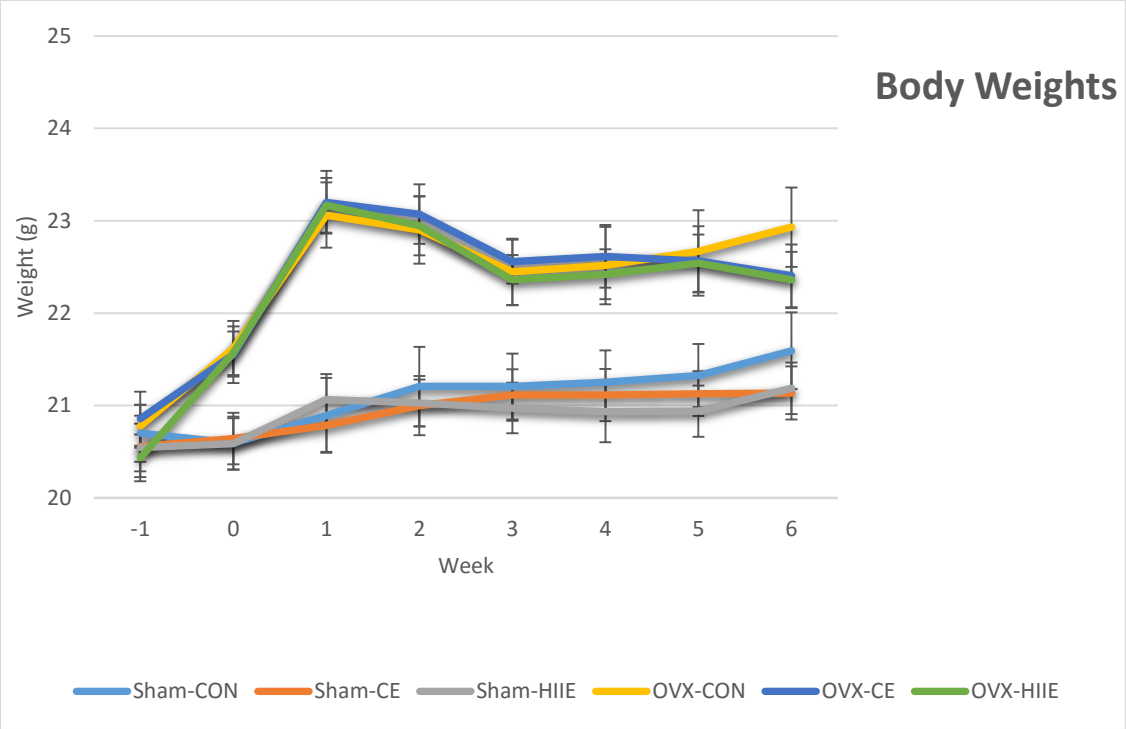


Figure 1.

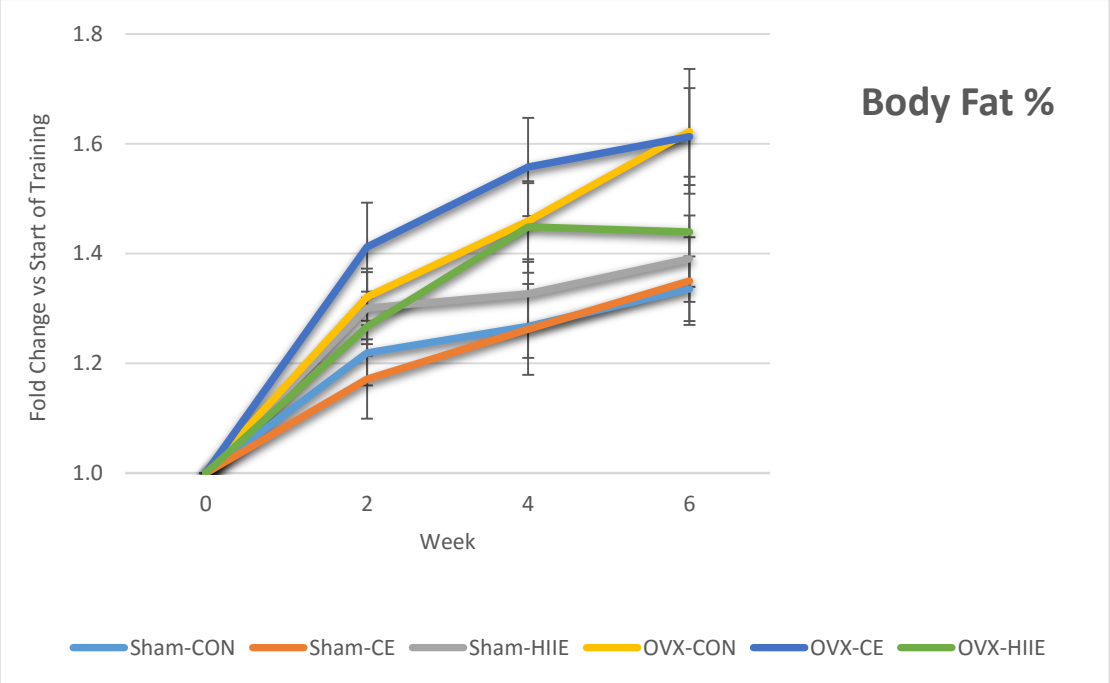


Figure 2.

