DIETARY IMPACTS ON IMMUNE COMPETENCE

by

Andrew Mittler Hydrusko

A thesis submitted to the Faculty of the University of Delaware in partial
fulfillment of the requirements for the degree Master of Science in Biological Sciences

Fall 2012

DIETARY IMPACTS ON IMMUNE COMPETENCE

by

Andrew Mittler Hydrusko

Approved: _____
Michael C. Moore, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
Randall Duncan, Ph.D.
Chair of the Department of Biological Sciences

Approved: _____
George H. Watson, Ph.D.
Dean of the College of Arts and Sciences

Approved: _____
Charles G. Riordan, Ph.D.
Vice Provost for Graduate and Professional Education

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Diet has a significant impact on the functioning of the immune system, but there is a lack of understanding specifically on how it affects the differing humoral and cellular components of the innate and acquired immune system. We used House Sparrows (*Passer domesticus*) as a model to look at the impact of dietary composition on these components. Thirty six wild birds of both sexes were collected and maintained on either a high or optimal fat diets. We tested two hypotheses for the impact of diet on gender, one proposing that immunocompetence is energy limited and one proposing that immunocompetence is nutrient limited. To assess the components of the immune system we used several different assays. Bacterial killing assays were used to gauge the humoral constituent of innate immunity while the hemolysis-hemagglutination (HH) assay was used to measure humoral innate immunity. Acquired immunity was tested with sheep red blood cell (SRBC) assays. Phytohemagglutination Assays (PHA) were used to gauge overall immune response. Diet affected PHA response differently in the two sexes with males responding more to calories and females to nutrients. Bacterial killing ability was not affected by diet but was tied to bird gender, with females exhibiting greater bactericidal ability than males. Females also exhibited stronger primary immune responses than males in the SRBC assays, with correlated secondary and primary immune responses. There was no interaction between any of the immune tests. This reinforces the resilience of the

house sparrow to changing dietary conditions while calling attention to a definite

separation of the components of the immune system. Further work involving greater

dietary challenges will be needed to understand the impacts on these individual

systems.

Chapter 1

INTRODUCTION

Immune responses are energetically costly to mount and other functions related to fitness can be impaired during immune responses (Martin, Scheuerlein & Wikelski, 2002), especially when resources are scarce. For example, in food restricted lizards, increased reproductive costs via follicle stimulating hormone injection led to decreased wound healing ability, a benchmark for general immune competence (French, DeNardo & Moore, 2007).

Resource limitation is a function of both the abundance and quality of food. Restrictions of specific dietary nutrients can reduce immune responses when resources are limited. Nestling Zebra Finches (*Taeniopygiag uttata*) consuming low quality diets exhibited lower growth rates, increased mortality, and decreased cellular immunity (Birkhead, Fletcher & Pellatt, 1999). Similarly, nestling Mountain Bluebirds (*Sialia currucoides*) receiving supplemental carotenoids in the diet exhibited enhanced t-cell mediated immune responses and increased rate of mass gain (O'Brien & Dawson, 2008). Female hamsters deprived of glucose showed compromised t-cell immunity (Martin, Weil, Bowers & Nelson, 2008), and mice fed vitamin A enriched diets exhibited enhanced t-cell mediated cytotoxicity and increased tumor resistance (Malkovsky, Dore, Hunt, Palmer, Chandler & Medawar, 1983).

1

We were specifically interested in examining the effects of high and low fat diets on immune responses. We chose House Sparrows (*Passer domesticus)* as our experimental subject because they consume a wide variety of food naturally. House sparrows are an omnivorous bird commensal with humans with a very wide spread invasive range, and are commonly found almost anywhere people settle (McKinney, 2002). Because of this, as the human diet has increasingly incorporated more fats, the diet of the house sparrow has also changed. For example, birds dwelling in urban settings have increased levels of blood cholesterol and urea nitrogen, and have diets documented to be higher in fat and protein than their rural counterparts (Gavett & Wakeley, 1986). We simulated this by feeding the birds one of two experimental diets, one consisting entirely of Mazuri, a commercial bird feed formulated to provide optimum nutrition for small birds, and a diet consisting of half Mazuri and half chopped up and dried french fries (purchased from a fast food restaurant). The latter experimental diet represents a lower quality diet simulating food available at fast food restaurants and dumpsters where these birds often feed in urban environments.

We compared House Sparrows fed these two diets to test two alternative hypotheses about the effect of diet on immunity. The first hypothesis, based on other studies (Butcher & Miles, 2011, Pap, Czirjak, Vagasi, Barta & Hasselquist, 2010), proposes that the lack of other nutrients in the high fat diet would compromise the immune system. This hypothesis, which we will call the Nutrient Limitation Hypothesis, predicts that subjects on the higher fat diet will exhibit weaker immune responses than those on the lower fat diet. Alternatively, immune function may be

limited by availability of calories (French, Denardo, & Moore, 2007), which is greater in the high fat diet. This hypothesis, which we will call the Energy Limitation Hypothesis, predicts greater immune responsiveness on the high fat diet.

House Sparrows offer additional specific advantages that make them good subjects for this research. As wild birds, House Sparrows retain individual genetic diversity, compared to classic lab models which have eliminated genetic variation (Smale, Heideman, & French 2005). In addition, House Sparrows adapt well to captivity, allowing us to control the diet and environmental conditions, ensuring as little variability as possible in experimental treatments between individuals. Additionally, all birds were captured during the same time of year, ensuring that no birds were undergoing molt or reproducing, two events documented to impact immune function via energetics **(**Pap, Czirjak, Vagasi, Barta & Hasselquist, 2010**)**.

The immune system is complex and has multiple facets, all of which have some separation of function and contribute to the defense of the organism in different ways. Many previous studies have used a single measure of the immune system, PHA, when assessing the immune competence of birds **(**Martin, Han, Lewittes, Kuhlman, Klasings & Wikelski, 2006**)**. Using only a single measurement may underestimate or overlook effects on immune function. To address this issue, we used several different immune assays targeted at different components of the immune system in an attempt to do more comprehensive assessment of immune function. Phytohemagglutinatin assay (PHA), which is a partial measure of overall immunity, measures localized swelling due to infiltration of immune cells originating both from the innate and

acquired immune system. Using this widely used technique also allows us to compare our results to others. However, we expanded on standard practice by including three additional assays that measure separate components of the immune system. The bacterial killing assay (BKA) is a humoral measure of innate immune function, measuring complement function. The Hemolysis-Hemagglutination assay (HH) gauges the humoral component of innate immune competency, utilizing naturally occurring levels of hemagglutinins. The sheep red blood cell assay (SRBC) gauges adaptive immune competency, examining the primary and secondary response to an antigen (sheep red blood cells) the birds have been innoculated with. We used these assay to assess the impact of our experimental diets on various components of immunity in House Sparrows. We were especially interested in determining if these responses were correlated, suggesting that these components are regulated together and that measuring one is a sufficient assessment of immune function, or not correlated, suggesting they are regulated independently, and have to be assessed independently.

Chapter 2

MATERIALS AND METHODS

Thirty six House Sparrows (*Passer domesticus,* 15 male, 21 female*),* were

collected via mist nets in Wilmington (1/16/2012) and Middletown (12/29/2011),

Delaware and in Oxford, Pennsylvania (1/9/2012, 1/19/2012). Birds were tagged with

numbered leg bands for future identification. Gender was identified by plumage.

Birds were transported to the laboratory and kept in individual cages at room

temperature on an 8:16 light:dark photo-period. Food and water were continually

provided and replenished on a daily basis. Food consumption was tracked by weighing

all food provided and weighing the remainder each day. Spillage was accounted for by

collecting paper rolls placed beneath the cages to catch waste on a bi-weekly basis and

manually separating feces and food. At the end of the experiment, all subjects were

gradually phased back on to a sunflower seed diet for several days and then released at

place of capture.

