EVALUATING THE NUTRITIONAL CONTENT OF INSECT DIETS FED TO FLORIDA GRASSHOPPER SPARROWS (*AMMODRAMUS SAVANNARUM FLORIDANUS*) AT WHITE OAK CONSERVATION

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Abstract
Avian insectivores obtain many essential nutrients that they cannot synthesize on their own through a diet of seeds and diverse wild insects. At White Oak Conservation, Florida grasshopper sparrows (*Ammodramus savannarum floridanus*) are fed a diet of feeder insects that aims to replicate the nutritional profile consumed by wild birds; however, plumage abnormalities in recent years may indicate nutrient deficiencies in the managed sparrows' diets. Specifically, duller adult plumage compared to wild counterparts and consistent fault-barring in young hatch-year birds were apparent beginning in 2019. In spring 2020, feeder insects and wild insect samples consistent with those fed to grasshopper sparrows were analyzed for nutrient content, specifically vitamin A, protein, and amino acid content. The goal was to determine if wild insects contained superior nutrient levels compared to feeder insects provided to the breeding population of sparrows at White Oak. These nutrients were selected due to their roles in plumage development, structure, and coloration. Results indicated that wild insects had higher levels of total vitamin A and beta-carotene compared to all feeder insects, while amino acids and proteins were comparable in feeder crickets and wild insects. Feeder mealworms and waxworms were insufficient in most nutrients, and vitamin A was completely undetectable in waxworm samples. This study suggests that wild insects should be prioritized to a greater extent in grasshopper sparrow diets to supplement feeder insect nutrients. Further solutions may involve dusting insects with supplements higher in total vitamin content than those currently provided to sparrows at White Oak to increase birds' intake of vitamin A.

Introduction
The Florida grasshopper sparrow (*Ammodramus savannarum floridanus)* is an insectivorous grassland passerine endemic to south-central Florida dry prairie habitat. Over the last two decades (1998-2017), the wild population has declined 90% across surveyed lands, edging the sub-species toward extinction (Florida grasshopper sparrow Working Group, unpublished data). In 2016, Florida grasshopper sparrows were brought to White Oak Conservation (hereafter White Oak) to establish an *ex-situ* breeding population for eventual reintroduction and release.

In 2017, the flock's first breeding season at White Oak, hatch-year (HY) birds were heavily affected by hypovitaminosis A. This lack of vitamin A was either a direct or compounded cause of mortalities among HY birds. Future mortalities surrounding hypovitaminosis A were avoided by dusting sparrow insect diets with NEKTON-S (Nekton-GMBH, Germany), a vitamin supplement high in vitamin A. Despite vitamin supplementation, in subsequent breeding seasons, plumage abnormalities were observed among the flock. Adult birds exhibited lighter, less vibrant feather coloration compared to wild birds, and many HY birds developed heavy fault-barring and pale-barring across flight feathers.

The goal for this nutritional analysis was to determine if the commercial feeder insect diets fed to Florida grasshopper sparrows at White Oak are deficient in nutrients that are critical for plumage coloration, health, and development. To examine this, gut-loaded feeder insects routinely fed to sparrows during breeding season were analyzed for essential nutrient content. Feeder insect nutrients were then compared to those of wild insects to determine if sparrow diets are nutritionally inferior to those consumed by wild birds and if so, to determine which specific nutrients are lacking in feeder insect diets.

Methods

Sparrow Diet Overview

During breeding season at White Oak, grasshopper sparrow diets consist of commercial passerine seed (Higgins Supreme Finch Seed, Higgins Premium Pet Foods, Miami, FL 33167), insectivore grain (Mazuri Exotic Animal Nutrition, Richmond, IN 47374) and three types of feeder insects: crickets (*Acheta domesticus*), mealworms (larval form of the darkling beetle, *Tenebrio molitor*), and waxworms (larval wax moth, *Achroia grisella*). All feeder insects are supplied by Premium Crickets (Winder, GA 30680). A combination of these insects is given twice a day to all sparrows. Amounts of each type of insect are dependent on the number of birds and age of chicks in each enclosure. Waxworms are only given to pairs with chicks and newly independent HY birds.

Feeder insects are dusted with NEKTON-S, a vitamin supplement, at every feeding to increase nutritional value. Calcium dust (Zoo Med Avian Calcium, Zoo Med Labs, Inc, San Louis Obispo, CA 93401) and ESB Plus dust (Pantex Holland, BV, The Netherlands), a coccidia-stat supplement, are given on insects on an alternating schedule throughout the breeding season.

Wild, native insects are provided as often as possible, with a goal of supplementing each enclosure with wild insects once every day. The amount provided is dependent upon staffing, weather conditions, and the insect population at collection sites. Enclosures with chicks or newly independent HYs are prioritized for wild insects if the whole flock cannot be supplemented. Native insects are only fed out during breeding season due to a decrease in insect populations during colder off-season months. Native insects are not dusted with vitamin supplements prior to feeding.

