Student Research Paper Archiving in Deep Blue

http://deepblue.lib.umich.edu/

| Paper Title: | The Effects of Captivity on Diet & Lifespan in White tailed Deer |
|---------------|---|
| Authors: | Sheldin Wilks, Madeleine Klemz, Erika Gumkouski, Alex Schipans |
| Collection: | Biological Station, University of Michigan (UMBS) |
| Course: | EEB 381: General Ecology |
| Instructor: | Jasmine Crumsey Forde |
| Teaching | Corbin Kuntze |
| Assistants: | Olivia Brinks |
| Date: | June 15, 2017 |
| Abstract: | |
| (no need to f | ill this in – I'll paste the final version in before giving it to Adam) |

AGREEMENT

I grant the Regents of the University of Michigan the non-exclusive right to retain, reproduce, and distribute my paper, titled in electronic formats and at no cost throughout the world.

The University of Michigan may make and keep more than one copy of the Paper for purposes of security, backup, preservation and access, and may migrate the Paper to any medium or format for the purpose of preservation and access in the future.

1. Sheldin Wilks

2. Alex Schipensky

3. Maddine Remy

4. Alex Schipensky

Madeleine Kleuz

Frica Gumkowski

{SIGN ABOVE}

The Effects of Captivity on Diet and Lifespan in White-tailed Deer (*Odocoileus virginianus*)

Erica Gumkowski Madeleine Klemz Alexandria Schipansky Sheldin Wilks

University of Michigan Biological Station EEB 381 - General Ecology June 14, 2018 Professor: Jasmine Crumsey-Forde

Abstract: White-tailed deer (*Odocoileus virginianus*) born and raised in captivity live, on average, three times longer than wild populations (Lopez et al., 2003). Raising white-tailed deer in captivity brings about significant changes to their diet. Nutrition has been shown to play an important role in survival and reproduction of white-tailed deer (Parker et al., 2009). This study seeks to determine the effects of captivity on diet and health in white-tailed deer and how this affects their lifespan. Fecal samples from captive and wild white-tailed deer populations were collected, in northern Michigan, and analyzed using gas chromatography-mass spectrometry and stable isotope analysis. The study results suggest that captive white-tailed deer consume significantly different diets and nutrients than wild populations, based on the stable isotope and principal component analysis.

I grant the Regents of the University of Michigan the non-exclusive right to retain, reproduce, and distribute my paper, titled in electronic formats and at no cost throughout the world.

The University of Michigan may make and keep more than one copy of the Paper for purposes of security, backup, preservation and access, and may migrate the Paper to any medium or format for the purpose of preservation and access in the future.

Signed,

Introduction

Throughout Michigan, white-tailed deer (Odocoileus virginianus) populations occupy every county in the state (Sargent & Carter, 1999). White-tailed deer inhabit young forests and brush, where food is readily accessible and close to the ground (Sargent & Carter, 1999). The deer in the Upper Great Lakes Region are found to eat mostly woody browse, conifer needles, evergreen forbs, non-evergreen forbs, deciduous leaves, fruit, and fungi (Rogers et al., 1981). However, their diet varies seasonally, as ground cover changes throughout the year (Rogers et al., 1981). More specifically, in the spring, they browse grasses, sedges, bracken fern, and wood anemone (Rogers et al., 1981). Classically considered an "edge species", white-tailed deer thrive in environments where cover and food are juxtaposed (VerCauteren & Hygnstrom, 2011). The ability to survive in a variety of habitats, due to their flexible diet, allows them to migrate seasonally and stray far from their home ranges (VerCauteren & Hygnstrom, 2011).

Due to the large presence of white-tailed deer throughout the state of Michigan, they are recognized as a valuable resource for hunting and tourism (VerCauteren & Hygnstrom, 2011). In the midwest, where hunter participation exceeds the national average (VerCauteren & Hygnstrom, 2011), white-tailed deer have become one of the most sought-after game animals in North America ("Why Deer Farming? | NADeFA," 2018). Driven primarily to raise bucks for hunting purposes, the captive deer industry has grown significantly in the Midwest, over the last 25 years (VerCauteren & Hygnstrom, 2011). In this study, captivity is defined as having limited space, high population density, low vegetation, human disturbance, and supplementary feed (Li et al., 2007). The captive white-tailed deer industry profits most from selling breeding stock and antlers but also profits from tourism on the deer "farm" (VerCauteren & Hygnstrom, 2011). As

captive and wild white-tailed deer populations continue to grow throughout Michigan, their economic contributions will, as well.

Raising white-tailed deer in captivity brings about significant changes to their diet. In captivity, supplemental feed is used because the deer feed on native plants faster than they can regrow. Additionally, captive deer are not exposed to the same variety of vegetation as the wild population. In other studies, variations in diet have shown to affect overall body mass and the size of antlers on male deer (Jones et al., 2011). Low relative body mass can place substantial limitations on breeding success, in captivity, often leading to decreased fitness in the white-tailed deer population (Jones et al., 2011). Depending on the soil fertility, precipitation patterns, and nutrient supplementation in captivity, phenotypic characteristics can be expressed differently than wild populations (Demarais et al., 2016).