2.1   Experimental Design

Birds were fed an initial diet of sunflower seed, and gradually switched over to

Mazuri Complete Small Bird Diet during a two week acclimation period, moving from

100% sunflower seed to 100% Mazuri in 25% intervals. Once birds from a given

block were acclimated to captivity and the Mazuri diet, they were either kept on

Mazuri or switched to the experimental diet consisting of half Mazuri and half minced

dried french fries (purchased at a fast food restaurant) by weight. Fries were left

exposed to air for two or three days until completely dried, and then finely minced in a

food processor. An equal weight of Mazuri was then added, and the mixed diet was

blended together in the food processor to make it difficult for birds to consume the

food components seperately. Visual inspection of the food cups and spillage showed

that birds were eating equal amounts of both food components. All birds were

randomly assigned without respect to gender to either the Mazuri (control) or Mazuri

and French Fry diet (experimental) for a total N of 18 per group (Table 1). Birds were

run through the treatments staggered in three separate blocks based on date of capture.

The experiment for each block began at the end of the 14 day acclimation period when

experimental diets were begun for half the birds in the block (Experimental Day 0).

Experimental Day 0 began for block 1 on 18 January 2012, block 2 on 19 January

2012 and Block 3 on 08 February 2012. Phytohemagglutinin was injected into the

wing web on Experimental Day 30. Swelling measurements and blood were taken 24

hours post injection. Sheep red blood cell injections occurred on Day 32, and then

again on day 39. Primary responses were taken one week post injection and secondary

responses two weeks post initial injection. Body mass was recorded on day 0, day 30,

day 37 (primary response), and day 44 (secondary response).

2.2 Collection of Blood Samples

Blood was collected by puncturing the brachial artery with a 26g needle and collecting approximately 75-150uL blood into heparinized microcapillary tubes. Blood was then separated by centrifuge, and the plasma decanted and frozen at -81°C.

2.3 Bacterial Killing Assay

The bacteria killing assay protocol was adapted from Matson, Tieleman & Klasing, 2006. Collected plasma was thawed at room temperature. 10mL of pH 7.2 phosphate buffer was added to two 15mL falcon tubes and warmed to 37°C. A bacterial pellet with a known count of 1×10^7 of colony forming units was added to one falcon tube with sterile forceps and dissolved over 30 minutes, with mixing by inversion at the 15 minute mark. 100uL of the bacterial pellet solution was added to 10mL of the phosphate buffer to form a working solution. For each subject, 4uL 200mM L-glutamine, 10uL heat-inactivated fetal bovine serum, 183 uL CO_2-independent media, and 3uL plasma was vortexed in a micro centrifuge tube. 3 tubes per batch received 3uL extra media instead of plasma to act as no plasma controls. 20uL of the working solution was then added to each tube (20uL of extra media into 3 tubes to provide no bacteria controls), mixed via flicking, and incubated at 41°C for 30 minutes. After incubation, the tubes were vortexed for five seconds, plated onto labeled agar plates, allowed to dry for 2-3 minutes (3 plates were left open as procedural controls), and incubated for 18 hours at 37°C. The number of bacterial colonies per plate was then counted and recorded. Colony number was then compared

with and turned into a percentage of the no plasma controls to provide a measurement of bactericidal ability.

2.4 Phytohemagluttin Skin Swelling Measurement

Protocol was adapted from Martin, Han, Lewittes, Kuhlman, Klasings & Wikelski, 2006. Feathers were trimmed back from the left and right wing web with a small set of scissors and thickness was measured, with one person holding the bird while the other used an electronic screw micrometer placed over the wing web. Each wing was measured 3 times and averaged. Then 0.1mL of 1mg per mL of phytohemagluttin (PHA) was injected into the skin of the left wing web and marked with a black permanent marker. Measurements were taken again 3 times for each wing web 24 hours later. Tissue cores were then taken for future analysis using a metal tissue corer placed over and forced through the upper layer of the wing web.

2.5 Hemolysis-hemagglutination Assay

Hemolysis-hemagglutination assays were carried out on the house sparrows as detailed in Matson, Ricklefs & Klasing, 2004. Twenty five microliter of plasma samples taken from day 0 and day 30 birds were pipetted into the first two columns of a 96 well plate. 25uL of 0.01 M phosphate buffered saline was then added to coluns 2 through 12. Column two was then serially diluted at a 1:2 ratio through column 11. Column 12 was used to serve as a negative control. Post dilution, 25uL of 1% rabbit blood cell suspension was added to all wells. The plates were then gently mixed on using a vortex and incubated in an oven at 37°C for 90 minutes. Post incubation,

8

plates were rested at 45° angle for 20 minutes to allow for easier visualization, and then scanned on a flatbed scanner for analysis (quantification of lysis/agglutination), and then again after resting for 70 minutes to allow for cell lysis.

2.6   Sheep Red Blood Cell Assay

Birds were injected twice with 200uL 10% Sheep red blood cell suspension (previously washed 3 times in 1 x PBS solution) in the pectoral muscle, one initial injection and a secondary one a week later.. Blood was taken 1 and 2 weeks post initial injection from the brachial vein, which was cleaned pre-bleeding with 70% ethanol solution. Assays were performed in 96 well round bottomed assay plates (8 rows by 12 columns). Each well of column 1 and 2 received 25uL of plasma, with each row being a different subject. 25uL of 0.01 M phosphate buffered saline (PBS) was added to rows 2-12. Once this was done, serial dilutions were carried out at a ration of 1:2 up to column 11. The last column served as a negative control. All wells then received 25uL of 1% rabbit red blood cell suspension the pre-injection samples, and 1% sheep red blood cell suspension. Plates were then covered and taped shut, and incubated at 37°C for 90 minutes. Once this was completed, plates were rested at a 45° angle to allow for visualization, and scanned using a flatbed scanner for analysis (quantification of lysis/agglutination).

2.7   Statistical Analysis

Data was analyzed with two way ANOVA. Body weight was analyzed with repeated measures ANOVA. BKA 30 day colony forming units were averaged and

recorded as percentages against the blood-free controls, then transformed using arcsine square root to preserve normal distribution. HH and SRBC underwent log transformation prior to analysis. Multivariate analysis was used to determine correlations.

Chapter 3

RESULTS

3.1 Body Weight and Food Consumption

Birds apparently ate equal amounts of both diets and there was no significant

difference in overall food consumption by diet (Figure 2; F1 = 0.036, P = 0.85) or

gender (F1 = 0.00, P = 0.99), and no interaction between the two (F1 = 0.09, P =

0.77).   To determine how diet composition affected consumption of macronutrients,

we used the dietary information provided by the manufacturers (Table 1) and

multiplied these by the daily consumption of each bird. We did the same for calorie

consumption.

The biggest difference between the experimental and Mazuri diets was that the

former had three times as much fat as the latter (Table 1). It is therefore not surprising

that birds on the experimental diet consumed significantly more fat than birds on the

pure Mazuri diet (F1 = 514.56, P < 0.01). There was no difference between the sexes

(F1 = 0.15, P = 0.71) and no interaction between diet and gender (F1 = 0.14, P = 0.71)

for fat consumption. The two diets had almost identical levels of carbohydrate and

carbohydrate consumption did not vary between diets (F1 = 1.92, P = 0.18) or gender

(F1 = 0.09, P = 0.77) and that there was no interaction between diet and gender (F1 =

0.08, P = 0.78). The experimental diet contained somewhat less of both protein and

nondigestable matter. There was however no significant difference in protein

11

consumption between diet (F1 = 0.52, P = 0.48) or gender (F1 = 0.10, P = 0.75), and no interaction was found between diet and gender (F1 = 0.11, P = 0.75). Obviously, if birds on the experimental diet were consuming more fat, they had to be consuming less of something else. We assume they therefor consumed slightly less of both protein and nondigestable matter and because these small differences were spread out over these two categories, they were not detectable statistically. Consistent with their greater consumption of fat, birds on the experimental diet consumed significantly more calories than the birds on the Mazuri diet (F1 = 42.51, P < 0.01), with no significant impact from gender (F1 = 0.11, P = 0.74), and no interaction found between diet and gender (F1 = 0.11, P = 0.75).

