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Effects of Maternal Swine Appeasing Substance on Growth Performance of Nursery Pigs

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Summary

Two experiments using a total of 640 weanling pigs were conducted to determine the effects of a maternal swine appeasing substance (FerAppease, FERA Diagnostics and Biologicals, College Station, TX) on growth performance, salivary cortisol concentration, and fecal microbiome of nursery pigs. In exp. 1, a total of 360 weanling pigs (DNA 600 × 241; initially 12.6 lb) were used in a 24-d study with five pigs per pen. Due to the potential contact of pigs in adjacent pens transferring the test product, pens were grouped by treatment within the barn and groups were distributed in the barn to minimize this risk. In exp. 1, there were two blocks of pens for each treatment (four total blocks of pens), with each block having 18 pens. In exp. 2, a total of 280 weanling pigs (DNA 600 × 241; initially 12.1 lb) were used in a 45-d study with five pigs per pen in 56 pens split in two rooms. Treatment groups were located in blocks of seven pens to avoid contact between treatment groups, and each treatment was assigned to four blocks (eight blocks total). Block of like treatments was considered the experimental unit for analysis of growth performance data. In both experiments, pens were assigned either a control (placebo of mineral oil) or the appeasing substance (FerAppease, FERA diagnostics and Biologicals, College Station TX). Applications of 3 mL/pig were done at d 0 (weaning) and d 10 by spraying the substance on the forehead of each individual pig. To limit any cross-contamination, either empty pens or the walkway separated the groups to physically segregate treatments from each other. All pigs were fed the same two-phase diet regimens: from d 0 to 10 and d 11 to 24, with phase 3 added in exp. 2 from d 25 to 45. In phase 1 (d 0 to 10, Table 2), control and FerAppease pigs had similar ($P > 0.05$) ADG, ADFI, and F/G, as well as BW at d 10. Similar results were observed in phase 2 with no evidence of a difference observed between treatments ($P > 0.05$). For the overall period, when combining phases 1 and 2 (both experiments) and 1, 2 and 3 (only in exp. 2), no differences ($P > 0.05$) between the two treatments were found for ADG, ADFI, F/G, and BW at d 24 and d 45. Salivary cortisol was measured on d 1 post-weaning and again on d 11 (both 24 h after product administration). There was no evidence ($P > 0.10$) of a treatment × day interaction or main effect

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of treatment. Salivary cortisol was greater ($P < 0.0001$) on d 1 post-weaning compared to d 11 post-weaning. Numerically, on d 1 post-weaning pigs applied with the FerAppease had reduced salivary cortisol concentrations; however, there were no statistically significant differences observed. Fecal samples were collected from two pigs per pen on d 0 and 10, and fecal microbiome analysis did not result in any differences between treatments for alpha or beta diversity, and no meaningful differences were observed in evaluation of class and genus relative abundance data. In summary, the post-weaning application of maternal pheromone to the forehead of nursery pigs post-weaning did not result in any improvements in feed intake, growth rate, feed efficiency, or fecal microbial composition.

Introduction

Weaning induces stress in piglets due to significant environmental changes. Besides the change from milk to solid feed, the shift involves moving from the familiarity of litter mates and maternal presence to encountering unfamiliar odors and the presence of other weaned piglets in the post-weaning pen. The initial days at the nursery play a crucial role, as the weight changes immediately after weaning influence later performance.[4] Maternal Pheromone (MP) is a semiochemical emitted by a female toward her offspring, and as pigs are weaned from their dam and moved to the nursery or wean-finish facility, this represents another significant change. Research using synthetic maternal pheromones has demonstrated positive outcomes in reducing aggression and skin lesions in weaned pigs.[5] A recent study introduced MP to lactating sow enrichment tassels (strips of material for sows to play with peri-farrowing), revealing a decrease in piglet mortality when MP was present around the time of farrowing.[6] A relatively new, commercially available product called FerAppease is based on semiochemicals designed to calm the pig and reduce stress. However, little data is available to know its impact on newly weaned pigs. Thus, the objective of this study was to evaluate the effects of a maternal swine appeasing substance applied around the time of weaning and 10 d post-weaning on growth performance, salivary cortisol concentration, and fecal microbial profiles of nursery pigs.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen (4 × 4 ft) was equipped with a 4-hole dry self-feeder, and a nipple waterer to provide ad libitum access to feed and water.