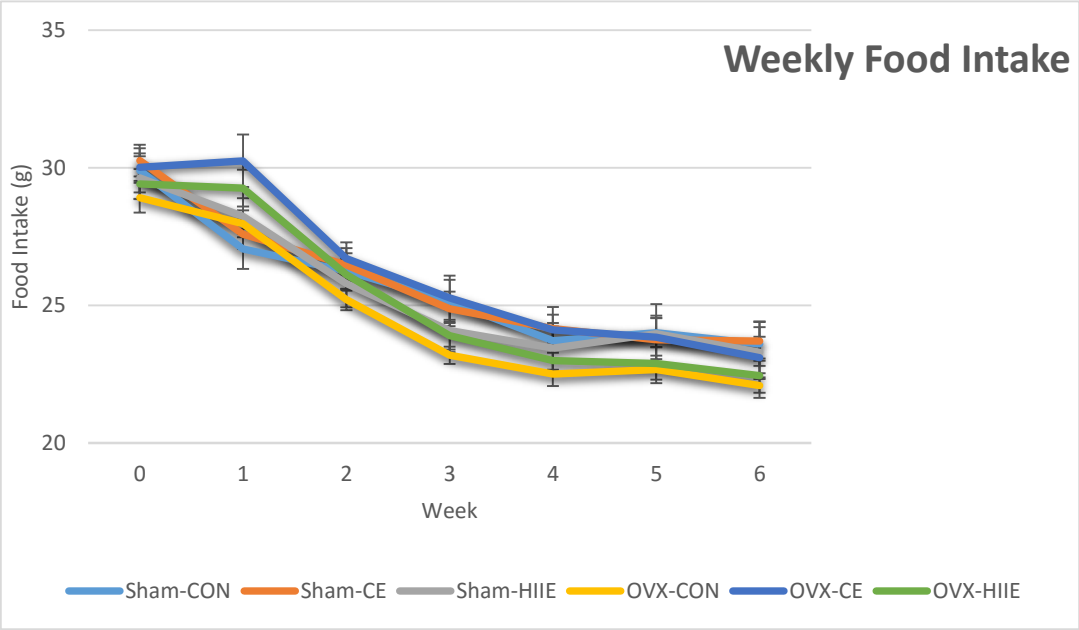

Weekly Food Intake
Food Intake (g)
35
30
25
20
0
1
2
3
4
5
6
Week
Sham-CON
Sham-CE
Sham-HIIE
OVX-CON
OVX-CE
OVX-HIIE

Figure 3.

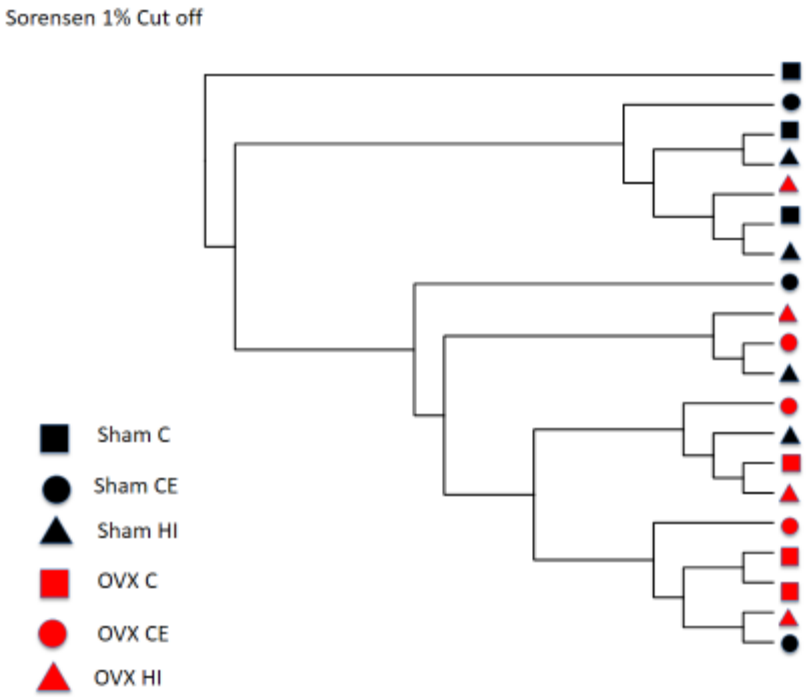


Figure 4.

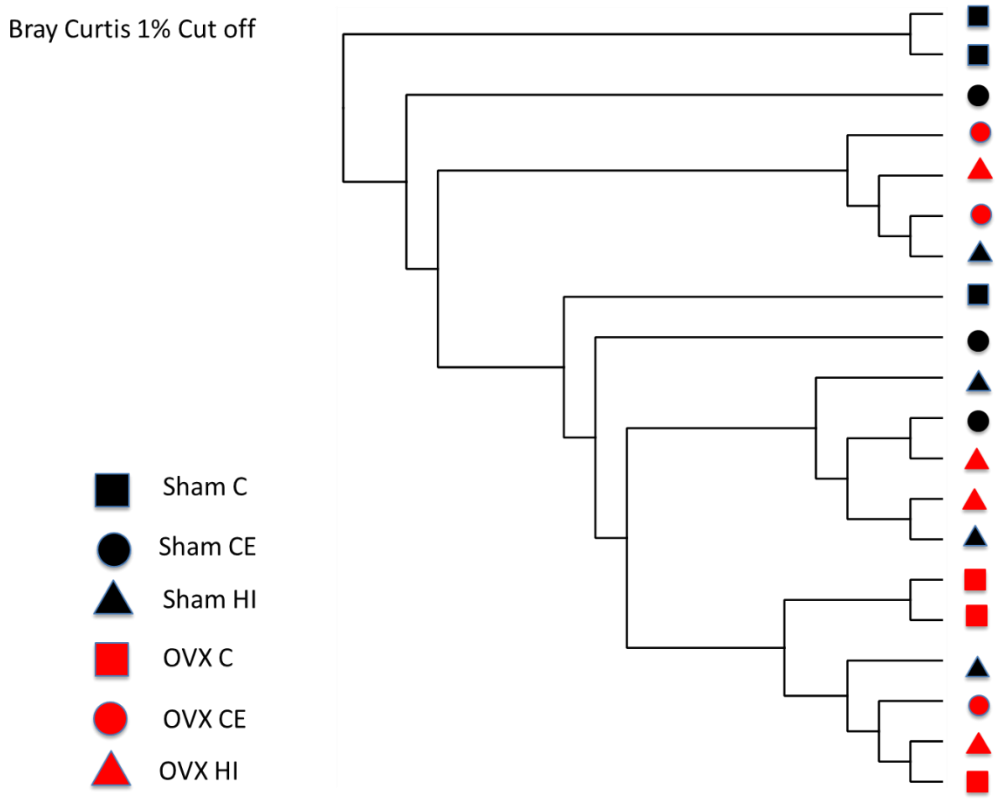


Figure 5.