Despite consuming almost three times as much fat and almost 30% more calories, House Sparrows on the experimental diet did not gain mass and remained the same body mass as birds on the Mazuri diet. For the four intervals that body mass was measured, mass was independent of sex (F1 = 0.61, P = 1.08) and diet (F1 = 0.61, P = 0.44), and no significant interaction was recorded between sex or diet (F1 = 0.04, P = 0.85) (Figure 1). Thus, body weight appears to be free of influence from sex and diet (Tables 2 & 3), while nutrient consumption is directly correlated with dietary content but not gender. While calorie consumption was higher in the experimental diet, this did not translate into increased body mass via fat storage, indicating that the excess calories may have been used to satisfy other energetic requirements.

3.2 Phytohemagluttin Assay Response

We measured swelling in response to PHA injection so we could compare our results to the results of others and, because the swelling involves several different immune components, to serve as a general measure of immune competence. In our experiment, there was no significant impact of gender ($F_1 = 0.43$, $P = 0.515$) or diet ($F_1 = 0.81$, $P = 0.375$) on swelling. However, there was a significant interaction between diet and gender ($F_1 = 5.38$, $P = 0.027$). Inspection of the graph (Figure 3) suggests that this interaction resulted from males increasing their immune response on the experimental diet, whereas females did not change.

3.3 Bacterial Killing Assay

The Bacterial Killing Assay serves as a general measure of innate immune competence because it determines the preexisting ability of the blood to fight bacterial infection. A stronger innate immune system is indicated when the blood kills greater numbers of bacteria when incubated on a plate. In our study, diet was found to have no impact on the bactericidal ability of the plasma ($F_1 = 1.06$, $P = .312$). However, females exhibited stronger bactericidal ability than males ($F_1 = 5.55$, $P = 0.025$), with no significant interaction recorded between gender and diet ($F_1 = 0.01$, $P = 0.929$). House sparrows on the Mazuri diet killed an average of 30.77 colonies, while birds on the experimental diet killed an average of 36.66. Females on Mazuri averaged 39.8 colonies killed, compared to 50.24 on fries, while males on average killed 18.47

colonies on Mazuri and 23.03 on fries. Thus, although diet had little effect on bactericidal capacity, our study revealed a difference between the sexes (Figure 4).

3.4 Hemolysis Hemagglutination

Hemolysis Hemagglutination serves as a measure of innate humoral immunity. The test integrates both naturally occurring antibodies and the complement system to agglutinate and lyse rabbit red blood cells. The amount of lysis/agglutination occurring down a blood concentration gradient against a constant number of red blood cells is then used to determine the strength of the immune response. Occurrence of agglutination was independent of sex ($F_1 = 0.89$, $P = 0.35$) and diet ($F_1 = 0.16$, $P = 0.69$). No significant interaction was recorded between gender and diet ($F_1 = 0.89$, $P = 0.35$). Lysis was also independent of sex ($F_1 = 0.07$, $P = 0.80$) and diet ($F_1 = 0.10$, $P = 0.75$). No significant interaction was recorded between the two ($F_1 = 0.14$, $P = 0.71$). Thus, diet appeared to have no effect on this parameter (Figures 5 & 6).

3.5 Sheep Red Blood Cell Assay

The SRBC assay tests the acquired immune system of the subject. Individuals are injected with sheep red blood cells, and given one week (primary response) and two weeks (secondary response) to build up acquired immunity to the injected red blood cells. The ability of blood to cause lysis or agglutination is used to determine the strength of the immune reaction. Females had stronger primary and secondary responses than males (Figures 7 & 8, $F_1 = 8.67$, $P = 0.01$) with secondary responses correlated to primary with weaker secondary responses (Figure 9, $F_1 = 0.81$, $P = 0.38$).

14

Diet had no significant impact ($F_1 = 0.63$, P = 0.24), and diet and gender were free of significant interaction ($F_1 = 0.22$, P = 0.64).