Nutrition has shown to play an important role in the survival and reproduction of wild and captive white-tailed deer populations (Parker et al., 2009). The main constraint for wild populations is food availability, while the needs of captive deer are met daily (Parker et al., 2009). The captive population used in this study was fed a diet of apples, carrots, crackers, straw, supplemental feed, and mineral blocks. Evidence show that protein constraints can be a nutritional challenge for wild populations (Parker et al., 2009). During periods of high body growth, such as fetal growth and lactation, protein requirements are highest, posing further challenges for wild populations (Parker et al., 2009). During winter, wild white-tailed deer discern differences in protein content of plants, in order to increase foraging rates on plants with high protein content (Tripler, et al., 2002). Body size and condition are a result of protein demands and food intake and have direct consequences on reproduction and overall fitness

(Parker et al., 2009). Wild populations with access to higher nutritional levels at the beginning of winter are shown to survive longer, as the regain of body mass in the spring is crucial for adult survivorship (Parker et al., 2009). Wild white-tailed deer populations, in general, live about four years; conversely, deer raised in captivity live six to fourteen years (Lopez et al., 2003). While diet is thought to influence the health of white-tailed deer, it is unclear whether the extended lifespan of captive populations is a result of differences in diet or other confounding variables.

Another factor contributing to the survivorship and health of wild white-tailed deer populations is exposure to predation. Many studies have documented that coyotes (*Canis latrans*) are the greatest source of natural mortality for neonate deer (Robinson et al., 2014). Coyotes have expanded into the eastern United States over the last 100 years, but their range expansion shows little evidence of declines in deer populations (Robinson et al., 2014). Another top predator that has expanded into the Great Lakes Region is the coywolf (*Canis latrans x Canis lupus*), a coyote-wolf hybrid (DeWeerdt, 2016). Due to wolf heritage, the coywolves are comfortable inhabiting forests, where they hunt in small packs. The size of coywolves leaves larger prey, such as white-tailed deer, more vulnerable to pursuit (DeWeerdt, 2016). Sources of predation, such as coyotes and coywolves, shorten the lifespan of wild white-tailed deer.

While white-tailed deer raised in captivity consume a different diet than wild populations, the extent of these differences and the effects they have on overall health and lifespan of the deer is unknown. This study explores how diet differs between captive and wild white-tailed deer populations, in northern Michigan. Differences in diet are used to analyze the potential effects on health and lifespan of white-tailed deer. Variation in diet are analyzed using stable carbon and nitrogen isotopes and a principal component analysis of fecal samples, from wild and captive

white-tailed deer populations. Stable carbon isotopes are used to distinguish differences in consumption of C_3 and C_4 plants. The principal component analysis allows us to make predictions of what the deer are eating, based on their habitat and the significant components identified. We expect to see larger values of stable carbon isotopes, indicating the consumption of more C_4 plants in captive populations, such as corn. Additionally, we expect to see larger values of stable nitrogen isotopes in the captive population because they are fed a mineral block, which is a potential source of protein. Differences in overall health are determined through further analysis of the principal components. In the analysis, the principal components are identified to show differences in diet. We predict that significant components found in the samples will differ between wild and captive populations.

Methods

Study Sites

Two study sites were chosen, to compare wild white-tailed deer and captive white-tailed deer populations in Northern Michigan. For the captive site, an anonymous white-tailed deer farm located in northern Michigan was chosen. Within the farm there were three separate enclosures, containing various amounts of both deer and vegetation. The first enclosure was 10,800 ft² and held 14 deer, some of which were pregnant. The second enclosure was 33,750 ft² and held 12 deer of various sexes. The third enclosure was 33,750 ft² and held eight deer, also varying in sex. It was noted that the second and third enclosures had a dense forest area with a dominant composition of coniferous trees. The wild sites consisted of a 1.2 km radius surrounding the University of Michigan Biological Station (45.558698, -84.677630) in Pellston, Michigan and a densely wooded area approximately 7.24 km northwest of the University of

Michigan Biological Station. The main vegetation in this area is *Populus grandidentata* and *Pinus strobus*. It is noted that the wild sample sites used were within a hunting sanctuary. The human population density of Pellston, MI is 435.37 p/mi² (Population of Pellston, MI, 2016), and the population density of the deer was unable to be determined for this area.

Sample Collection

Across two sampling sites, a total of 39 white-tailed deer fecal samples were collected and analyzed for principal components, specifically cholesterol, lactic acid, and stable isotopes of carbon and nitrogen. 21 samples were collected from the captive site and 18 samples were collected between the wild sites. While sampling, a GPS was used to map where samples were found and collected. Weather and temperature were recorded in the chance that these variables affected the data; the setting where the samples were found were also recorded. Each sample was stored in a 15mL centrifuge tube. To ensure that the chemical components in the samples were preserved and not affected by heat and other factors they were placed in a -20°C freezer, within two hours of collection.

Principal Component Analysis

After all samples were collected, they were freeze-dried for 24 hours in a vacuum chamber that was cooled to -50°C. After freeze-drying, samples were individually crushed using a mortar and pestle and returned to their respective 15 mL centrifuge tubes. The mortar and pestle were cleaned with methanol between each sample. A solvent of equal parts 99.5% acetonitrile and methanol was made and added to the crushed fecal samples. The samples were first placed in a Fisher Scientific FS60 Ultrasonic Cleaner for 15 minutes, to break up solid particles in the solution. Then placed into a Sorvall ST 40 Centrifuge for ten minutes at 3800 rpm

to separate the solid particles from the liquid solution. The liquid solution was extracted from each sample and put into a corresponding 15 mL centrifuge tube, for later use. All samples were extracted a total of three times. After this extraction process, acetonitrile was added to the 15 mL centrifuge tubes that contained the liquid extraction samples, to put the samples at a common volume. Then extraction samples were placed in the Sorvall ST 40 Centrifuge for ten minutes at 3800 rpm.