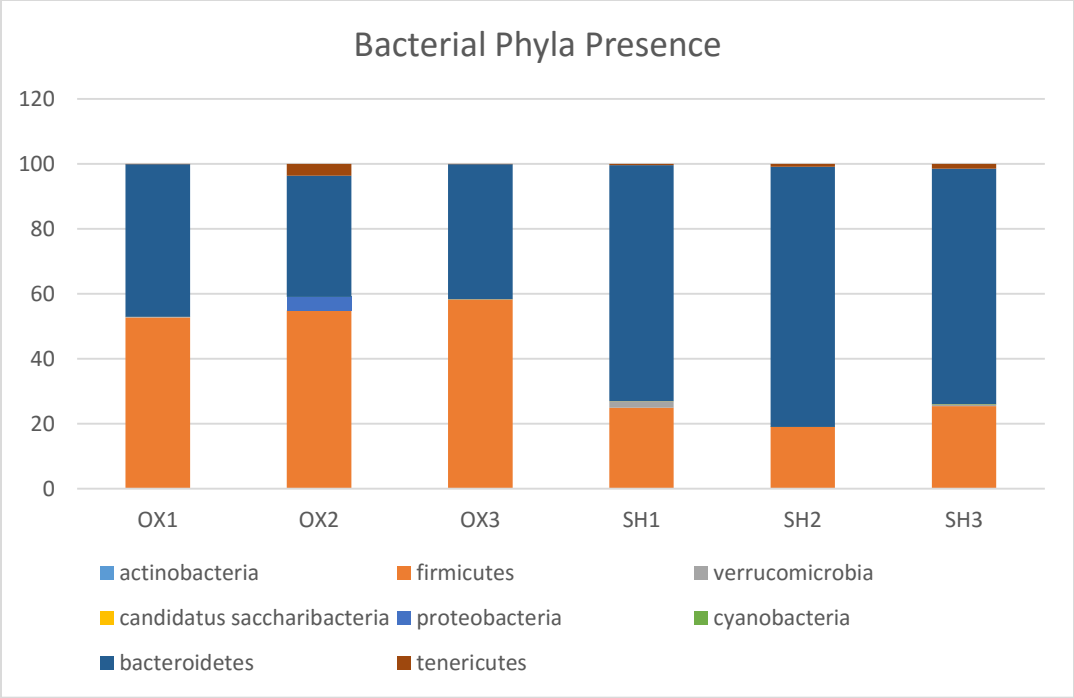

Bacterial Phyla Presence
120
100
80
60
40
20
0
OX1
OX2
OX3
SH1
SH2
SH3
actinobacteria
firmicutes
verrucomicrobia
candidatus saccharibacteria
proteobacteria
cyanobacteria
bacteroidetes
tenericutes

Figure 6.

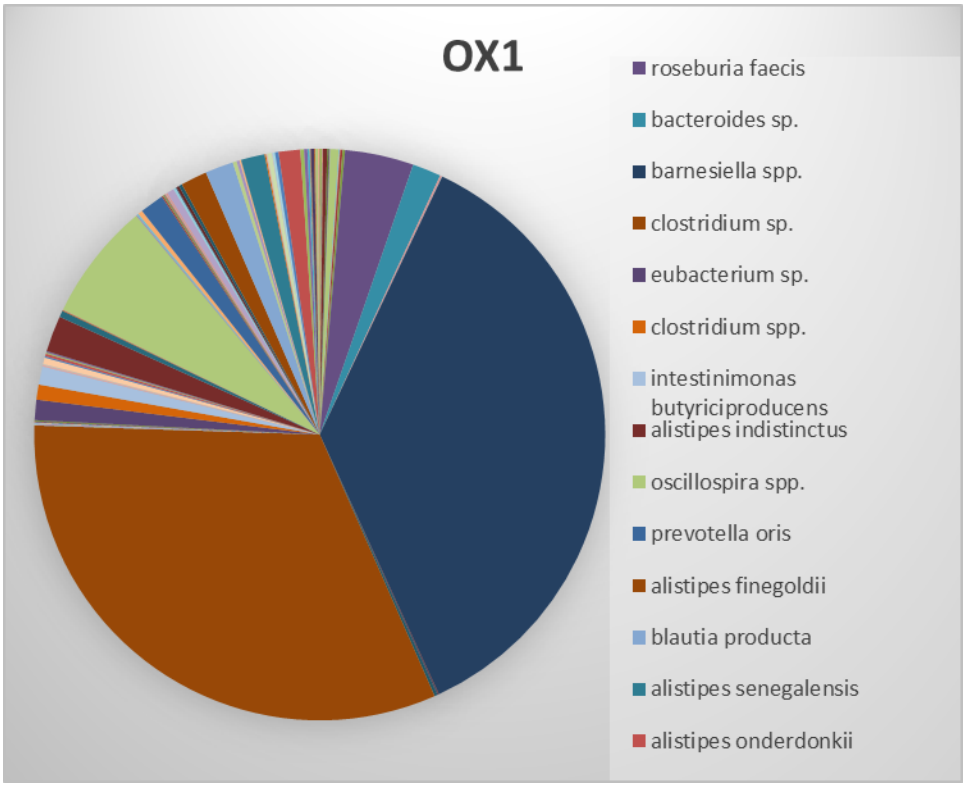


Figure 7.