3.6 Immune System Component Interactions

There was almost no significant correlation between any of the individual assays. PHA response did not correlate with BKA (P = 0.46), HH lysis (P = 0.07)/agglutination (P = 0.18) titers, or SRBC primary (P = 0.92)/secondary (P = 0.87) response titers. BKA did not correlate with HH lysis (P = 0.48)/agglutination (P = 0.14) titers or SRBC primary (P = 0.10)/secondary (P = 0.59) response titers. HH agglutination titers correlated with SRBC primary response titers (P = 0.47), but not with SRBC secondary response titers (P = 0.90). HH lysis titers did not correlate with either SRBC primary (P = 0.31)/secondary (P = 0.39) response titers. This suggests that these tests were measuring separate components of the immune system that are regulated independently.
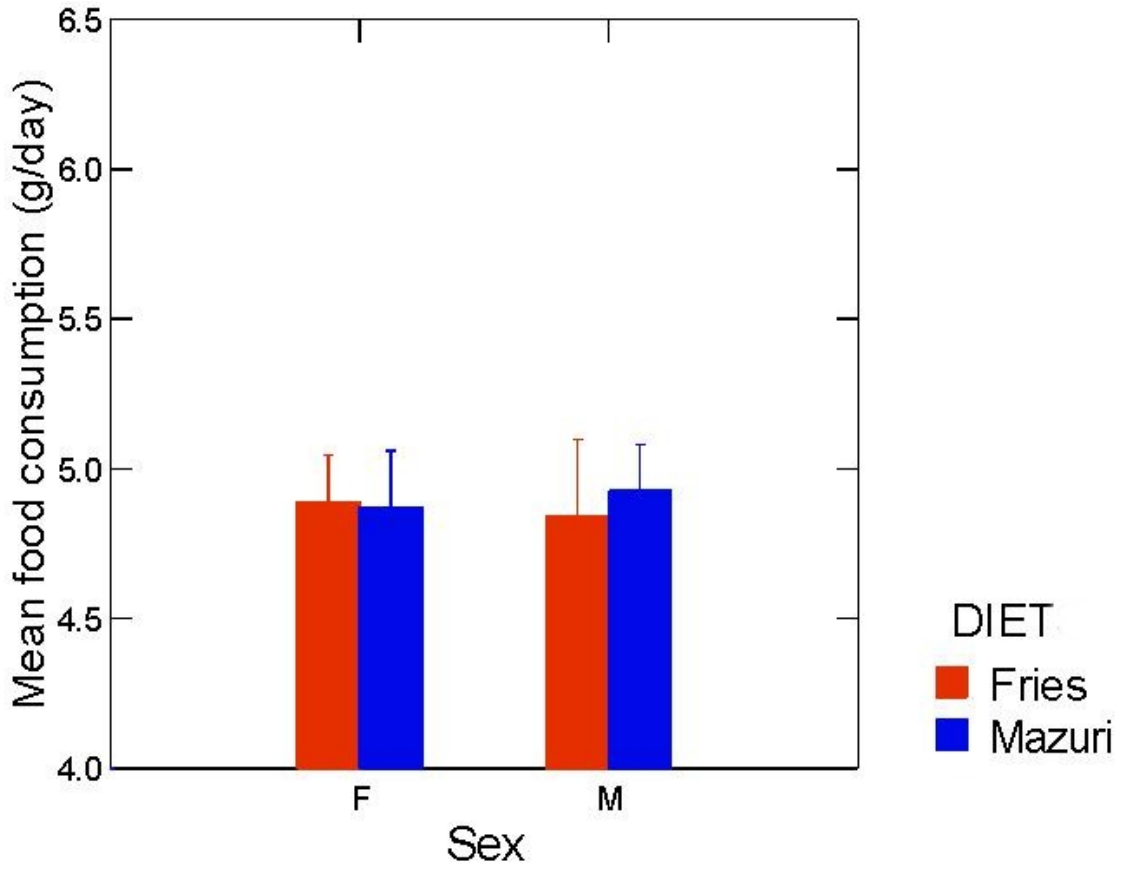
Figure 1.   Average daily consumption of food for male and female House Sparrows over 30 days by diet.
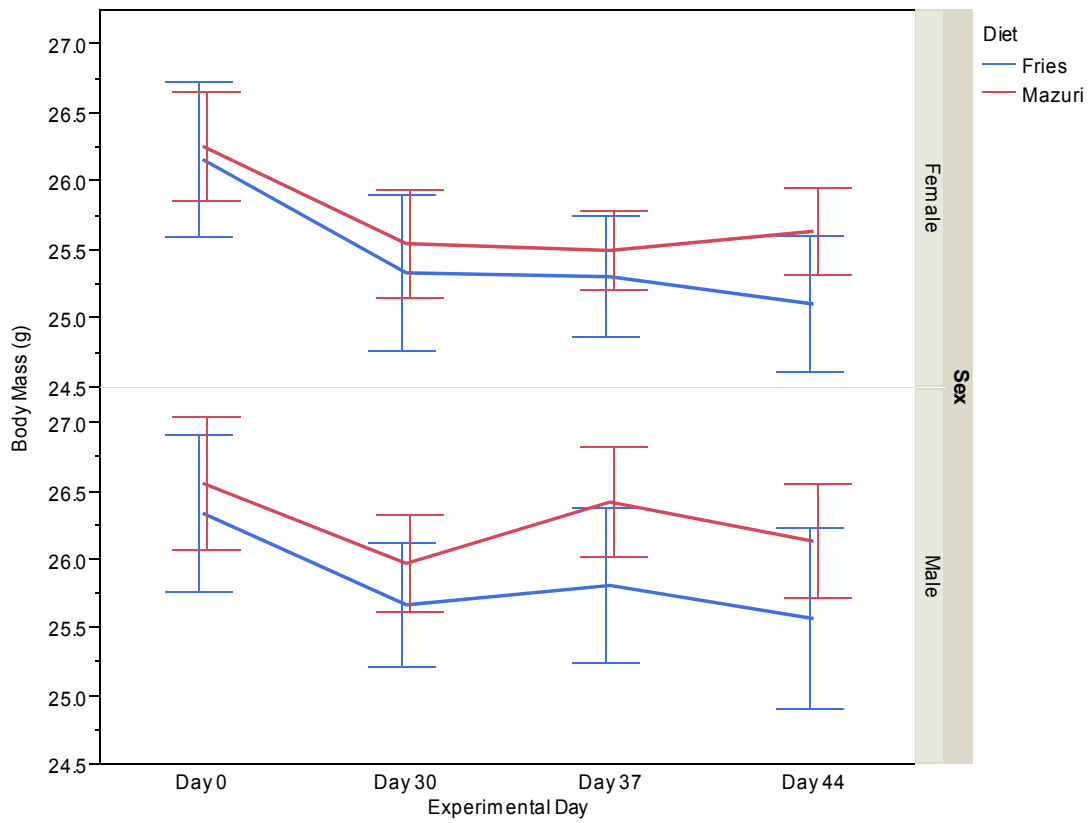
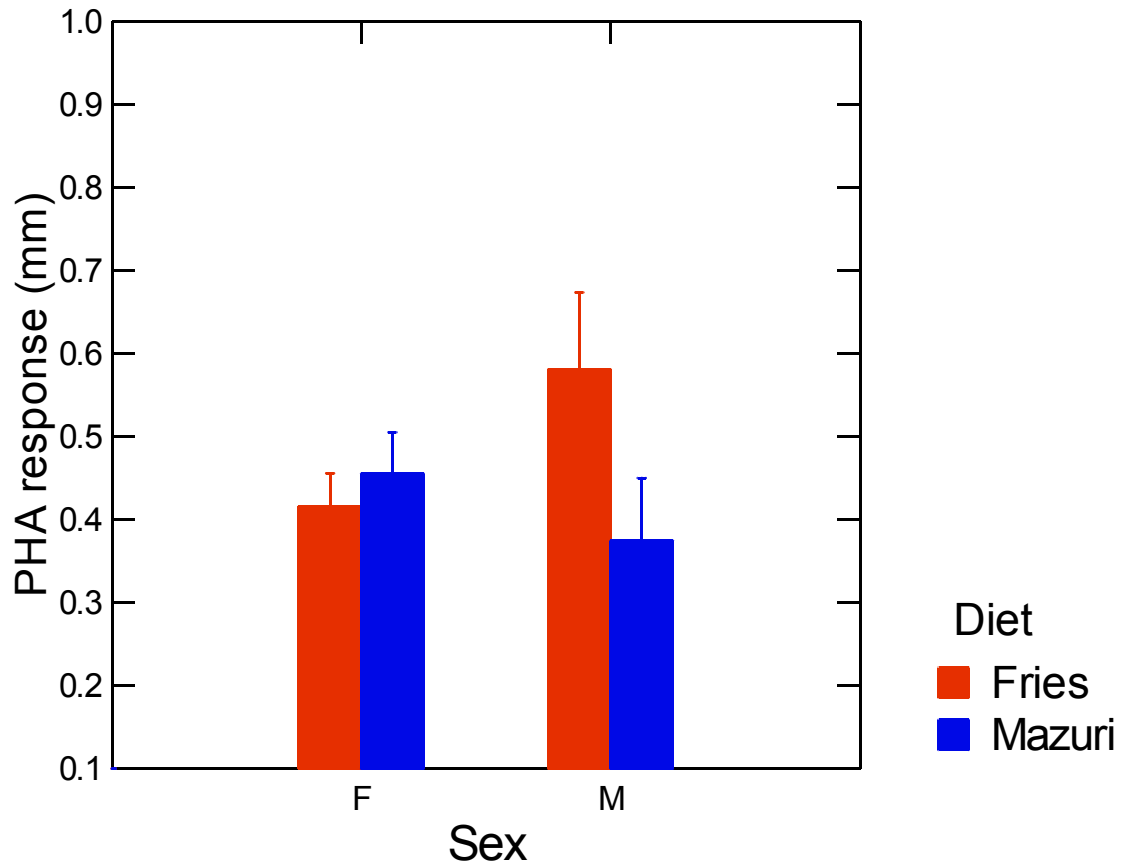Figure 2.   House Sparrow body weight over time according to diet and gender.