To concentrate any remaining solutes left in the extractions, 1500μl of the scat extraction was removed from the 15 mL centrifuge tubes and placed into a sterile 2000μl microcentrifuge tube. Then placed into a SavantTM SPD111 SpeedVacTM for 45 minutes. During the final preparation stages, 100μl of dimethyl sulfoxide and 100μl of acetonitrile were added to the microcentrifuge tubes. The samples were placed in Fisher Scientific FS60 Ultrasonic Cleaner for one minute to dissolve any remaining loose particles in the solution. 200μl of acetamide with trimethylchlorosilane was added to each sample before being placed in a Fisher Scientific Isotemp 200 Series 230F Conventional Oven at 50°C for 30 minutes, to allow the reaction to finish. 1500μl of the solution was transferred into 2000μl glass vials. These vials were placed into the Thermo AI/AS 1310 Series Autosampler and sent through the Thermo TRACETM 1310 Gas Chromatograph and the Thermo ISQTM LT Single Quadrupole GC-MS System, for principal component analysis.

Stable Isotopes Analysis

The freeze-dried samples were pulverized using a SPEX SamplePrep 8000D Mixer/Mill®. Each sample was returned to its respective 15 mL centrifuge tube and weighed for stable isotope analysis. Samples were analyzed for percent carbon, percent nitrogen, and carbon

and nitrogen stable isotopes. The laboratory used "in-house" standards, due to the cost of the certified international materials. Compounds used for the "in-house" standard include caffeine, acetanilide, and bovine serum albumin, a serum protein derived from cows. The values are certified with the laboratory and the same materials are run alongside the international standards. In the end, there is confidence in the actual isotopic composition of the "in-house" material.

An independent sample t-test of the average percent carbon and nitrogen content and average cholesterol levels was performed, to compare averages between the wild and captive populations. A Mann-Whitney test was used to evaluate average carbon and nitrogen stable isotopes and average lactic acid levels from each population of deer. The standard error used for nitrogen was 0.10 per mil δ^{15} N (versus air). The standard error used for carbon was 0.05 per mil δ^{13} C VPDB (versus Vienna Pee-Dee Belemnite).

Results

Fecal Carbon and Nitrogen Content

Fecal samples of the captive population of white-tailed deer contained significantly more nitrogen than the wild white-tailed deer population (Table 1; t=-3.487, P=0.001). δ ¹⁵N values of captive populations were enriched by 3.23 ± 0.25%, relative to wild population δ ¹⁵N values, showing significant differences. Captive populations have significantly larger average δ ¹⁵N values (Table 3; U=3.000, P=0.0001).

The average percentage of carbon was significantly larger in the wild deer population, as compared to the captive population (Table 2; U=0.000, P=0.0001). Additionally, captive deer populations had significantly lower average δ^{13} C values (Table 4; U=0.000, P=0.0001).

Fecal Cholesterol and Lactic Acid Content

Looking at lactic acid values, the captive deer population had a significantly higher average amount of lactic acid detected, $3.33 \times 10^7 \pm 8.94 \times 10^7$, compared to the wild population, $4.72 \times 10^6 \pm 9.54 \times 10^6$ (Table 5; U=76.000, P=0.001).

In regard to cholesterol values, the captive deer population had a significantly higher average amount of cholesterol detected, 2.26×10^7 , compared to the wild population, 1.77×10^7 (Table 6; t=2.73; P=0.007).

Principal Component Analysis

The principal component analysis identified 201 different components, from the samples. Three significant components were chosen, from both the wild and captive populations, for further analysis. The significant components from the wild population were bilobol ($C_{21}H_{34}O_2$), stigmastanol ($C_{29}H_{52}O$), and valerenic acid ($C_{15}H_{22}O_2$). The significant components from the captive population were hydrocortisone acetate ($C_{23}H_{32}O_6$), trehalose ($C_{12}H_{22}O_{11}$), and 1-octacosanol ($C_{28}H_{58}O$).

Discussion

Diets

The results from the stable nitrogen isotope data revealed that the captive deer population had a significantly larger average $\delta^{15}N$ value than the wild deer population. Due to the fact that wild deer consume leaves of small trees and shrubs, which tend to have lower $\delta^{15}N$ values than other non-woody plant types, this could explain why wild white-tailed deer populations have a lower average $\delta^{15}N$ value (Cormie & Schwarcz, 1994). The low values of $\delta^{15}N$ found in the wild population could also be a result of browsing on agricultural lands treated with artificial

fertilizers, which have lower δ^{15} N values (Cormie & Schwarcz, 1994). Nitrogen content has been shown to have a strong association with forage quality, indicating that the captive deer could have access to higher quality forage (Gil-Jiménez et al., 2015). Additionally, larger δ^{15} N values are an indication of a higher protein diet, indicating that the captive deer are being fed more protein than they would normally forage on in the wild (Robbins et al., 2010). Diet quality can be characterized by protein content because nitrogen determines animal growth (Gil-Jiménez et al., 2015). Different soils also influence protein levels of vegetation, which could indicate that wild populations are foraging on plants in lower quality soils (Jones et al., 2008). Additionally, the captive population was fed a mineral supplement, which could cause higher δ^{15} N values, although further isotope analysis of the mineral supplement would need to be done in order to confirm this prediction.