[4] Faccin, J. E. G., F. Laskoski, H. S. Cemin, A. P. G. Mellagi, M. L. Bernardi, R. R. Ulguim, F. P. Bortolozzo, and M. D. Tokach. 2020. Evaluating the impact of weaning weight and growth rate during the first week post weaning on overall nursery performance. J Swine Health Prod. 28(2):70-78. doi:10.54846/jshap/1138.

[5] Guy, J., S. Burns, J. Barker, and S. Edwards. 2009. Reducing post-mixing aggression and skin lesions in weaned pigs by application of a synthetic maternal pheromone. Anim Welf. 18:249–55. doi:10.1017/S096272860000049X.

[6] Sundman, E. R., N. K. Gabler, S. T. Millman, K. J. Stalder, L. A. Karriker, and A. K. Johnson. 2022. The use of attractants to stimulate neonatal piglet interest in rope enrichment. Animals. 12:211. doi:10.3390/ani12020211.

Animals and treatments

Two experiments were conducted to determine the effects of a maternal swine appeasing substance on growth performance, salivary cortisol concentration, and fecal microbiome of nursery pigs. In exp. 1, a total of 360 weanling pigs (DNA 600 × 241; initially 12.6 lb) were used in a 24-d study with five pigs per pen in 72 pens. Pigs were randomly assigned to pens and four groups of 18 pens next to each other were assigned to the same experimental treatment. Either empty pens or the corridor separated the groups of 18 pens on each side of the rooms to physically segregate treatments from each other. This pattern was reversed on the other side of the walkway. Block of like treatments was considered the experimental unit with two replicates per treatment).

In exp. 2, a total of 280 weanling pigs (DNA 600 × 241; initially 12.1 lb) were used in a 45-d study with five pigs per pen in 56 pens split in two rooms. Pigs were randomly assigned to pens and groups of seven pens next to each other were assigned to the same experimental treatment. An empty pen separating the groups of seven pens on each side of the rooms served to segregate treatments from each other. This pattern was reversed on the other side of the walkway, and the entire layout was flipped in the second room. Thus, there were four blocks of 14 pens with two blocks/room. Block of like treatments was considered the experimental unit with four replicates per treatment).

Pigs were applied with one of two treatments: control (placebo of mineral oil) or an appeasing substance (FerAppease, FERA diagnostics and Biologicals, College Station TX), both applied at 3 ml/pig treatment. Applications were done at d 0 (weaning) and d 10 by spraying the substance on the forehead of each individual pig. To limit cross contamination between FerAppease and control, during any procedures that require handling of pigs (applying placebo, applying FerAppease, weighing pigs, collecting saliva samples, and fecal samples), activities were completed by first working with the control pigs. Additionally, a different drench applicator was used for each of the treatment groups. In both experiments, all pigs were fed the same two-phase diet regimen: from d 0 to 10 and d 11 to 24, with a phase 3 diet fed from d 25 to 45 in exp. 2 (Table 1). Individual pig weights and feed disappearance were measured on days: 0, 10, 17, and 24 in both experiments, and d 31, 38, and 45 in exp. 2 to calculate ADFI, ADG, and F/G.

Salivary cortisol samples were collected from two pigs per pen at 24 h post-weaning and on d 11 post-weaning. Samples were collected using a sterile polyester-tipped applicator that was swabbed around the inside of the mouth to collect saliva. The swab was then placed into a sterile polystyrene tube with a cap and sealed. Following collection, all saliva samples were frozen at -4 °F until analysis. Following collection of the d 11 samples, salivary cortisol was measured using a commercially available enzyme immunoassay kit following manufacturer recommendations (Salimetrics, State College, PA).

Fecal samples were collected on d 0 and 10 post-weaning to characterize fecal microbial populations using 16S analysis. Samples were collected from the same two pigs per pen as salivary sample collection. Following collection, fecal samples were frozen at -4 °F within 2 h of sample collection for each collection. After 3 d in the -4 °F freezer, fecal samples were transferred to a -112 °F freezer until processed for 16S metagenomics analysis.

