5-2022

Macronutrient Availability Shapes Host Response to Infection and Feeding Behavior

Weston Gerald Perrine
University of Arkansas, Fayetteville

Macronutrient Availability Shapes Host Response to Infection and Feeding Behavior

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology

by

Weston Gerald Perrine
University of Nebraska
Bachelor of Science in Biology, 2017

May 2022
University of Arkansas

This thesis is approved for recommendation to the Graduate Council

_____

Sarah DuRant, Ph.D.

Thesis Director

_____

Jeff Lewis, Ph.D.

Committee Member

_____

Kris Forbes, Ph.D.

Committee Member

Abstract

Macronutrients in the diet are vital to the physiological processes necessary for an organism to effectively clear a pathogen. Diet can be important to a host's susceptibility to infection and severity of pathology, though results can vary across host-pathogen systems (Sen et al. 2016). Manipulating the ratio of specific macronutrients in the diet is an effective method to begin understanding how individual macronutrients, rather than food types, have on immune responses. Using an avian host-pathogen system, I explored the effects of dietary macronutrient composition, specifically lipid and protein content, on disease pathology and behavior of canaries (*Serinus canaria)* infected with *Mycoplasma gallisepticum* (MG). To do this I conducted two experiments. In experiment one, I provided isocaloric diets comprised of identical ingredients that varied in macronutrient content (20:80 lipid:protein [high protein} or 80:20 lipid:protein [high lipid]) to female canaries, then measured several possible disease outcomes in birds either infected with MG or that were sham infected. In a second experiment, MG- and sham-infected canaries were offered both the high protein and high lipid diets prior to and during infection to assess whether birds exhibit macronutrient specific feeding behaviors during infection. In the first experiment, canaries fed a protein-rich diet, whether infected or not, consumed more calories per week than canaries fed a lipid-rich diet. All birds that were infected exhibited illness-induced anorexia in the first week post infection and experienced a significant decline in body mass. Infected birds fed the high protein diet had a significant decline in fat stores post infection. Diet did affect visible pathology of the infection; infected birds fed the high lipid diet exhibited clinical signs of infection (swollen eye conjunctiva) 5 ± 3 days longer than birds on the protein diet, as measured by eye score. Despite these differences in eye score over time, the post-infection prevalence of MG specific antibody and pathogen load was not

significantly different between infected birds in either diet treatment, suggesting that a high protein diet leads to greater tolerance of MG-infection than a high lipid diet. Results of the second experiment indicated that when birds had access to both the high lipid and high protein diets, the protein diet was consumed in higher quantities than the lipid diet prior to infection but declined after infection, while lipid consumption remained consistent. Physical recovery of birds in experiment two was similar to birds in the first experiment that were only fed the protein diet. These data indicate that diet macronutrients play an important role in individual variation in disease severity among hosts infected with a pathogen. This could have important implications on disease transmission in populations and could reveal anthropogenic food solutions that could improve community health.

Acknowledgments

I would like to acknowledge and give sincere appreciation to my undergrad mentor Scott Gardner for working so hard to provide research experiences to undergraduate students that were truly remarkable. His encouragement and passion while working in the lab and field revealed the sort of career I would aspire to obtain. I would also like to express the gratitude I will always have to my master's advisor Sarah DuRant for allowing me to join her lab right as it was beginning in Arkansas. Without her, I would have missed out on so much priceless experiences I have had here and all the incredible moments of learning new things. I would also like to thank our first post-doc Ashley Love for all the guidance and assistance you provided me when I started my first real experiment. In addition, I would like to thank Will Kirkpatrick, Erin Sauer, Sarah Heissenberger, and Madeline Sudnick for being such amazing lab-mates that are always ready to help everyone out and to be an incredible group of friends. I would also like to acknowledge all the undergraduate collaborators that put in so much work and enthusiasm into our projects, who include, Ashley Morris, Johnathan Novotny and Chloe Connelly. I would like to thank the biology faculty for constantly helping me progress forward as a scientist and person. I would like to acknowledge the Biology Graduate Student Association for allowing me the opportunity to form many meaningful relationships with other graduate students and to engage with the greater community outside of our department. Thank you to my parents, siblings, and the rest of my family for supporting me throughout my life and academic career. Finally, I would like to thank my partner, Alexis O'Callahan, for constantly being an enjoyable, engaging, understanding, and loving presence.