Sparrow winter diets consist of commercial passerine seed (Higgins Supreme Finch Seed), insectivore grain, and feeder mealworms only. Like wild sparrows (Skipper & Kim, 2013), Florida grasshopper sparrows at White Oak adapt winter consumption to rely heavily on seed and decrease insect intake. Because the nutrition contents of commercial seed are readily available, the current study focuses on feeder and wild insect nutrition content and does not discuss seed nutrients.

Sampling Methods

Feeder Insects

Prior to sampling for analysis in Spring 2020, feeder insects delivered to White Oak in a single shipment were gut-loaded following White Oak's diet protocol. This requires all insects to be gut-loaded for at least 24 hours before being fed to sparrows. Crickets were fed Fluker's® High-Calcium Cricket Diet and Fluker's® Cricket Quencher Calcium Fortified (Fluker Farms, Port Allen, LA 70767) for 48 hours prior to sampling. Mealworms were gut-loaded with thinly sliced sweet potato rounds for 24 hours prior to collection. Waxworms were gut-loaded with a "Gold Grub Mix" medium (A. Leiberman, personal communication) for 48 hours prior to sampling.

All feeder insects were housed identically to those fed to the sparrows and were collected by selecting a random subset of each type of insect from the larger population. One-hundred and thirty grams of gut-loaded crickets, 130g of mealworms, and 130g of waxworms were each sampled and frozen for two days prior to shipment for analysis. Insects were not dusted with any supplements prior to shipment.

Wild Insects

Wild insects were captured using sweep nets at sites on White Oak's property that have been designated for wild insect collection for sparrow diets. These grassland sites typically have an abundant variety of wild arthropods and are free of pesticides and herbicides. Sweeping occurred across four days, with sampling lasting about 20 minutes across multiple frequented sweep sites. Captured insects were then frozen and sorted to remove foliage and other unwanted material. Insects were kept on ice during sorting to avoid thawing and re-freezing until samples could be sent out. Collection continued until 130g of native insects were obtained to be sent out for analysis.

Nutritional Analysis

All samples were sent to MidWest Laboratories (Omaha, NE, 68007) for nutritional testing. All commercial feeder and wild insect samples were subjected to the same three analyses: vitamin-A content, including both retinol and beta-carotene, protein content, and amino acid panel content. These were selected based on these nutrients' critical roles in plumage health, coloration, and structure (Hill, 2003; Strivastava *et al*., 2011; Mendes-Pinto *et al.*, 2012).

Results and Discussion

Vitamin A Content

Total vitamin A (retinol and beta-carotene content combined) was higher in wild insects (14,055 IU/100 g) than in their commercially-raised counterparts. Gut-loaded mealworms had higher total vitamin A content (3,700 IU/100 g β-carotene) than gut-loaded crickets (650 IU/100 g retinol). Feeder waxworms contained no detectable vitamin A. All vitamin A content reported as dry weight measurements (Table 1).

Wild insects had the highest levels of beta-carotenoids compared to all feeder insects. Retinols were low in all insect types, with only crickets containing more retinol than beta-carotene. Beta-carotene was only present in wild insects and mealworms.

Sparrows, like all vertebrates, cannot synthesize vitamin A and its precursor, β-carotene, and as a result are reliant on dietary resources such as insects to obtain these nutrients (Perera & Yen, 2007). Given that vitamin A plays a critical role in integumentary coloration and health, including that of feathers (McGraw *et al.*, 2005), reproductive success, and immune function (Navarro *et al.*, 2010; Green & Fascetti, 2016), it is vital that birds' diet be rich in this essential nutrient. Although the optimal level of vitamin A for a Florida grasshopper sparrow is currently undefined, as most journals describe vitamin A levels necessary for household *Psittacines* (Stahl & Kronfield, 1998), it is clear the wild insects are the superior source of the vitamin. Our results are consistent with other studies that have found feeder insects to be insufficient in nutrients such as vitamin A and its precursors (Finke, 2002).

Protein Content

Crickets had the highest ratio of protein to water content (78%), followed by wild insects (68.9%). Moisture content and protein content were almost identical in both crickets and wild insects. Mealworms and waxworms both had noticeably lower protein content (52.3% and 48.1%, respectively) than crickets or wild insects.

Studies on chick growth in commercial Galliformes concluded that consuming an adequate percentage of protein is imperative for chick development, including feather development and feather structure (Leeson & Walsh, 2003). Although protein requirements for Florida grasshopper sparrows are not known, literature suggests passerines have a higher turnover rate of body proteins compared to non-passerines and require a high-protein diet for proper development (Allen & Hume, 2001).