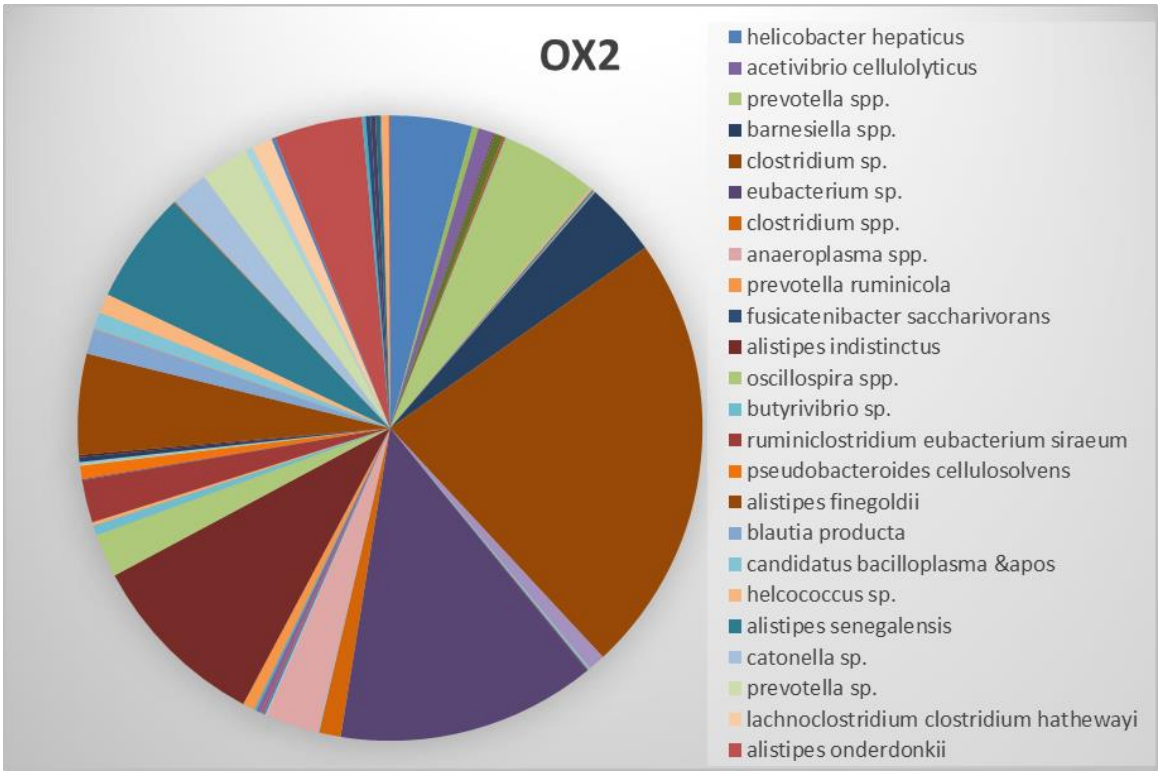


Figure 8.

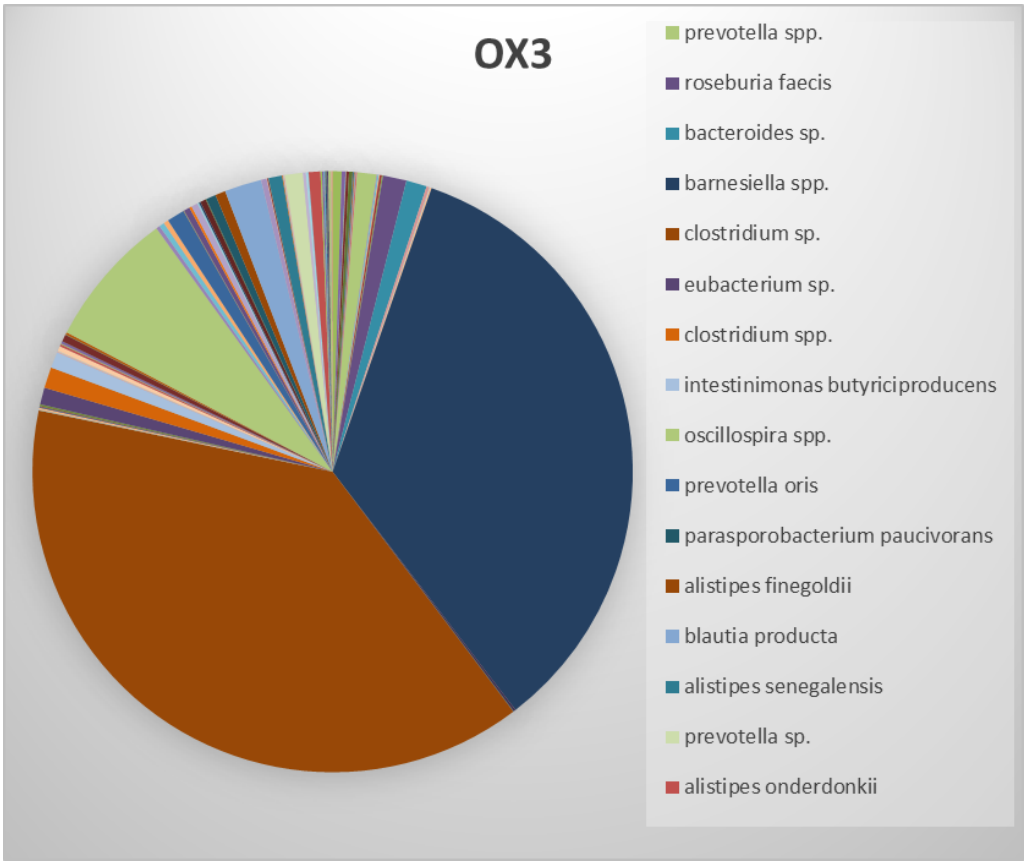

OX3
prevotella spp.
roseburia faecis
bacteroides sp.
barnesiella spp.
clostridium sp.
eubacterium sp.
clostridium spp.
intestinimonas butyriciproducens
oscillospira spp.
prevotella oris
parasporobacterium paucivorans
alistipes finegoldii
blautia producta
alistipes senegalensis
prevotella sp.
alistipes onderdonkii

Figure 9.