16S Analysis

Amplicons of the V4 region of 16s rRNA were subjected to single end Illumina sequencing to produce 300 base reads (Illumina MiSeq, version 2.5.36.11, San Diego, CA). The 5' primer was trimmed during initial processing. These partially trimmed reads (approximately 6.6 million) were imported into QIIME2 (version 2021.8) for analysis. Sequences were truncated at 250 bases to remove 3' primer and adapter sequences and then denoised with DADA2 to correct sequencing errors and produce a set of representative sequences. The representative sequences were aligned using qiime alignment mafft and the alignment was masked with qiime alignment mask. The masked alignment was used as input for qiime phylogeny fasttree to construct a phylogenetic tree, which was then rooted using qiime phylogeny midpoint-root. Representative sequences were classified using a trained Naïve-Bayes classifier based on the 99% Greengenes reference database.

Two samples with less than 500 reads each were excluded from analysis. Barplots of representative sequences by sample were created using the qiime taxa barplot function. Alpha and beta diversity indices were calculated using the qiime diversity core-metrics_phylogenetic function. Statistical significance of alpha and beta diversity indices between groups was assessed with pairwise Kruskal-Wallis and PERMANOVA tests, respectively. Classified representative sequence taxa were collapsed to specific taxonomic levels before differential abundance calculation with ANCOM using the qiime composition ancom function. All results were visualized using QIIME2 View.

Statistical analysis

Data from the two experiments were combined and analyzed as a completely randomized design. Group of like pens based on location in the barn (block) was considered the experimental unit and block within experiment was used as random effect to properly specify the level of experimental replication. Treatment was included in the model as a fixed effect. Growth performance data were analyzed using the lme4 package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria). Salivary cortisol data were analyzed using the GLIMMIX procedure of SAS (version 9.4, Cary, NC). Treatment, day, and the associated interaction were considered fixed effects, pen was included in the model as a random intercept to account for two salivary cortisol measurements per pen on each sampling day, and data were analyzed using repeated measures using an unstructured covariance matrix. All results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

In phase 1 (d 0 to 10, Table 2), control and FerAppease pigs had similar ($P > 0.05$) ADG, ADFI, and F/G as well as BW at d 10. Similar results were found in phase 2 with no evidence of a difference between treatments. For the overall period, when combining phases 1 and 2 (both experiments) and 1, 2 and 3 (only in exp. 2), no differences ($P > 0.05$) were found for ADG, ADFI, F/G, and BW at d 24 and d 45.

For salivary cortisol, there was no evidence ($P > 0.10$) of a treatment × day interaction or main effect of treatment. Salivary cortisol was greater ($P < 0.0001$) on d 1 post-weaning compared to d 11 post-weaning. Numerically on d 1 post-weaning, pigs provided the FerAppease had reduced salivary cortisol concentrations; however, there were no statistically significant differences observed ($P > 0.05$).

Fecal microbiome analysis did not result in any observable differences between treatments for alpha (Figure 2) or beta (Figure 3) diversity, and no meaningful differences were observed in evaluation of class and genus relative abundance data (Figure 4).

In conclusion, the post-weaning nasal application of maternal pheromone did not result in any improvements in feed intake, growth rate, feed efficiency, or fecal microbial composition up to 10 d post-weaning.

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Table 1. Composition of the diets (as-fed basis)

	Diet Phase		
Item	**Phase 1**	**Phase 2**	**Phase 3**
Ingredients, %			
Corn	43.0	58.4	67.4
Soybean meal, 46% CP	17.5	21.8	28.2
Fish meal	4.5	-	-
Whey powder	25.0	10.0	-
Enzymatically treated soybean meal[1]	5.0	5.0	-
Soybean oil	2.0	0.50	0.50
Calcium carbonate	0.30	0.65	0.75
Monocalcium P, 21% P	0.50	1.2	0.90
Salt	0.30	0.55	0.60
L-Lys-HCl	0.41	0.55	0.55
DL-Met	0.21	0.22	0.21
L-Thr	0.18	0.23	0.23
L-Trp	0.04	0.05	0.05
L-Val	0.13	0.16	0.16
Trace mineral premix	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
Choline chloride, 60%	0.05	-	
Phytase[2]	0.08	0.08	0.08
Zinc oxide	0.39	0.25	-
Total	100	100	100

continued

Table 1. Composition of the diets (as-fed basis)