Table of Contents

Introduction

Diet is vital to the function of the immune system and a host's ability to efficiently clear an infection (Shils et al. 1994). The impact of the quantity and composition of diet on disease dynamics within and across species has become increasingly critical to understand as more anthropogenic food resources are introduced to natural and altered environments (Restani et al. 2001). Changes in land-use and availability of resources can result in emerging disease hotspots, primarily by reducing species diversity and changing reservoir host population behavior (Hosseini et al 2017). For instance, availability of a new food source from humans can cause hosts or potential hosts to increase contact with conspecifics or with other species (Plowright et al. 2011). Studies on the emergence of disease resulting from anthropogenic influences often focuses on changes within and among species interactions as a driver of disease spread and emergence, however, shifts in food consumption can also be important to individual disease outcomes, which can be important to epidemic dynamics (Hite and Cressler 2018). Exploring the effects of diet, for instance nutritional composition of the diet, on disease dynamics should be explored to better understand anthropogenic influences on wildlife disease.

The nutritional composition of the diet contributes to how effectively immune processes eliminate pathogens (Cunningham-Rundles et al. 2005, Amar et al. 2007). Manipulative studies in an ecological context show the importance of dietary macronutrients on immune processes (Povey et al. 2014, Cotter et al. 2011), emphasizing how resource quality in an area can influence individual disease outcomes. For instance, altering ratios of dietary proteins causes hormonal stimulation and regulation of immune mechanisms (Klasing 2007, Povey 2009). In one study, caterpillars experimentally infected with a baculovirus, *Spodoptera exempta*, preferred high protein diets when given the option. The increase in consumption of high protein diets led to

higher rates of survival from infection than the caterpillars given a high carbohydrate diet (Povey et al. 2013). Another study in poultry demonstrated how variation in dietary lipids affected immune responses by modifying leukocyte production (Friedman and Sklan 1995). However, high lipid diets have been shown to increase mortality rates during some infections (Adamo 2008). Lipid composition also has direct effects on inflammatory responses, which are modulated by the ratios of n-6 to n-3 fatty acids in the diet (Klasing 1998). Variation in immune responses based on macronutrient composition in the diet is likely a result of a host requiring different nutrition to produce robust immune responses for clearing specific pathogens (Hite 2018).

While nutritional resources are critical to host immune function, those resources are also accessible to pathogens for growth and replication (Grieger and Kluger 1978). Hosts can restrict pathogen access to nutrients (i.e., starve the pathogen) by undergoing self-induced anorexia. By reducing nutritional intake, the host can outcompete an infection for nutritional requirements and improve their recovery time (Wang et al. 2005). Altering dietary composition and intake are two strategies for efficiently clearing an infection (Carvajal-Lago 2021, Love 2013, Povey 2009, Griffioen-Roose et al. 2012).

The goal of this study is to explore the effects of macronutrients on disease outcomes, e.g., disease severity and recovery time after exposure to a bacterial pathogen, in a vertebrate host-pathogen system. Similar research has been done with non-pathogenic novel antigens, but a true pathogenic infection could lead to very different results because the immunological pathways used to clear the antigen by the host can be different (Koch et al. 2018, Macpherson et al. 2001). Additionally, many studies that investigate the relationship between dietary macronutrient content and infection do not record caloric intake or the ingredients vary across

the artificial diets, making it difficult to pinpoint the effects of macronutrients on disease outcomes. This study will improve our understanding of why some animals exhibit selective or reduced feeding during illness. To do this, we conducted two experiments aimed at answering different but complementary questions: 1) How will macronutrient specific diets affect disease severity and immune response in a host-pathogen system and 2) Will dietary preference shift during an infection? In the first experiment we predicted that birds fed a high lipid diet would clear infection of *Mycoplasma gallisepticum* faster than birds fed the high protein diet based on previous work in our lab (Love 2013). The previous study used similar diets and an avian host system that closely match the protocol for these experiments. Because the earlier studies in our lab indicated that birds reduce protein consumption but maintain lipid consumption during a non-pathogenic immune challenge (Love 2013), we predicted that birds in the second experiment would prefer a high lipid diet during *Mycoplasma gallisepticum* infection more than the high protein diets post infection.

Materials and Methods

Experimental Design and Timeline

Experiment 1: Individually housed canaries (N=37) were provided either a high lipid or high protein diet 17 days prior to inoculations to acclimate treatment groups to the diets. Following acclimation to the diets, birds were inoculated with either FREY's media or MG. We weighed birds, measured fat scores, assessed eye inflammation, and measured various immune endpoints (e.g. white blood cell counts, pathogen load, MG-specific antibody levels) throughout infection. Eyes were scored for inflammation and swabbed to measure pathogen load prior to infection and every other day post inoculation until 35 days post infection. Body mass and fat scores were collected prior to infection and at days 7-, 14-, 21- and 35-days post infection. We

collected a blood sample from birds immediately prior to infection and at days 7-, 14-, and 21-days post infection. The blood samples were used to assess hematocrit and white blood cells, and to quantify MG antibody concentrations in birds.