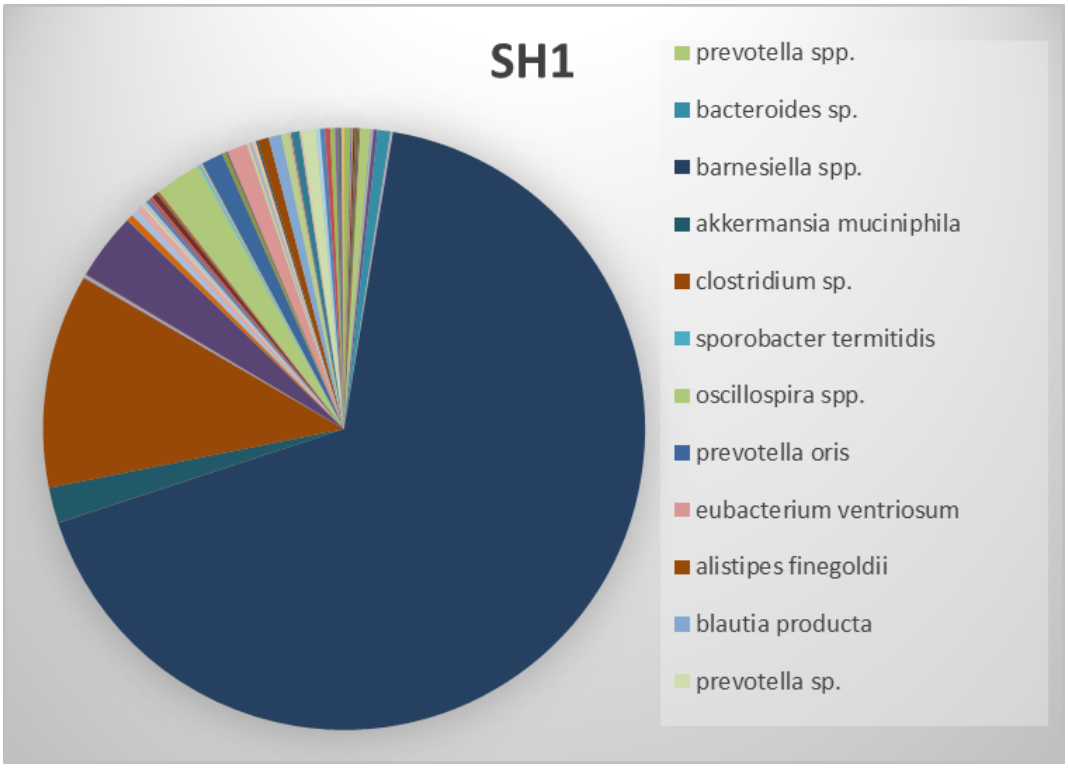


Figure 10.

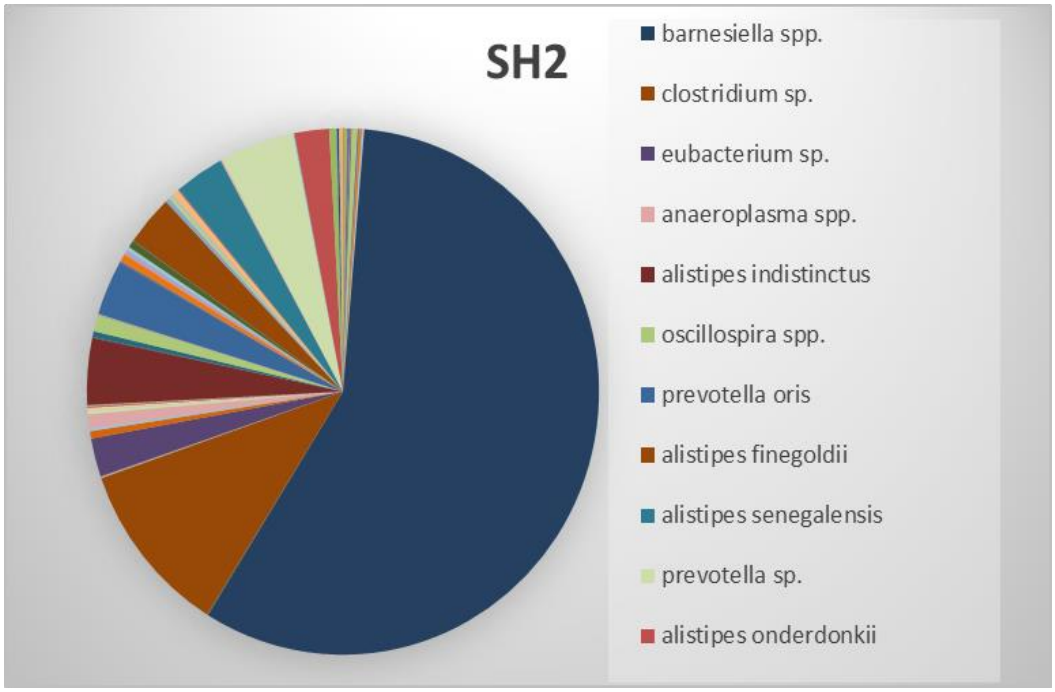


Figure 11.