The stable isotope results also revealed that the captive deer population had significantly lower average δ^{13} C values, as compared to the wild deer population. The δ^{13} C values are expected to reflect the δ^{13} C of the plants in the deer's diet (Cormie & Schwarcz, 1994). C_3 plants are significantly depleted in δ^{13} C, compared to C_4 plants, in terrestrial environments (Cormie & Schwarcz, 1994). Also, the canopy effect in deep forests can greatly decrease the δ^{13} C of the food chain, due to recycling of isotopically light CO_2 from plant respiration and decomposition in soil litter, causing the wild deer to have lower δ^{13} C values (Cormie & Schwarcz, 1994). Conversely, higher δ^{13} C values are a result of consuming more C_4 plants, such as corn (Cormie & Schwarcz, 1994). It can be predicted that the captive deer population was being fed a diet with more C_4 plants. Further stable isotope tests on the food supplements given to the captive deer population could be analyzed to support this prediction.

The results showed that the captive white-tailed deer population had significantly higher cholesterol levels than the wild white-tailed deer population. This could be a result of the captive deer being fed more saturated fats in their diet, specifically in the supplemental feed fed to the deer. Further tests would need to be analyzed on the supplemental feed, collected from the deer farm, to determine the saturated fat percentages, before making any additional conclusions. The lower cholesterol levels in the wild deer could also be due to seasonal factors. In a previous study, it was found that cholesterol levels in does, were higher in the fall and winter months (Rule & McCormick, 1998). This study was conducted in late May, which has different vegetation available to the deer. The length of this study was not long enough to conclude that cholesterol plays a role in the lifespan of wild and captive white-tailed deer.

The principal component analysis of GC/MS data shows two clearly distinct metabolomic profiles, corresponding to the wild and captive white-tailed deer populations (Figure 7). Upon analysis, three significant components from both the wild and captive populations were chosen. In the captive deer population, the significant components were bilobol (C₂₁H₃₄O₂), stigmastanol (C₂₉H₅₂O), and valerenic acid (C₁₅H₂₂O₂). Bilobol is an irritant found in poison ivy (*Toxicodendron radicans*), a plant that grows in almost every habitat and is commonly consumed by white-tailed deer in northern Michigan ("Toxicodendron radicans | CLIMBERS," 2013). The high concentration of bilobol found in captive populations could be due to a greater prevalence of poison ivy in the enclosures or a greater preference for it. Stigmastanol is a phytosterol found in a variety of plant sources. While it is known for its ability to inhibit the absorption of cholesterol, the captive population had higher levels of cholesterol, in comparison to the wild population (Figure 6) (Heinemann et al., 1991). Valerenic acid is found

in Valerian plants (*Valeriana officinalis*), which usually grows in lacustrine habitats (O'Neal & Soulliere, 2006). While the captive deer enclosures were not lacustrine habitats, it is possible that Valerian plants were present.

In the wild population, the significant components related to diet were hydrocortisone acetate ($C_{23}H_{32}O_6$), trehalose ($C_{12}H_{22}O_{11}$), and 1-octacosanol ($C_{28}H_{58}O$). Hydrocortisone acetate is the synthetic acetate salt form of cortisol, a "stress" steroid hormone ("Hydrocortisone acetate | C23H32O6 - PubChem"). This glucocorticoid is commonly used to assess stress in mammals. The diet consumed by wild deer can have an effect on the cortisol level excreted in the fecal samples (Keay et al., 2006). Diet, in itself, can often be a cause of stress in wild populations; on the other hand, diet stress is not usually a concern for captive populations (Keay et al., 2006). Trehalose is a mycose sugar that serves as a protectant to many Arabidopsis species. Arabidopsis species found in Michigan are the sand cress (Arabidopsis lyrata) and the mouse-ear cress (Arabidopsis thaliana) ("Arabidopsis - Michigan Flora"). Due to the high concentration of trehalose found in the wild population samples, it can be inferred that white-tailed deer are consuming one or both of these species. 1-Octacosanol is commonly found in the epicuticular waxes of plants, including the leaves of many species of Eucalyptus, of most forage and cereal grasses, of Acacia, Trifolium, and Pisum ("1-Octacosanol"). The high concentrations of 1-octacosanol found in the wild white-tailed deer samples, suggests these deer are consuming a lot of forage in their daily diet. Overall, knowledge of these six components allows us to make predictions about how the diets of wild versus captive populations differ. Based on the principal components analysis of GC/MS data, wild and captive populations of white-tailed deer in northern Michigan have significantly different diets.

Health

In the wild population, a significant component identified was hydrocortisone acetate $(C_{23}H_{32}O_6)$. The high concentration of hydrocortisone acetate found in the wild population could be due to living in a higher stress environment. For example, wild populations must face the stress of scavenging for food and escaping predators daily, while captive populations do not have exposure to this challenge.

It was found that captive deer had a significantly higher average of lactic acid than the wild deer population. The consumption of plants with high secondary compounds contributes to lactic acid accumulation. Secondary compounds are compounds that are not essential for the survival of the plant, however, they can act as a defense mechanism against herbivores (Liu et al., 2012). Based on the data, it is possible that captive deer are consuming plants with higher concentrations of secondary compounds (Campbell & Hewitt, 2000). Diets that are rich in secondary compounds can result in metabolic acidosis in herbivores, where excess acid is excreted or buffered to maintain acid-base homeostasis in the deer (Campbell & Hewitt, 2000). Metabolic acidosis is a characteristic found in capture myopathy, which occurs as a result of damage to the skeletal and cardiac muscles after a pursuit, capture, handling, and manipulation of an animal (Blumstein et al., 2015). Even though the captive deer are not exposed to predation, because they were raised in captivity, the higher lactic acid levels found could be due to frequent human interaction. This could affect the overall health of the deer, and as a result, the lifespan of captive deer.