	Diet Phase		
Item	**Phase 1**	**Phase 2**	**Phase 3**
Calculated analysis			
SID AA, %			
Lys	1.40	1.35	1.30
Ile:Lys	57	54	53
Leu:Lys	111	110	112
Met:Lys	37	36	36
Met and Cys:Lys	57	56	57
Trp:Lys	64	63	63
Thr:Lys	19.5	19.3	19.3
Val:Lys	70	69	70
NE, kcal/lb	1,178	1,125	1,120
Ca, %	0.73	0.72	0.65
STTD P, %	0.61	0.56	0.45
Zn, ppm	2,918	1,910	110
CP, %	21.2	20.1	20.0

[1] HP 300 (Hamlet Protein, Findlay, OH).
[2] Ronozyme 2700 (DSM Nutritional Products, Inc, Parsippany, NJ) provided an assumed 0.13% release of STTD P with 567 FTU/lb in the final diet.

Table 2. Effects of the application of a maternal appeasing substance on nursery pig performance[1]

	Treatments			
Item	Control	FerAppease	SEM	*P* =
BW, lb				
d 0	12.3	12.4	0.27	0.596
d 10	15.4	15.5	0.38	0.917
d 24	28.5	28.6	0.76	0.755
d 45[2]	57.3	56.4	0.55	0.292
d 0 to 10				
ADG, lb	0.35	0.35	0.050	0.936
ADFI, lb	0.40	0.42	0.031	0.253
F/G	1.18	1.24	0.091	0.276
d 11 to 24				
ADG, lb	0.87	0.87	0.039	0.864
ADFI, lb	1.25	1.24	0.021	0.796
F/G	1.45	1.43	0.077	0.592
d 0 to 24				
ADG, lb	0.67	0.67	0.019	0.819
ADFI, lb	0.93	0.93	0.054	0.897
F/G	1.39	1.38	0.048	0.692
d 0 to 45[2]				
ADG, lb	1.00	0.98	0.011	0.291
ADFI, lb	1.44	1.45	0.021	0.883
F/G	1.44	1.47	0.014	0.152

[1]A total of 640 pigs were used in two experiments with five pigs/pen. Due to the potential contact of pigs in adjacent pens transferring the test product, pens were grouped by treatment within the barn, and groups were distributed in the barn to minimize this risk. Treatment block was considered the experimental unit. In exp. 1, there were a total of four blocks (18 pens per block), with two blocks per treatment. In exp. 2, there were a total of eight blocks (seven pens per block), with four blocks per treatment. Pigs were applied a 3 mL/pig application of either a control (placebo of mineral oil) or an appeasing substance (FerAppease, FERA diagnostics and Biologicals, College Station TX) on the forehead at d 0 (weaning) and d 10. Exp. 1 was a 24 d study post-weaning, while exp. 2 was a 45-d study post-weaning.

[2]Data accounting only for exp. 2.

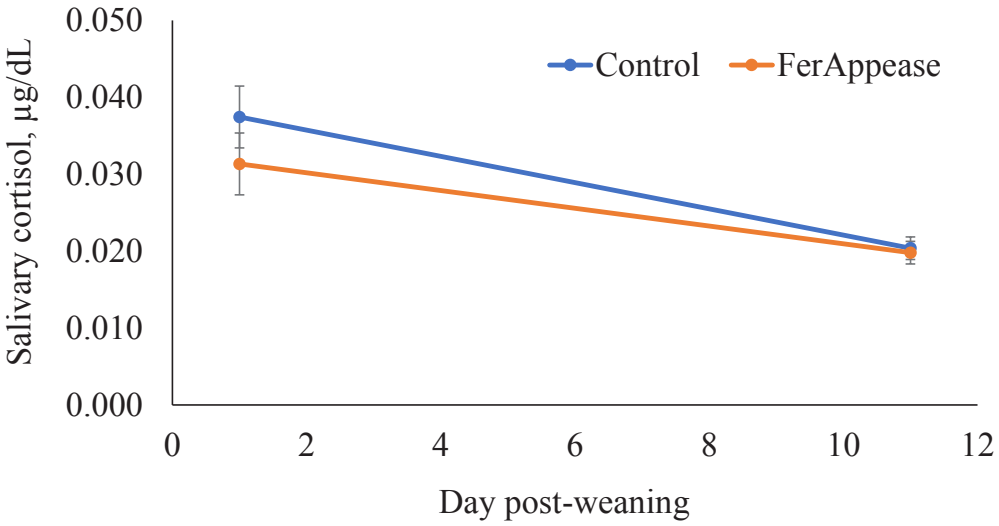

Figure 1. Salivary cortisol concentration of pigs 24 h following application of placebo or FerAppease on d 0 and 10 post-weaning. Treatment × day, *P* = 0.352; Treatment, P = 0.284; Day, *P* < 0.0001. Error bars represent +/- SEM.