Figure 3. Phytohemagluttin response of male and female House Sparrows according to diet.
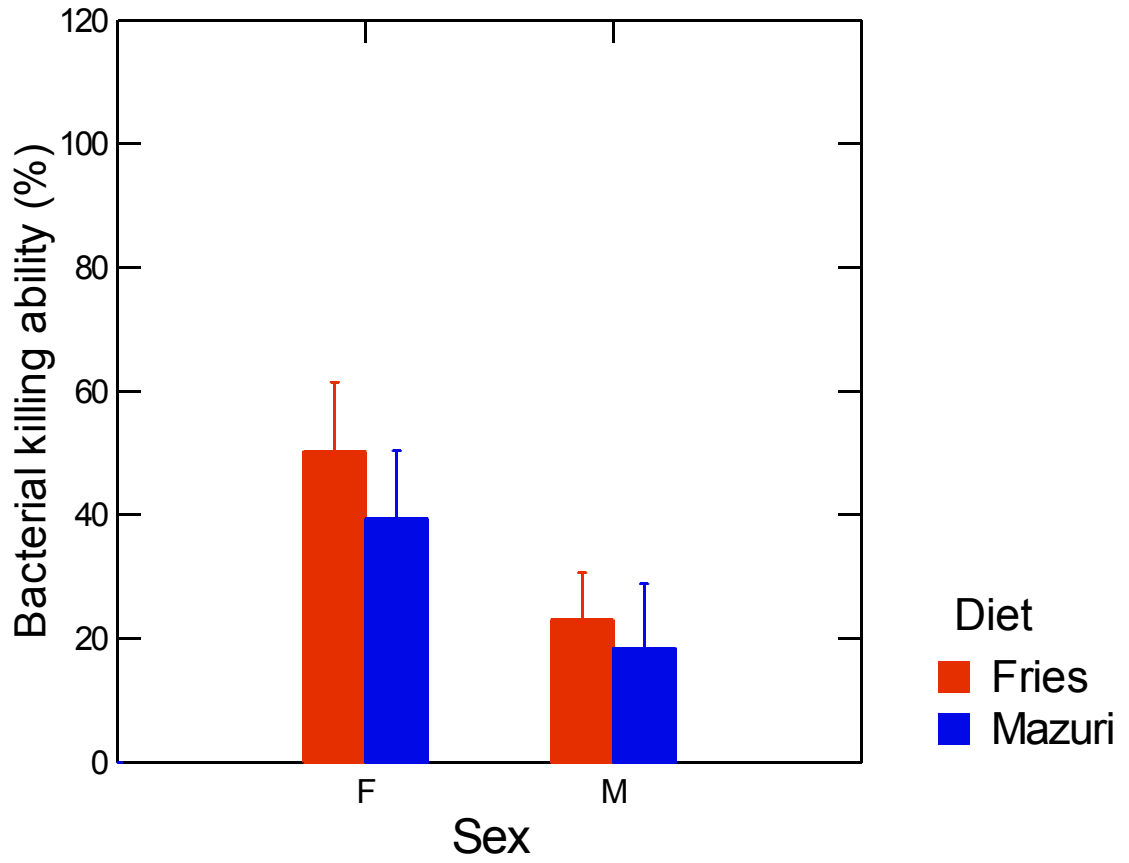
Figure 4   Bacterial killing assay response of male and female House Sparrows according to diet.
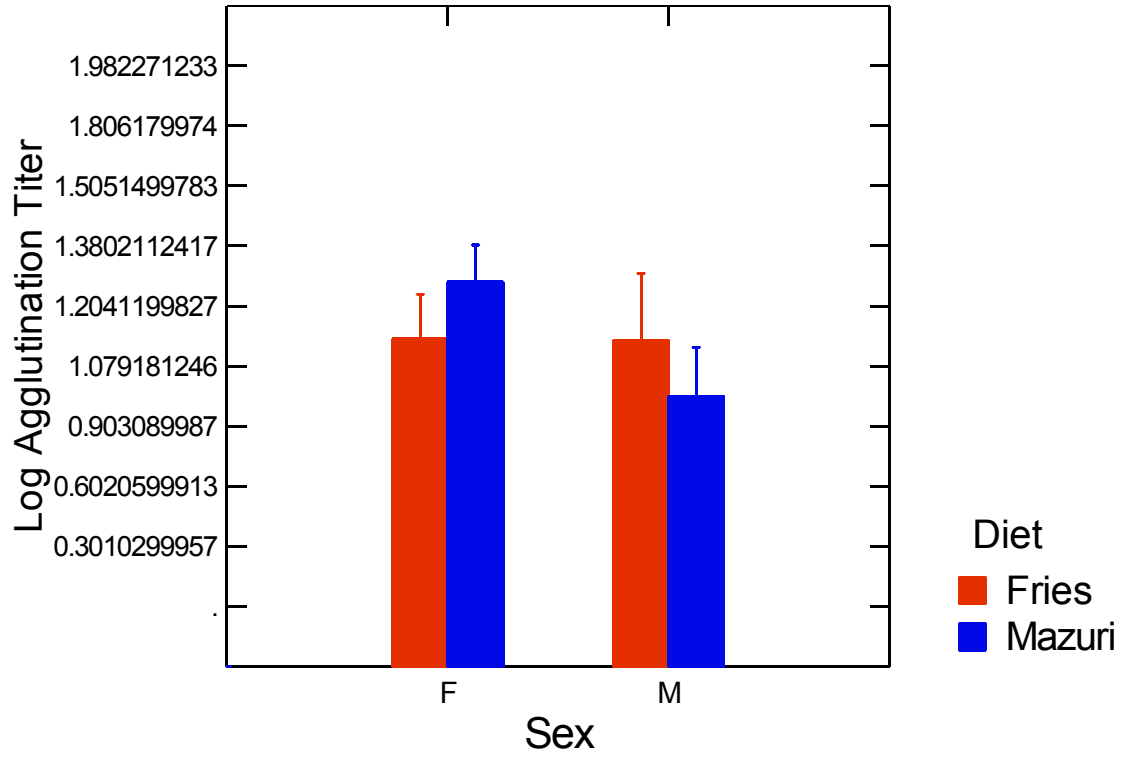
Figure 5. Hemolysis hemagglutination agglutination response of male and female House Sparrows according to diet.
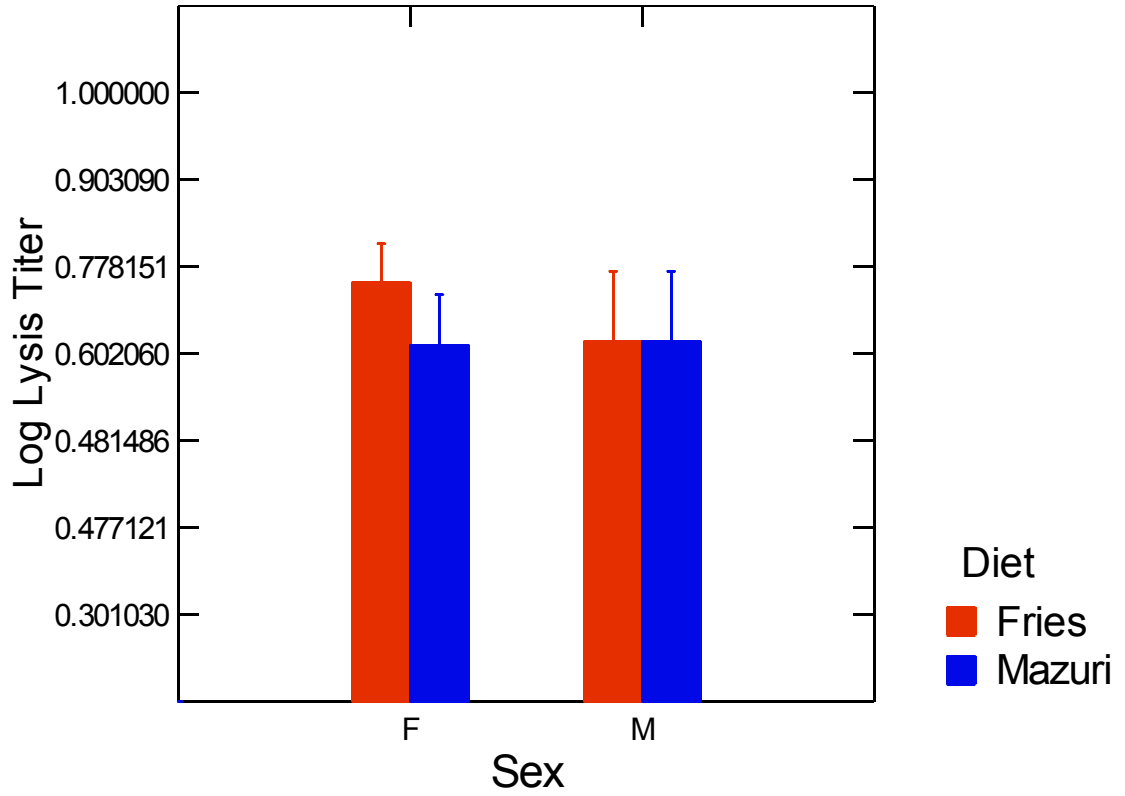
Figure 6.   Hemolysis hemagglutination lysis response of male and female House Sparrows according to diet.
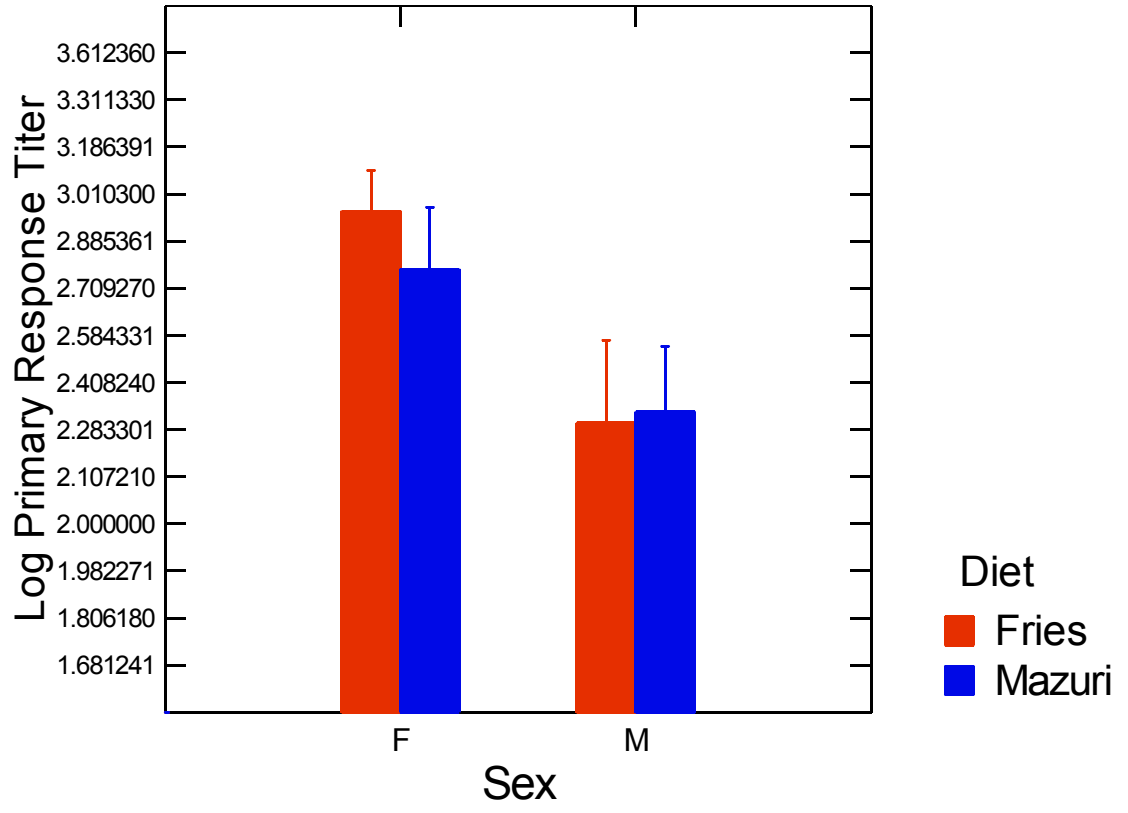
Figure 7.   Sheep red blood cell assay primary response of male and female House
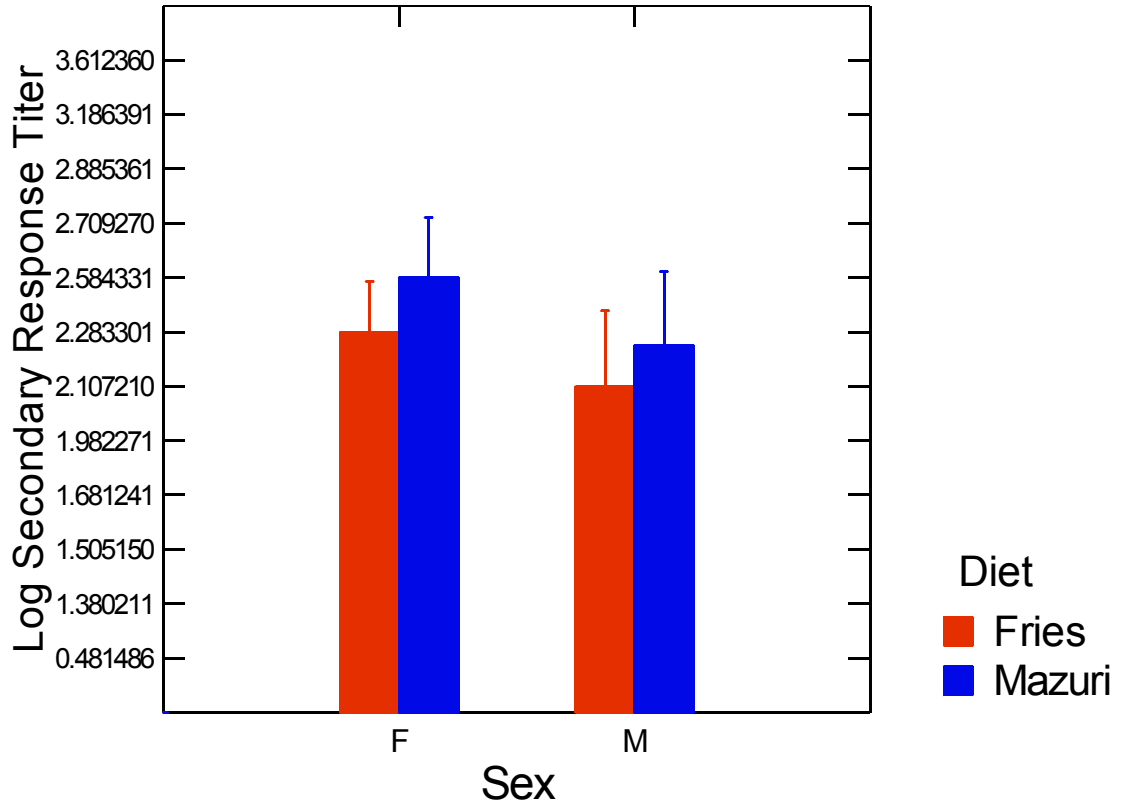Sparrows according to diet.