Errors and Improvements

Due to the limited time frame while conducting this study, a small sample size for both wild and captive populations was used. Given more time, more samples could have been collected from the wild sites to better represent the wild white-tailed deer population in northern Michigan. Additionally, further sampling at additional deer farms would have allowed the study to make broader conclusions about captive deer enclosures and assess true differences.

The locations selected were quite specific and could also factor into the results. The two wild locations attempted to be located outside of a white-tailed deer's home range, however, deer are not limited to a defined radius. Thus a mixture of samples from the same herd are possible, regardless of the distance between the two wild sites. The distance between the captive herd and wild herd was not a factor in this study because the captive herd is born and raised in captivity, thus eliminating any possibility of herd interbreeding.

Accuracy in lab prep is a factor for this study. When weighing fecal samples for GC/MS analysis, the weights were recorded for one pellet of each sample, rather than using a consistent weight amongst all samples. The various sample weights could have resulted in mathematical error during the calculation of cholesterol and lactic acid area count per gram sample ratio.

The variable of seasonal food sources could also be addressed, as the data received was primarily spring resources. Cholesterol levels are said to have seasonal fluctuations and the sample collection time does not accredit for these seasonal changes (Rule & McCormick, 1998).

Conclusion

Based on the fecal analysis of captive and wild populations of white-tailed deer in northern Michigan, it can be concluded that these populations have significantly different diets.

The differences in diet could be attributed to supplementary feed and mineral blocks fed to the captive deer, that wild populations do not consume, as well as differences in forage surrounding the habitats of wild and captive populations. Given the results of this study, it can be inferred that white-tailed deer populations living in captivity consume a higher quality diet than wild populations. The greater nutritional quality of food supplied to captive populations could be a potential reason for the longer lifespan seen in captive populations. In further studies, it would be interesting to use stable isotope analysis to determine the age of each of the samples gathered from the wild and captive populations. Comparing the age of the samples to their predicted diet would help to determine the role that diet plays in lifespan.

Acknowledgements

We would like to thank our General Ecology Professor Jasmine Crumsey-Forde and our Teaching Assistants Corbin Kuntze and Olivia Brinks for their constant feedback and support throughout our project. We would also like to thank UMBS Laboratory Manager Timothy Veverica and the other lab techs for their help in analyzing all of our fecal samples. Additional thanks to Adam Schubel; the Resident Biologist, and Sherry Webster; the Stockroom Manager for assisting us in our experimental design and materials. Lastly, we would like to thank the deer farm, in which we collected samples, for giving us permission to analyze samples from their white-tailed deer.

Literature Cited

- Arabidopsis Michigan Flora. Retrieved June 13, 2018, from https://michiganflora.net/genus.
 aspx?id=Arabidopsis
- Blumstein, D. T., Buckner, J., Shah, S., Patel, S., Alfaro, M. E., & Natterson-Horowitz, B. (2015). The evolution of capture myopathy in hooved mammals: a model for human stress cardiomyopathy? *Evolution, Medicine, and Public Health*, 2015(1), 195–203. http://doi.org/10.1093/emph/eov015
- Campbell, T., & Hewitt, D. (2000). Effect of Metabolic Acidosis on White-Tailed Deer Antler

 Development. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, 73(6), 781-789. doi:10.1086/318096
- Card, W., Kirkpatrick, R., Webb, K., & Scanlon, P. (1985). Nutritional Influences on Nefa,

 Cholesterol, and Ketones in White-Tailed Deer. *The Journal of Wildlife Management*,

 49(2), 380-385. doi:10.2307/3801537
- Cormie, A. & Schwarcz, H. (1994). Stable isotopes of nitrogen and carbon of North American white-tailed deer and implications for paleodietary and other food web studies.

 *Palaeogeography, Palaeoclimatology, Palaeoecology, 107(3-4), 227-241. Retrieved from ArticlesPlus (https://doi.org/10.1016/0031-0182(94)90096-5).
- Demarais, S., Strickland, B. K., Webb, S. L., Smith, T. and Mcdonald, C. (2016). Simulated effects of releasing pen-raised deer into the wild to alter population-level antler size.

 Wildl. Soc. Bull., 40: 41-49. doi:10.1002/wsb.626

- DeWeerdt, S. (2016). Part Coyote, Part Wolf, Part Dog: Enter the Coywolf; A hybridized canid made up of coyote, wolf and dog genes is expanding across the eastern U.S. Newsweek, 166(1). Retrieved from http://link.galegroup.com.proxy.lib.umich.edu/apps/doc/A438412661/STND?u=lom_umichanna&sid=STND&xid=025e4940
- Gil-Jiménez, E., Villamuelas, M., Serrano, E., Delibes, M., & Fernández, N. (2015). Fecal Nitrogen Concentration as a Nutritional Quality Indicator for European Rabbit Ecological Studies. *PLOS ONE*, *10*(4), e0125190. https://doi.org/10.1371/journal.pone.0125190
- Heinemann, T., Kullak-Ublick, G. A., Pietruck, B., & von Bergmann, K. (1991). Mechanisms of action of plant sterols on inhibition of cholesterol absorption. Comparison of sitosterol and sitostanol. *European Journal of Clinical Pharmacology*, 40 Suppl 1, S59-63.
- Hydrocortisone acetate | C23H32O6 PubChem. Retrieved June 13, 2018, from https://pubchem.ncbi.nlm.nih.gov/compound/Cortell#section=Top
- Jones, P., Demarais, S., Strickland, B., & Edwards, S. (2008). Soil Region Effects on White-Tailed Deer Forage Protein Content. *Southeastern Naturalist*, 7(4), 595-606. Retrieved from http://www.jstor.org.proxy.lib.umich.edu/stable/20204034
- Jones, P., Strickland, B., Demarais, S., DeYoung, R., & Maher, C. (2011). Inconsistent association of male body mass with breeding success in captive white-tailed deer.