Amino Acid Content

Feeder crickets and wild insects had the highest dry weight percentages across all amino acids except tryptophan and serine (Table 2). Serine was most prevalent in waxworms, while tryptophan was highest in both mealworms and wild insects. Glutamic acid and alanine had the highest percentages across all four insect types, while tryptophan had the lowest dry weight percentages across all insect types. Sulphur-containing amino acids cystine and methionine were highest in feeder crickets and wild insects. Mealworms and waxworms contained similar dry-weight percentages of almost all eighteen amino acids.

Sulfur-containing amino acids (methionine and cystine), have critical implications in feather development. Lack of these amino acids can result in poor quality feathers and could lead to pale barring or fault barring in developing passerines (Murphy *et al.*, 1988; Kahn, 2005). Leucine and serine, amino acids essential in the protein Keratin, are highly involved in feather structure, specifically at the rachus (Strivastava *et al.*, 2011; Mendes-Pinto *et al.*, 2012), and could cause constitutional feather weakness if not present in adequate amounts. Aside from their structural significance, amino acids also combine with proteins and carotenoids in pigment-protein interactions, resulting in species-specific feather coloration and patterning (Mendes-Pinto *et al.*, 2012). The amino acid and protein levels in our current study are consistent with other studies that have analyzed feeder insect nutrition (Finke, 2002; Bednarov *et al.*, 2014).

Conclusions

The current study found that feeder insects are deficient in essential levels of vitamin A and β-carotene and are most likely insufficient without wild insect supplementation to provide adequate protein and amino acid content as well. As a result, the most obvious course of action to mitigate plumage abnormalities at White Oak is to increase the amount of wild insects fed to grasshopper sparrows. The availability of wild insects, however, can be limited by inclement weather and insect population density constraints.

One key adjustment in addition to prioritizing wild insects could be providing more vitamin A in the form of dusting commercial feeder insects with powdered supplements. While insect diets are already supplemented with NEKTON-S powder, alternatives, such as NEKTON-Gelb, are higher in carotenoids and may provide a better source of critical vitamins. A comparative study of plumage brightness and quality in Florida grasshopper sparrows at White Oak between birds given

NEKTON-S and NEKTON-Gelb is currently in progress (Crenshaw *et al.*, manuscript in preparation).

Acknowledgments

Thank you US Fish and Wildlife Service, Fish and Wildlife Foundation of Florida, Florida Fish and Wildlife Conservation Commission, and White Oak Conservation for supporting the Florida grasshopper sparrow program and therefore this project. Thanks to all of the White Oak interns that helped make this project happen and who cared for the Florida grasshopper sparrow flock at White Oak. Thank you to the White Oak health team for their support. Thank you to Heidi Bissell for consultation and encouraging us to investigate Florida grasshopper sparrow diet and nutrition.

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Table 1. Raw break down of vitamin A content across all four insect sample types fed to Florida grasshopper sparrows (*Ammodramus savannarum floridanus*) at White Oak Conservation. All nutrients presented as dry weight IU/100g units.

Insect Sample	β-carotene	Retinol	Total vitamin A
Feeder crickets (*Acheta domestica*)	n.d.[1]	650	650
Feeder mealworms (larval *Tenebrio molitor*)	3700	n.d.	3700
Feeder waxworms (larval *Achroia grisella*)	n.d.	n.d.	n.d.
Wild Insects (mainly arthropods)	14000	55	14055

[1]Nutrient unable to be detected in sample

Table 2. Raw break down of eighteen essential amino acids across all four insect sample types fed to Florida grasshopper sparrows (*Ammodramus savannarum floridanus*) at White Oak Conservation. All nutrients presented as dry weight percentages (% DM).

Amino acid	Feeder crickets	Feeder mealworms	Feeder waxworms	Wild Insects
Aspartic acid	5.29	3.84	4.35	4.60
Threonine	1.59	1.20	1.21	1.59
Serine[2]	3.02	2.27	3.88	2.76
Glutamic acid	8.56	5.81	5.57	7.26
Proline	3.86	3.50	3.02	3.79
Glycine	4.11	2.67	2.66	3.89
Alanine	5.75	3.97	3.58	6.91
Cystine[1]	1.93	1.04	0.86	1.20
Valine	3.57	3.00	2.34	4.28
Methionine[1]	3.57	1.60	1.72	2.14
Isoleucine	2.73	2.24	1.92	2.79
Leucine[2]	4.62	3.47	2.99	4.96
Tyrosine	3.65	3.04	2.90	3.83
Phenylalanine	2.43	1.77	1.72	2.17
Lysine (total)	4.20	2.84	2.66	3.73
Histidine	1.64	1.60	0.98	1.56
Arginine	4.53	2.40	2.31	4.02
Tryoptophan	0.53	0.37	0.30	0.44

[1]Sulpher amino acids
[2]Amino acids essential in the protein keratin