Figure 8.   Sheep reed blood cell assay secondary response of male and female House Sparrows according to diet.
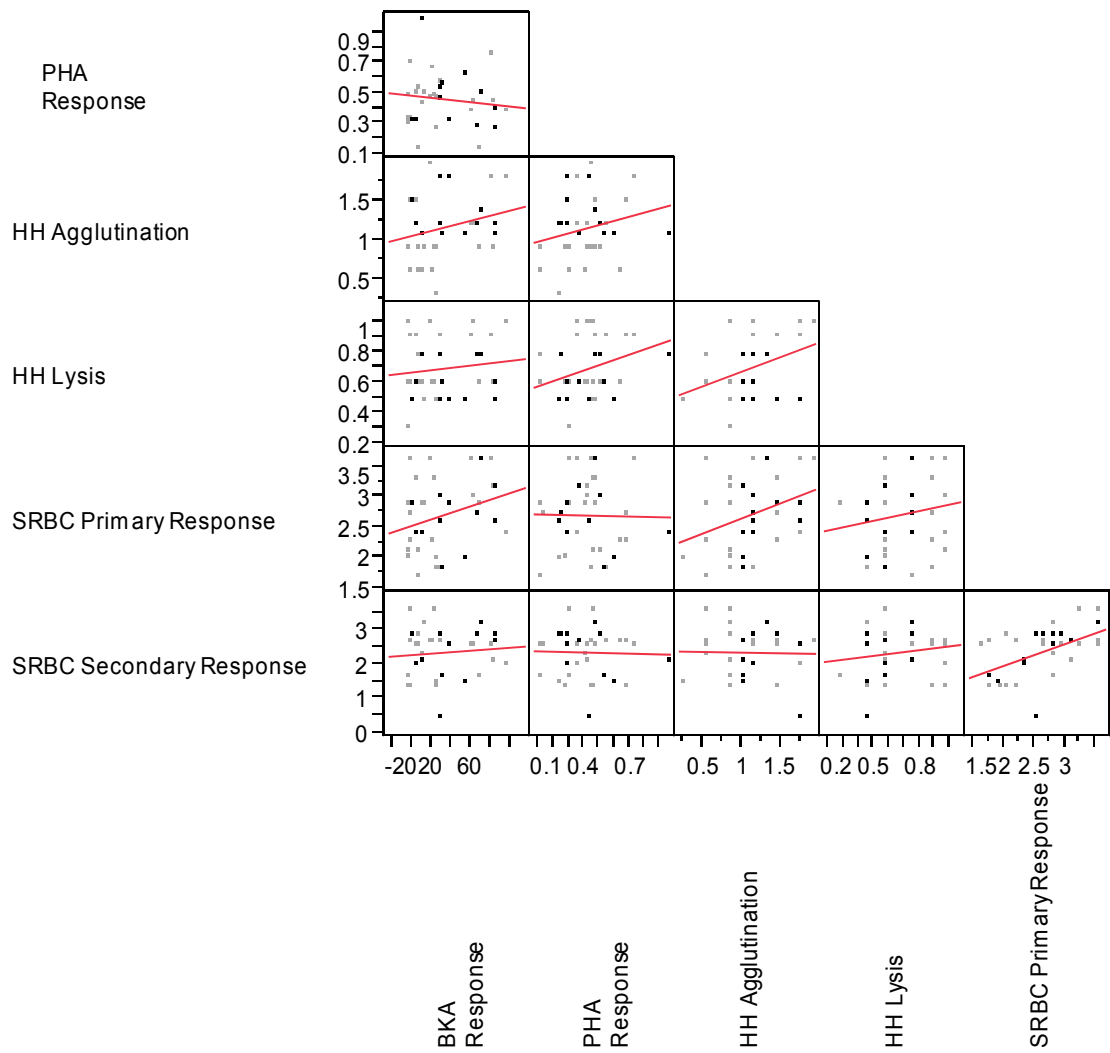
Figure 9.　　Correlations between immune assays.