 *Journal of Mammalogy, 92(3), 527-533.

- Keay, J. M., Singh, J., Gaunt, M. C., & Kaur, T. (2006). Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review.
 Journal of Zoo and Wildlife Medicine: Official Publication of the American Association of Zoo Veterinarians, 37(3), 234–244. https://doi.org/10.1638/05-050.1
- Lopez, R. R., Vieira, M. E. P., Silvy, N. J., Frank, P. A., Whisenant, S. W., & Jones, D. A. (2003).
 - Survival, Mortality, and Life Expectancy of Florida Key Deer. *The Journal of Wildlife Management*, 67(1), 34. https://doi.org/10.2307/3803059
- Li, C., Jiang, Z., Tang, S., & Zeng, Y. (2007). Influence of enclosure size and animal density on fecal cortisol concentration and aggression in Pere David's deer stags. *General and Comparative Endocrinology*, 151(2). doi:10.1016/j.ygcen.2007.01.014
- Liu, X., Jia, Y., Li, N., Gong, H., Zhang, Y., & Ma, W. (2012). Research Progress on Ways of Secondary Metabolism in Plants. *Applied Mechanics and Materials*, 178-, 1004. doi:http://dx.doi.org.proxy.lib.umich.edu/10.4028/www.scientific.net/AMM.178-181.100
- Michigan Flora Online. A. A. Reznicek, E. G. Voss, & B. S. Walters. (2011). University of Michigan. Web. June 12, 2018. https://michiganflora.net/genus.aspx?id Arabidopsis.
- National Center for Biotechnology Information. PubChem Compound Database; CID=5744, https://pubchem.ncbi.nlm.nih.gov/compound/5744 (accessed June 12, 2018).
- National Center for Biotechnology Information. PubChem Compound Database; CID=68406, https://pubchem.ncbi.nlm.nih.gov/compound/68406 (accessed June 12, 2018).
- O'Neal, R. P., & Soulliere, G. (2006, March). Conservation Guidelines for Michigan Lakes and

- Associated Natural Resources. State of Michigan Department of Natural Resources.
- Parker, K. L., Barboza, P. S., & Gillingham, M. P. (2009). Nutrition integrates environmental responses of ungulates. *Functional Ecology*, 23(1), 57–69. https://doi.org/10.1111/j.1365-2435.2009.01528.x
- Population of Pellston, MI. (2016). In *Population.us*. Retrieved June 12, 2018, from http://population.us/mi/pellston/
 - Robbins, C. T., Felicetti, L. A., & Florin, S. T. (2010). The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. *Oecologia*, *162*(3), 571–579. https://doi.org/10.1007/s00442-009-1485-8
- Robinson, K., Diefenbach, D., Fuller, A., Hurst, J., & Rosenberry, C. (2014). Can Managers

 Compensate for Coyote Predation of White-Tailed Deer? *The Journal of Wildlife Management, 78*(4), 571-579. Retrieved from

 http://www.jstor.org.proxy.lib.umich.edu/stable/43188182
- Rogers, L. L., Mooty, J. J., & Dawson, D. (1981). Foods of White-tailed Deer in the Upper Great

 Lakes Region- A Review. . Retrieved from

 https://www.fs.fed.us/nrs/pubs/gtr/gtr_nc065.pdf
- Rule, D. C., & McCormick, R. J. (1998). Fatty acid composition and cholesterol concentration in tissues of white-tailed deer (Odocoileus virginianus) as influenced by lactation, age, and season of the year. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 119(3), 563-570. Retrieved from ArticlesPlus (https://doi-org.proxy.lib.umich.edu/10.1016/S0305-0491(98)00029-7).

- Sargent, M. S., & Carter, K. S. (1999). Landowner's Guide: White-tailed Deer. Retrieved May 30, 2018, from http://www.michigandnr.com/publications/pdfs/hunting wildlifehabitat/landowners guide/species mgmt/Deer.htm
- Toxicodendron radicans | CLIMBERS. (2013). Retrieved June 13, 2018, from http://climbers.lsa.umich.edu/?p=167
- Tripler, C., Canham, C., Inouye, R., & Schnurr, J. (2002). Soil nitrogen availability, plant luxury consumption, and herbivory by white-tailed deer. *Oecologia*, *133*(4), 517–524. https://doi.org/10.1007/s00442-002-1046-x
- VerCauteren, K., & Hygnstrom, S. E. (2011). Managing White-tailed Deer: Midwest North America. *Papers in Natural Resources*, 37.
- Why Deer Farming? | NADeFA. (2018). Retrieved May 24, 2018, from http://www.nadefa.org/why-deer-farming

Appendix

Table 1. Group statistics from independent sample t-test of percent nitrogen values of fecal samples from wild and captive white-tailed deer populations. Values represent means ± 1 standard deviation.