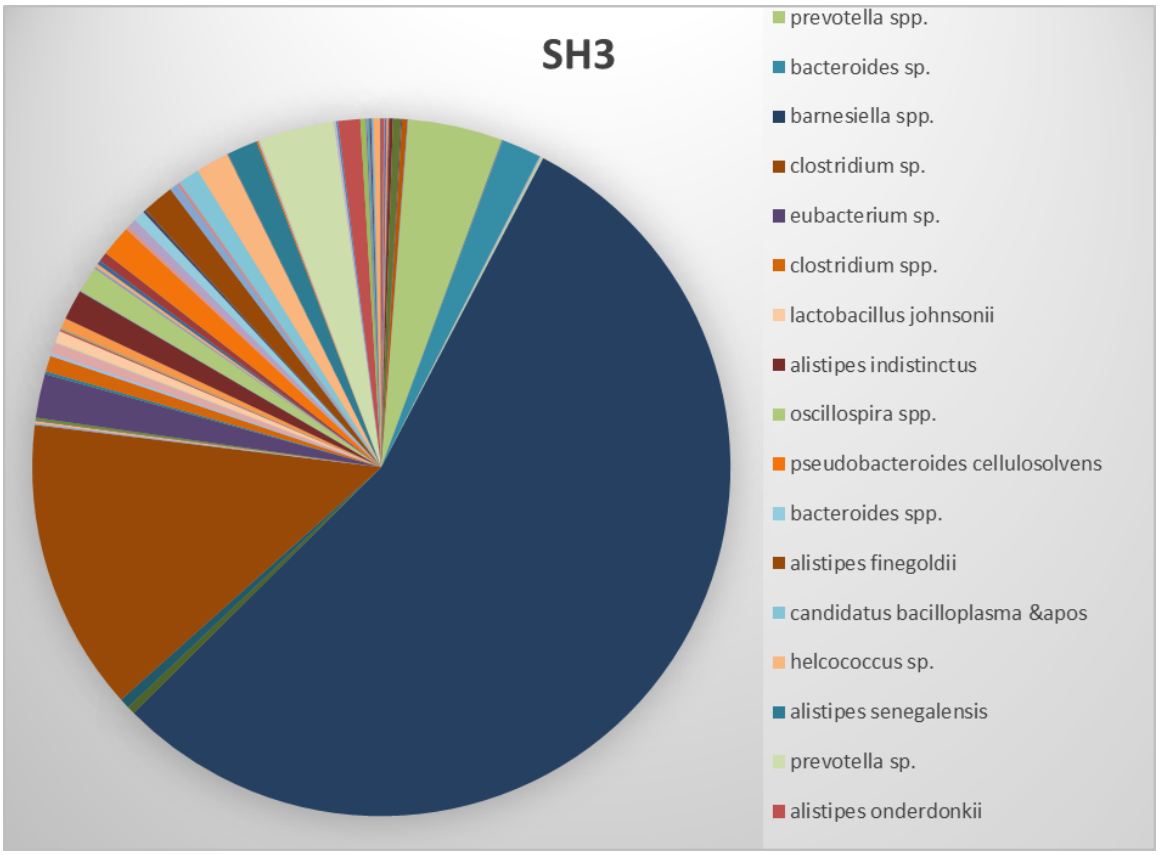
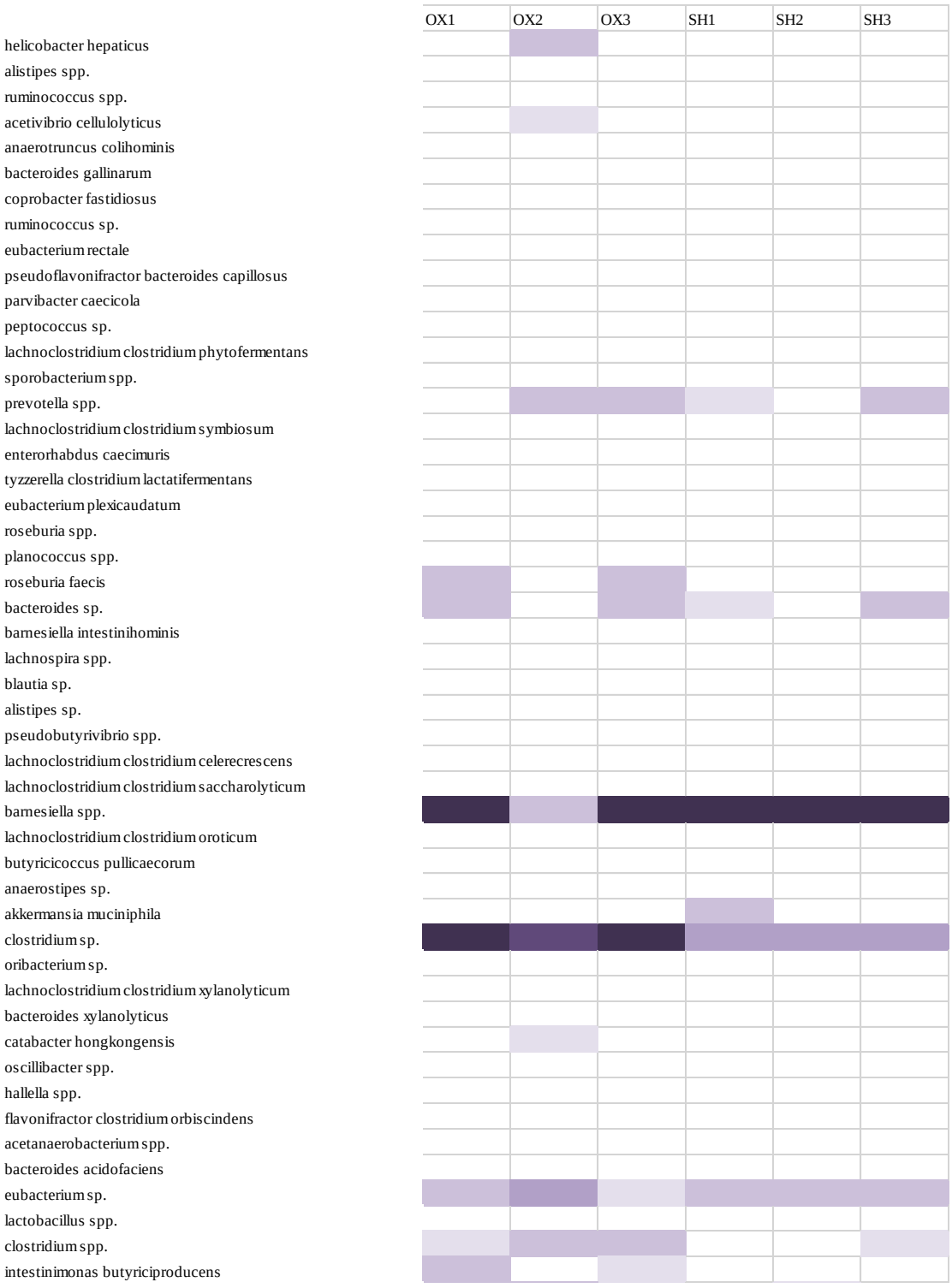
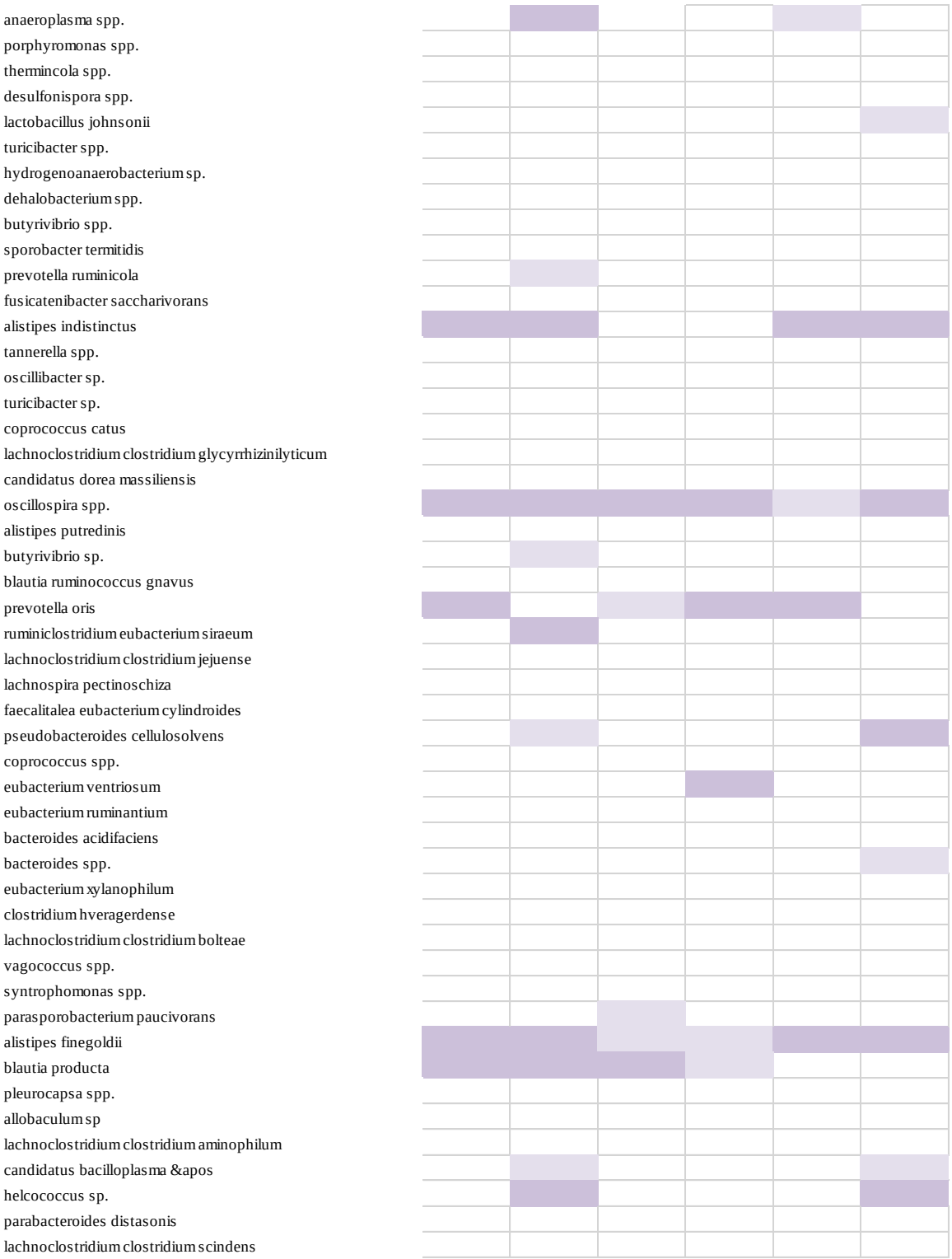