Table 1.   Dietary composition of food provided per gram (dry weight).
            Experimental diet is a 50/50 mixture of Mazuri and dried Fries so the
            values were averaged from those two diets to obtain values for the
            Experimental Diet.

	Mazuri	Fries	Experimental Diet
Protein	0.15g	0.07g	0.11g
Fat	0.05g	0.24g	0.15g
Carbohydrate	0.66g	0.63g	0.65g
Nondigestable or Moisture	0.14	0.06	0.09
Calories	3.18kcal	5.00kcal	4.09kcal

Table 2.   Estimated daily consumption of nutrients for female House Sparrows.

Females				
Diet	Protein	Carbohydrates	Fat	Calories
Mazuri	0.73g	3.21g	0.24g	15.45kcal
Fries	0.53g	3.13g	0.70g	19.87kcal

Table 3.   Estimated daily consumption of nutrients for male House Sparrows.

Males				
Diet	Protein	Carbohydrates	Fat	Calories
Mazuri	0.74g	3.24g	0.25g	15.61kcal
Fries	0.54g	3.17g	0.71g	20.08kcal

Chapter 4

CONCLUSION

In this experiment, we maintained 36 wild-caught house sparrows under constant environmental conditions, and fed them either a nutritionally complete control diet or an experimental diet high in fat and carbohydrates. The goal of this was to determine the impact of a high fat/carbohydrate diet on the function of the different aspects of the immune system, which we broke down into the humoral and cellular components of the acquired and innate immune systems. These systems were tested by PHA, BKA, HH, and SRBC immune assay, and results were analyzed by two way anova.

Our most interesting result was the interaction between diet and gender during the PHA test, with the males mounting stronger responses on the experimental diet and females responding stronger on the control. This suggests that there are potentially different nutritional requirements for mounting an immune response by gender. Different nutrients also may be limited by gender, requiring one gender to need it in greater quantities to sustain optimal fitness. The response of males was consistent with the Energy Limitation Hypothesis since they showed a stronger PHA response on the high fat, high calorie diet whereas the response of females was more consistent with the Nutrient Limitation Hypothesis since they showed a stronger response on the more micronutrient rich Mazuri diet. For males, it therefore appears that the additional

calories were invested in some components of the immune system. It is unknown how the females used the additional calories, although increased activity levels would be the most obvious hypothesis.

There is a notable lack of literature concerning gender differences in diet as it pertains to immune function, particularly for birds, and highlights a need for further investigation into this area. Differing dietary requirements for optimal growth in birds (Dozier III, Corzo, Kidd & Schilling, 2007) has been documented for protein requirements, with males requiring greater amounts of protein to maintain increased muscle mass. Additionally, non-human primates have been observed to have gender differences in inflammatory response related to a calorie restricted diet (Ebersole, Steffen, Reynolds, Branch-Mays, Dawson, Novak, Gunsolley, Mattison, Ingram & Novak, 2008).

Significant gender differences in immune function were found in the BKA test and SRBC assay, with females exhibiting stronger responses for both. Existing literature has tied testosterone to weakened immune response, linking it with increased levels of "stress hormone" corticosterone. Corticosterone has documented immunosupressive effects, including inhibition of the inflammatory response, a classic measure of immune competence (Evans & Goldsmith, 2000). When corticosterone was controlled for, however, testosterone was actually found to increase antibody levels, through a suggested change in testosterone influenced behavior (aggression and dominance) leading to increased access to resources, enhancing immune capability. Our birds were offered unlimited access to food resources, potentially blunting these

affects that would otherwise occur in a wild environment with competition. Appropriately, these immune changes, are restricted by the annual behavioral cycles of the house sparrow, specifically the mating and breeding stages (both which incur significant energetic cost to the bird through physiological and behavioral changes) with no significant gender variation during the molt and winter stages (Pap, Czirjak, Vagasi, Barta & Hasselquist, 2010).

Our birds were captured during the non-breeding season and held in constant conditions to prevent molting (which incurs additional energetic costs on the bird). While the seasonality is at odds with our findings, this may be explained by a hold-over effect from the breeding season. Nesting birds, particularly cavity nesting birds like house sparrows, are exposed to various parasites. Females spend greater amounts of time on the nest, and thus have greater exposure to parasites (O'Brien & Dawson, 2008). This could lead to elevated immune response that carried over to the winter season.

Our results did not consistently support our hypothesis, and most immune measures showed no effect of diet. Diet only drew a significant response for PHA response. Previous work has directly tied diet to the function of the avian immune system and has demonstrated both nutrient and system specific effect, for example, vitamin E's inhibition of prostaglandin synthesis, preventing prostiglandin inhibition of of inflammatory response (Butcher & Miles, 2011). We chose the experimental diet of 50% Mazuri and 50% french fries in order to avoid serious malnourishment of the birds and to reduce mortality over the course of the experiment. It is possible that this

approach was too conservative to show major effects of diet on immunity that might be revealed by putting birds on an even lower quality diet.

There is a dietary "quality" threshold for nutrient levels, above which there is relatively little impact on the fitness of the organism. For wild Snowshoe Hare (*Lepus americanus*), even with decreasing quality of diet, weight loss did not occur until crude dietary protien dropped beneath 11% (Sinclair, Krebs & Smith, 1982). Akin to this, it is likely that the nutrition provided by the Mazuri in the experimental diet was sufficient to meet the resource needs of the house sparrow immune system, and thus above this quality threshold, with the birds suffering no ill effects. House sparrows are a widespread invasive species and have documented lower costs of mounting immune responses compared to similar birds. For example, PHA and killed bacteria injections decreased metabolic output and egg production in tree sparrows, but not at all in house sparrows. (Lee, Martin II & Wikelski, 2005). House sparrows also may be better equipped to deal with the potential deleterious effects of a high fat diet. This could be tied to their ability to mount successful immune responses on the experimental diet and widespread invasive range.