| Location | Sample Size (N) | Average %N | t-value | p-value |
|----------|-----------------|-----------------|---------|---------|
| Wild | 18 | 2.61 ± 0.77 | -3.487 | 0.001 |
| Captive | 21 | 3.23 ± 0.25 | | |

Table 2. Group statistics from Mann-Whitney test and t-test of percent carbon values of wild and captive white-tail deer populations. Values represent means ± 1 standard deviation.

| Location | N | Average %C | U value | p-value |
|----------|----|------------------|---------|---------|
| Wild | 18 | 49.29 ± 1.67 | 0.000 | 0.0001 |
| Captive | 21 | 39.46 ± 1.94 | | |

Table 3. Group statistics from Mann-Whitney test of $\delta^{l5}N$ values of wild and captive white-tailed deer populations. Values represent means ± 1 standard deviation.

| Locatio n | N | Mean Rank (δ ¹⁵ N) | Average δ ¹⁵ N | U value | p-value |
|--------------|----|-------------------------------|---------------------------|---------|---------|
| Wild | 18 | 9.67 | -1.17±1.41 | 3.00 | 0.0001 |
| Captive | 21 | 28.86 | 2.49±0.65 | | |

Table 4. Group statistics from Mann-Whitney test of $\delta^{13}C$ values of wild and captive white-tailed deer populations. Values represent means ± 1 standard deviation.

| Locatio n | N | Mean Rank (δ ¹³ C) | Average δ ¹³ C | U value | p-value |
|--------------|----|-------------------------------|---------------------------|---------|---------|
| Wild | 18 | 9.50 | -30.63±0.97 | 0.00 | 0.0001 |
| Captive | 21 | 29.00 | -26.15±0.46 | | |

Table 5. Group statistics from Mann-Whitney test of lactic acid area values of wild and captive white-tailed deer populations. Values represent means ± 1 standard deviation.

| Location | N | Mean Rank Lactic Acid Area (area counts/ g sample) | Average Lactic Acid Area (area counts/ g sample) | U value | p-value |
|----------|----|--|--|---------|---------|
| Wild | 18 | 13.72 | 4723077.47±9.54x10 ⁶ | 76.00 | 0.001 |
| Captive | 21 | 25.38 | 33459431.34±8.94x10 ⁷ | | |

Table 6. Group statistics from t-test of cholesterol area values of wild and captive white-tailed deer populations. Values represent means ± 1 standard deviation.

| Location | N | Cholesterol Area (area counts/ g sample) | t-value | p-value |
|----------|----|--|---------|---------|
| Wild | 18 | $17707511.02 \pm 12833347.68$ | 2.73 | 0.007 |
| Captive | 21 | $22594336.34 \pm 31130830.83$ | | 0.007 |

Table 7. The GC/MS found over 200 significant chemical compounds in 39 fecal samples. The following table shows five of the highest and lowest principle compounds within two principle tests.

| PC1 Highest and | l Lowest 5 Compounds | |
|---|----------------------|--------------|
| | PC1 | PC2 |
| Lowest Compounds | | - |
| Bilobol C15:1 (2TMS) | -0.44003 | 0.3567 |
| Stigmastanol TMS Derivative - silane, trimethyl | -0.42541 | 0.43823 |
| Valerenic Acid, TMS | -0.39503 | -0.19166 |
| 11-Octadecenoic acid,(E)-,TMS | -0.39173 | 0.35865 |
| Valerenic Acid, TMS 2 | -0.38287 | 0.49086 |
| | PC1 | PC2 |
| Highest Compounds | | |
| Pimaric Acid | 0.80182 | -0.15343 |
| Isopimaric Acid | 0.81342 | -0.10582 |
| Dehydroabietol cinnamate | 0.82063 | -0.08752 |
| Silane | 0.82534 | -0.21272 |
| 2-(p-(Dimethylamino)phenyl)benzimidazole | 0.90437 | 0.15262 |
| PC2 Highest and | l Lowest 5 Compounds | |
| | PC1 | PC2 |
| Lowest Compounds | | |
| 24-Methylenecycloartan-3-one | 0.25657 | -0.69577 |
| D-(+)-Trehalose,octakis(trimethylsilyl)ether | 0.36113 | -0.61737 |
| 1-Octacosanol, TMS Derivative | 0.52604 | -0.59951 |
| Hydrocortisone Acetate | 0.25303 | -0.52098 |
| beta-Amyrone | 0.09197 | -0.51705 |
| | | |
| | PC1 | PC2 |
| Highest Compounds | | |
| Stigmastanol, TMS Derivative | 0.42129 | |
| Stigmastanol- trimethylsiyl ether | 0.65178 | |
| Diosgenin | 0.35412 | |
| Bilobol C15:1 (2TMS) 2 | 0.15747 | |
| Stigmastanol TMS Derivative | -0.13816 | 0.71562 |

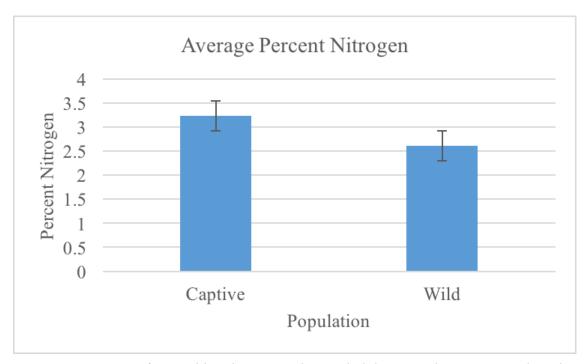


Figure 1. Average %N from wild and captive white-tailed deer populations. Error bar show 1 standard deviation.