SH3
prevotella spp.
bacteroides sp.
barnesiella spp.
clostridium sp.
eubacterium sp.
clostridium spp.
lactobacillus johnsonii
alistipes indistinctus
oscillospira spp.
pseudobacteroides cellulosolvens
bacteroides spp.
alistipes finegoldii
candidatus bacilloplasma &apos
helcococcus sp.
alistipes senegalensis
prevotella sp.
alistipes onderdonkii

Figure 12.


OX1
OX2
OX3
SH1
SH2
SH3
helicobacter hepaticus
alistipes spp.
ruminococcus spp.
acetivibrio cellulolyticus
anaerotruncus colihominis
bacteroides gallinarum
coprobacter fastidiosus
ruminococcus sp.
eubacterium rectale
pseudoflavonifractor bacteroides capillosus
parvibacter caecicola
peptococcus sp.
lachnoclostridium clostridium phytofermentans
sporobacterium spp.
prevotella spp.
lachnoclostridium clostridium symbiosum
enterorhabdus caecimuris
tyzzerella clostridium lactatifermentans
eubacterium plexicaudatum
roseburia spp.
planococcus spp.
roseburia faecis
bacteroides sp.
barnesiella intestinihominis
lachnospira spp.
blautia sp.
alistipes sp.
pseudobutyrivibrio spp.
lachnoclostridium clostridium celerecrescens
lachnoclostridium clostridium saccharolyticum
barnesiella spp.
lachnoclostridium clostridium oroticum
butyricicoccus pullicaecorum
anaerostipes sp.
akkermansia muciniphila
clostridium sp.
oribacterium sp.
lachnoclostridium clostridium xylanolyticum
bacteroides xylanolyticus
catabacter hongkongensis
oscillibacter spp.
hallella spp.
flavonifractor clostridium orbiscindens
acetanaerobacterium spp.
bacteroides acidofaciens
eubacterium sp.
lactobacillus spp.
clostridium spp.
intestinimonas butyriciproducens
0-0.5 %
0.51-1.0%
1.01-10.0%
10.01-20.0%
20.01-30.0%
>30.01