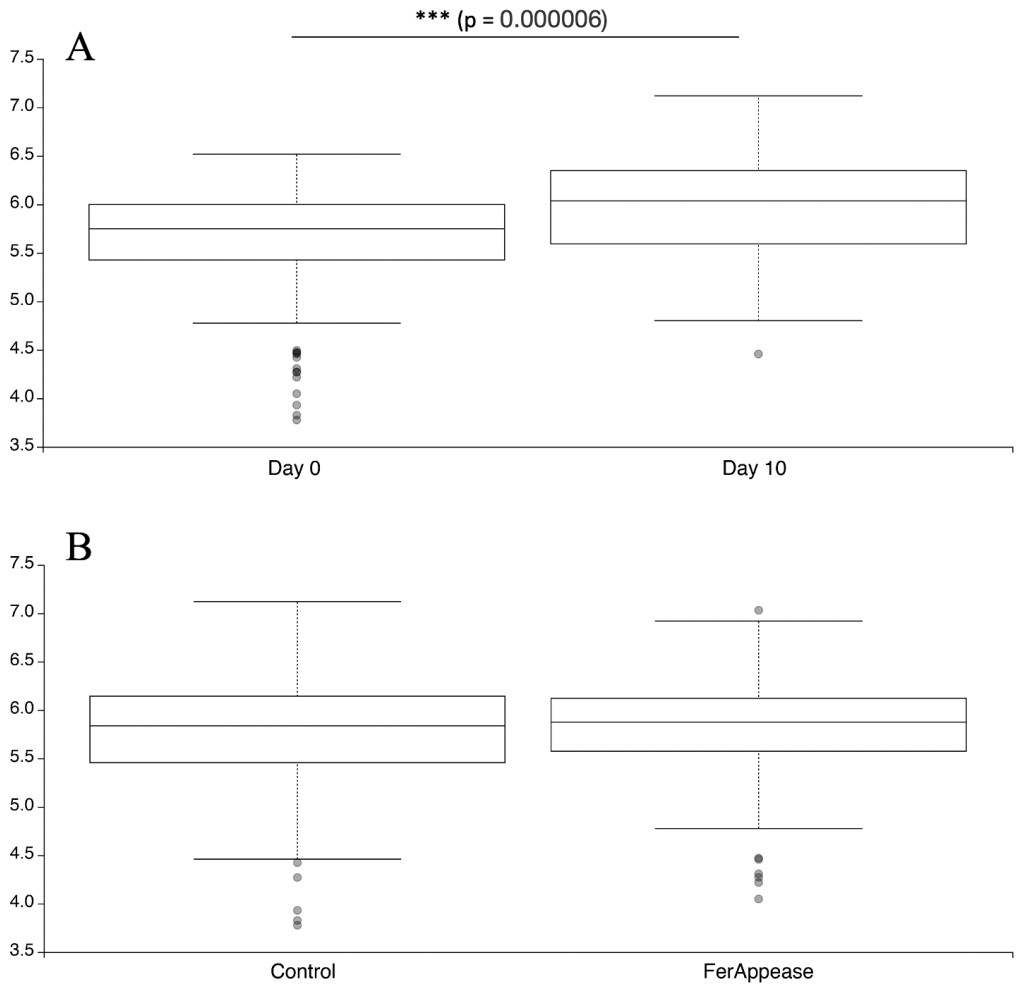

Figure 2. Shannon Alpha Diversity index for the effect of day (A) and effect of treatment (B). Day, *P* < 0.0001; Treatment, *P* > 0.05.