Also important is the lack of significant interaction between the recorded immune tests. This highlights the separation of the immune components. BKA and HH served as innate immunity tests, while SRBC served as an acquired immune test, and PHA a general competence test. The separation between BKA and HH is particular interesting, however, as it indicates possible separation between the humoral and cellular components of the innate immune system. This echos the findings in

Matson, Cohen, Klasing, Ricklefs, & Scheuerlein, (2005), where there was no correlation between observed plasma and leukocyte immunity at species or individual level. Not only does our finding strengthen their statement that a measure for each type of assay is needed to best characterize the immune competence of a species, but strengthens our decision to do so in our study and highlights the need for multiple assay characterization in future work.

This reinforces the need for understanding of dietary impact on the differing aspects of immune system. In future work, we will need to exert greater levels of dietary stress upon the house sparrows to magnify any potential changes in response strength. Once impacts on specific immune components are identified, we can begin to look at what nutrients are tied to these impacts, opening up paths of research with the potential for great impacts on immunology and medicine.

REFERENCES

Birkhead, T. R., Fletcher, F., & Pellatt, E. J. (1999). Nestling diet, secondary sexual traits and fitness in the zebra finch. Proceedings of The Royal Society: Biological Sciences, 226(1417), 385-390.

Boa-Amponsem, K., Dunnington, E. A., & Siegel, P. B. (1998). Diet and humoral responsiveness of lines of chickens divergently selected for antibody response to sheep red blood cells . Avian Diseases, 42(3), 565-571.

Butcher, G. D., & Miles, R. D. (2011). Interrelationship of nutrition and immunity. Manuscript submitted for publication, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.

Dozier III, W. A., Corzo, A., Kidd, M. T., & Schilling, M. W. (2007). Dietary digestible lysine rquirements of male and female broilers from forty-nine to sixty-three days of age. Poultry Science, (87), 1385-1391. doi: 10.3382/ps.2007-00529

Ebersole, J. L., Steffen, M. J., Reynolds, M. A., Branch-Mays, G. L., Dawson, D. R., Novak, K. F., Gunsolley, J. C., Mattison, J. A., Ingram, D.K., & Novak, M.J. (2008). Differential gender effects of a reduced-calorie diet on systemic inflammatory and immune parameters in nonhuman primates. Journal of Periodontal Research, (43), 500-507. doi: 10.1111/j.1600-0765.2008.01051.x

Evans, M. R., & Goldsmith, A. R. (2000). The effects of testosterone on antibody production and plumage coloration in male house sparrows (passer domesticus). Behavioral Ecology and Sociobiology, (47), 156-163.

French, S. S., DeNardo, D. F., & Moore, M. C. (2007). Trade-offs between the reproductive and immune systems: Facultative responses to resources or obligate responses to reproduction?. The American Naturalist, 170(1), 79-89.

Gavett, A. P., & Wakeley, J. A. (1986). Blood constituents and their relation to diet in urban and rural house sparrows. The Condor, 80(3), 279-284.

Lee, K. A., Martin II, L. B., & Wikelski, M. C. (2005). Responding to inflammatory challenges is less costly for a successful avian invader, the house sparrow (passer domesticus), than its less-invasive congener. Oecologia, 145(2), 244-251. doi: 10.1007/s00442-005-0113-5

Malkovsky, M., Dore, C., Hunt, R., Palmer, L., Chandler, P., & Medawar, P. B. (1983). Enhancement of specific antitumor immunity in mice fed a diet enriched in vitamin a acetate. Proceedings of the National Academy of Sciences of the United States of America, 80(20), 6332-6326.

Martin II, L. B., Han, P., Lewittes, J., Kuhlman, J. R., Klasings, K. C., & Wikelski, M. (2006). Phytohemagglutinin-induced skin swelling in birds: histological support for a classic immuneocological technique. Functional Ecology, (20), 290-299.

Martin, L. B., Scheuerlein, A., & Wikelski, M. (2002). Immune activity elevates energy expenditure of house sparrows: A link between direct and indirect costs?. Proceedings of the Royal Society, (270), 153-158. doi: DOI 10.1098/rspb.2002.2185

Martin, L. B., Weil, Z. M., Bowers, S. L., & Nelson, R. J. (2008). Sex-specific effects of glucose deprivation on cell-mediated immunity and reproduction in siberian hamsters (phodopus sungorus). The Journal of Comparative Physiology B, (178), 623-628. doi: 10.1007/s00360-008-0253-0

Matson, K. D., Cohen, A. A., Klasing, K. C., Ricklefs, R. E., & Scheuerlein, A. (2005). No simple answers for ecological immunology: relationships among immune indices at the individual level break down at the species level in waterfowl. Proceedings of The Royal Society B, (273), 815-822. doi: 10.1098/rspb.2005.3376

Matson, K. D., Ricklefs, R. E., & Klasing, K. C. (2004). A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Developmental & Comparative Immunology, (29), 275-286.

Matson, K. D., Tieleman, B. I., & Klasing, K. C. (2006). Capture stress and the bactericidal competence of blood and plasma in five species of tropical birds.Physiological and Biochemical Zoology, 79(3), 556-564

McKinney, M. L. (2002). Urbanization, biodiversity, and conservation. BioScience, 52(10), 883-890.

O'Brien, E. L., & Dawson, R. D. (2008). Parasite-mediated growth patterns and nutritional constraints in a cavity-nesting bird. Journal of Animal Ecology, (77), 127-134. doi: 10.1111/j.1365-2656.2007.01315.x

Pap, P. L., Czirjak, A., Vagasi, C. I., Barta, Z., & Hasselquist, D. (2010). Sexual dimorphism in immune function changes during the annual cycle in house sparrows. Naturwissenschaften, (97), 891-901. doi: 10.1007/s00114-010-0706-7

Sinclair, A. R. E., Krebs, C. J., & Smith, J. N. M. (1982). Diet quality and food limitation in herbivores: the case of the snowshoe hare. Canadian Journal of Zoology, 60(5), 889-897. doi: 10.1139/z82-121

Smale, L., Heideman, P. D., & French, J. A. (2005). Behavioral neuroendocrinology in nontraditional species of mammals: Things the 'knockout' mouse can't tell us. Hormones and Behavior, (48), 474-483. doi: 10.1016/j.yhbeh.2005.05.002

Appendix A

PERMISSION LETTER

University of Delaware
Institutional Animal Care and Use Committee
Application to Use Animals in Research and Teaching

RECEIVED
FEB 1 4 2011
IACUC
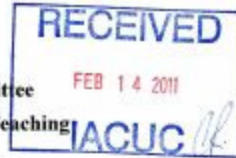
(Please complete below using Arial, size 12 Font.)

Title of Protocol: Tradeoffs between Reproduction and Immunity

| AUP Number: 1218-2011-0 | ← (4 digits only — if new, leave blank) |

Principal Investigator: Michael C. Moore

Common Name: House Sparrow

Genus Species: *Passer domesticus*

Pain Category: *(please mark one)*

USDA PAIN CATEGORY: *(Note change of categories from previous form)*

Category	Description
☐ B	Breeding or holding where NO research is conducted
☐ C	☐ Procedure involving momentary or no pain or distress
☐ D	Procedure where pain or distress is alleviated by appropriate means (analgesics, tranquilizers, euthanasia etc.)
☒ E	Procedure where pain or distress cannot be alleviated, as this would adversely affect the procedures, results or interpretation

Official Use Only

IACUC Approval Signature: _Steven D Bar_

Date of Approval: _21 Feb 2011_

34