anaeroplasma spp.
porphyromonas spp.
thermincola spp.
desulfonispora spp.
lactobacillus johnsonii
turicibacter spp.
hydrogenoanaerobacterium sp.
dehalobacterium spp.
butyrivibrio spp.
sporobacter termitidis
prevotella ruminicola
fusicatenibacter saccharivorans
alistipes indistinctus
tannerella spp.
oscillibacter sp.
turicibacter sp.
coprococcus catus
lachnoclostridium clostridium glycyrrhizinilyticum
candidatus dorea massiliensis
oscillospira spp.
alistipes putredinis
butyrivibrio sp.
blautia ruminococcus gnavus
prevotella oris
ruminiclostridium eubacterium siraeum
lachnoclostridium clostridium jejuense
lachnospira pectinoschiza
faecalitalea eubacterium cylindroides
pseudobacteroides cellulosolvens
coprococcus spp.
eubacterium ventriosum
eubacterium ruminantium
bacteroides acidifaciens
bacteroides spp.
eubacterium xylanophilum
clostridium hveragerdense
lachnoclostridium clostridium bolteae
vagococcus spp.
syntrophomonas spp.
parasporobacterium paucivorans
alistipes finegoldii
blautia producta
pleurocapsa spp.
allobaculum sp
lachnoclostridium clostridium aminophilum
candidatus bacilloplasma &apos
helcococcus sp.
parabacteroides distasonis
lachnoclostridium clostridium scindens

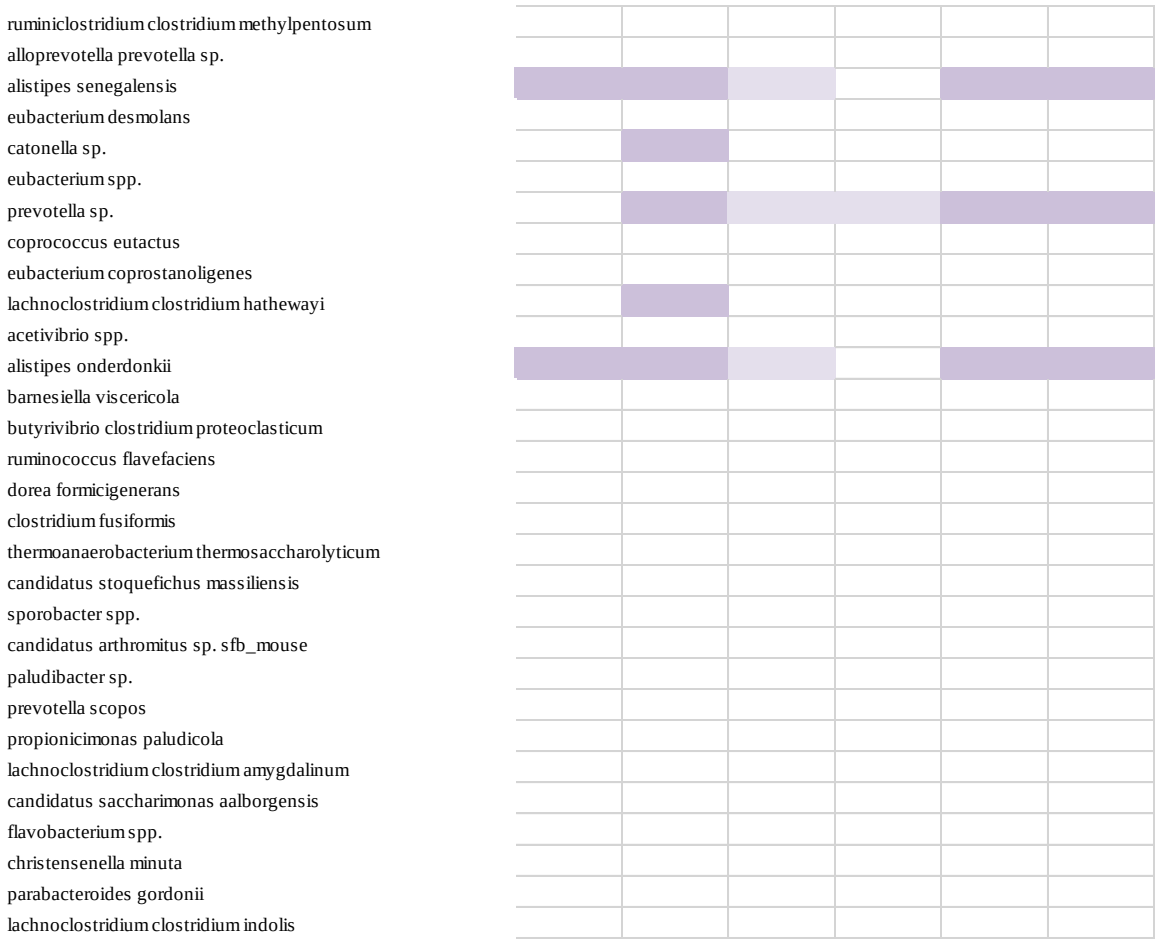

ruminiclostridium clostridium methylpentosum
alloprevotella prevotella sp.
alistipes senegalensis
eubacterium desmolans
catonella sp.
eubacterium spp.
prevotella sp.
coprococcus eutactus
eubacterium coprostanoligenes
lachnoclostridium clostridium hathewayi
acetivibrio spp.
alistipes onderdonkii
barnesiella viscericola
butyrivibrio clostridium proteoclasticum
ruminococcus flavefaciens
dorea formicigenerans
clostridium fusiformis
thermoanaerobacterium thermosaccharolyticum
candidatus stoquefichus massiliensis
sporobacter spp.
candidatus arthromitus sp. sfb_mouse
paludibacter sp.
prevotella scopos
propionicimonas paludicola
lachnoclostridium clostridium amygdalinum
candidatus saccharimonas aalborgensis
flavobacterium spp.
christensenella minuta
parabacteroides gordonii
lachnoclostridium clostridium indolis

Figure 13.

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