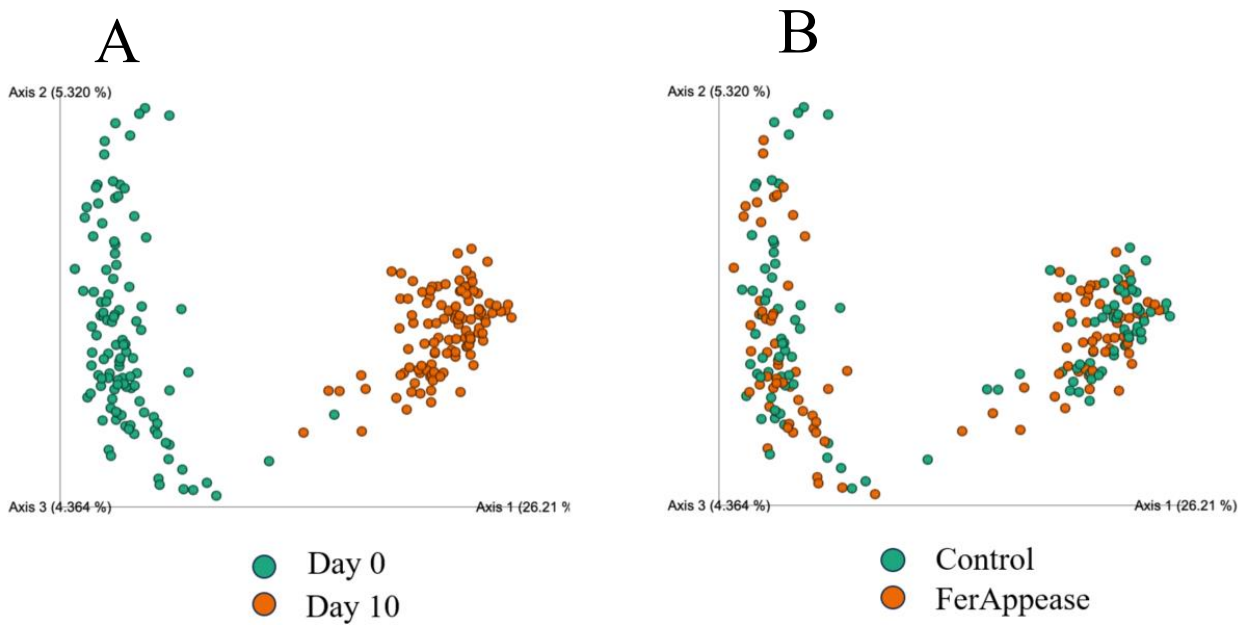

Figure 3. Bray-Curtis Beta Diversity index for the effect of day (A) and effect of treatment (B). Similarity in microbial composition between groups shown as overlapping observations, whereas distinct populations indicate dissimilarity in microbial communities between groups. Day, $P = 0.001$; Treatment, $P = 0.616$.

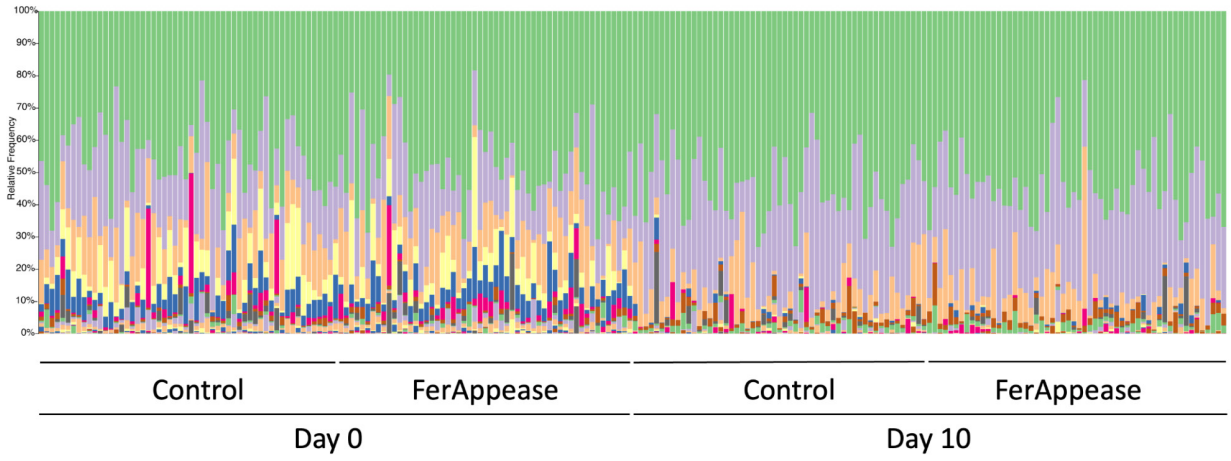

Figure 4. Relative abundance of class level by treatment and day.