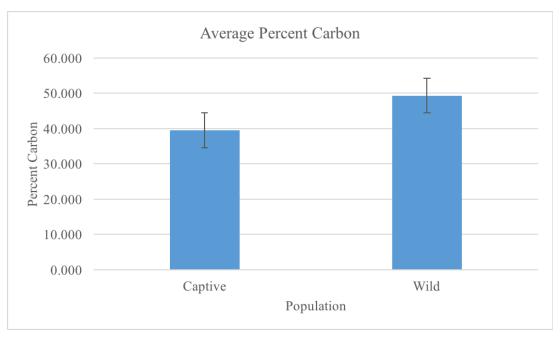


Figure 2. Average %C from wild and captive white-tailed deer populations. %C identifies plant based chemical compounds found in the diets of white-tailed deer. Error bar show 1 standard deviation.

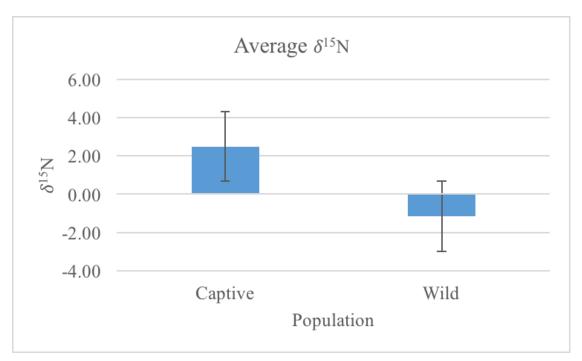


Figure 3. Average $\delta^{l5}N$ from wild and captive white-tailed deer populations. Error bars show 1 standard deviation.

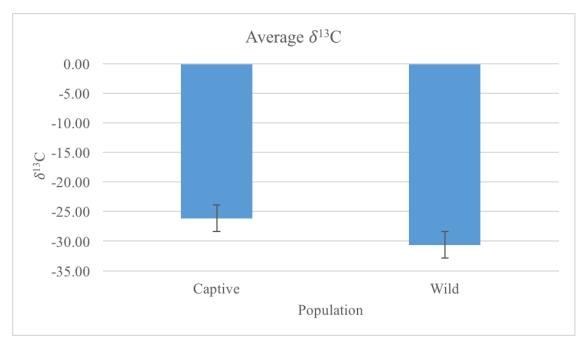


Figure 4. Average $\delta^{l3}C$ from wild and captive white-tailed deer populations. Error bars show standard deviation.

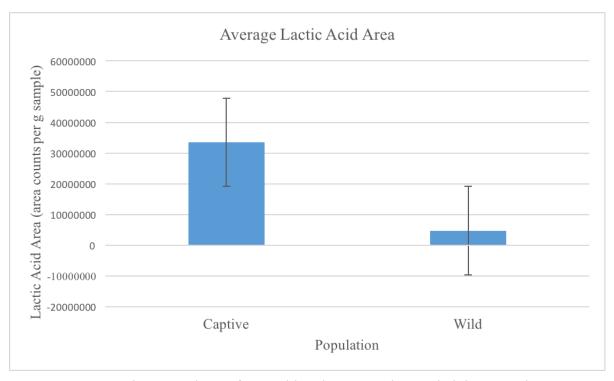


Figure 5. Average lactic acid area from wild and captive white-tailed deer populations. Error bars show 1 standard error.

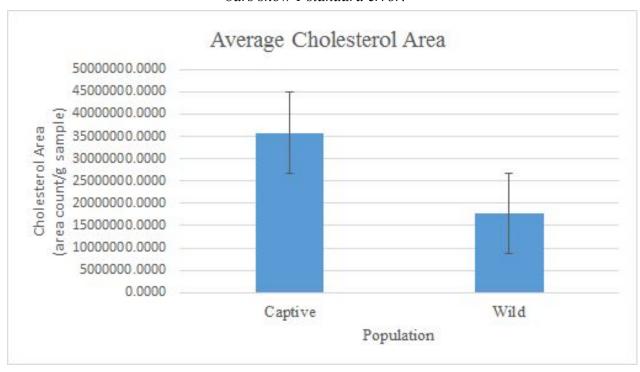


Figure 6. Average cholesterol area from wild and captive white-tailed deer populations. Error bars show 1 standard deviation.

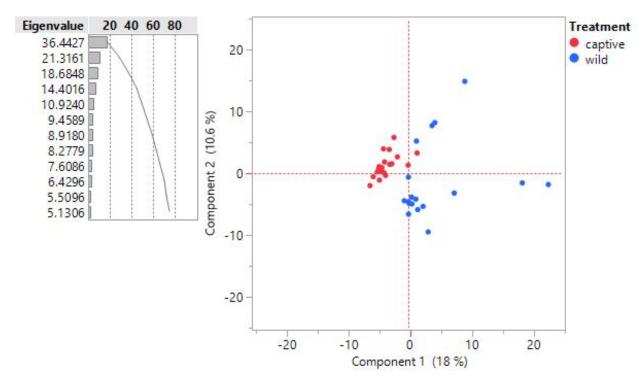


Figure 7. Principal components analysis of GC/MS data shows to clearly distinct metabolomics profiles corresponding to the two sample populations. Note that there are four "wild" samples that appear to have predominantly "captive" metabolomic signatures.