Experiment 2: Canaries (N=25) were housed individually and provided both a high lipid and high protein diet daily throughout the experiment. Birds were acclimated to the diet for 17 days, then inoculated with either FREY's media or MG. We recorded bird's body mass, fat stores, conjunctiva inflammation, and other immune endpoints (e.g. white blood cell counts, pathogen load, MG-specific antibody levels) throughout infection. Prior to and every 2-3 days after infection, we scored eye inflammation, then bilaterally swabbed eye conjunctiva to quantify pathogen load. Body mass and fat scores were collected prior to infection and 7-, 14-, 21-, and 35 days post infection. We collected blood samples prior to infection and at days 7-, 14-, and 21-days post infection. Similar to experiment 1, blood samples were used to assess hematocrit, determine white blood cell distributions, and to quantify concentrations of MG antibodies in birds.

Bird Housing

Canaries were housed in an ABSL-1 biosafety room on a 14L:10D light cycle throughout both experiments. Birds were housed in wire cages (24"x16"x16") that are divided into two units with each unit containing one bird to allow assessment of individual food consumption. Each housing space contained two plastic perches, a water dish, and a food dish in the first experiment. The housing space contained an additional food dish for the second experiment (diet preference) and the water dish was placed completely within the cage. To prevent contamination of control birds by MG-infected birds, a plastic partition divided the room with controls held on one side of the partition and MG-infected birds kept on the other.

Diet Composition and Monitoring Feeding

In the first experiment, birds were fed daily either a 24 g isocaloric food bar that was lipid-rich (80:20 lipid to protein ratio) or protein-rich (20:80 lipid to protein ratio). Both diets contained varying proportions of egg whites, egg yolks, hulled millet, cod liver oil, and were congealed together with agar. To monitor feeding behavior throughout the experiment, diets were weighed and replaced daily at the start of each light cycle. In the second experiment, birds were offered the same 24 g isocaloric food bars as described for experiment one, except that they received one bar of both the lipid rich diet and the protein rich diet. The two bars were placed in separate food dishes located on either side of the front of the cage and for each rack of cages we randomized which side would receive which diet. Placement of diets was determined at the beginning of the experiment and remained unchanged throughout the duration of the experiment. For both experiments, three food bars for both diets were placed in the bird room and weighed daily to account for desiccation. An average of the three controls were calculated and subtracted from the amount of diet that was consumed by each bird to get a more accurate value of diet consumption.

Inoculations and Monitoring of Disease Severity

After acclimation to diets, birds were inoculated with MG or a control solution. We inoculated MG-treated birds in both experiments bilaterally with MG inoculum (VA1994; stock ID 2009.7994-1-7P; D. H. Ley, North Carolina State University, College of Veterinary Medicine, Raleigh, NC) in their palpebral conjunctiva with 25 μL containing 5*107 CCU/ml of MG inoculum diluted 16.9% in Frey's media. Birds assigned to the control treatments in both experiments were inoculated with 25 μL of Frey's media. Throughout the duration of infection, inflammation of the conjunctiva, a measure of disease severity, was scored on a scale of 0-3

(Hawley et al. 2011); higher scores represent a greater degree of disease pathology. Both eyes of each bird were given a score and were summed together to determine a total eye score value, which is a measure of MG disease severity in house finches (Hawley et al. 2011). Disease severity was also monitored by recording changes in body mass and fat scores. Fat scores were measured on a scale of 0-3 and categorized by how much visible adipose tissue was present in the interclavicular fossa of the birds. A low fat score value indicates only a small trace or lack of visible fat tissue and higher values indicate more fat tissue present.

Antibody Assays

After blood samples were collected, they were microcentrifuged at 3500 rpm, the plasma was removed and stored in a -20°C freezer for future analysis of MG antibody concentrations. MG-specific antibody concentrations were examined by separating plasma from blood samples. Serum antibodies were quantified using the IDEXX MG antibody enzyme-linked immunosorbent assay test kit (IDEXX, Cat#99-06729). A blocking step was added to the original assay kit's protocol, with the addition of 300 μL of 1% bovine serum albumin (Pierce 10X BSA; Thermo Fisher Scientific) in phosphate-buffered saline to room temperature plates before they were incubated. All plates were washed three times with phosphate-buffered saline containing 0.05% Tween 20 using an ELx50 plate washer (BioTek). Serum samples were diluted 1:50 in sample buffer and were then plated to be run in duplicate. Intensity of light absorbed by the serum samples was measured at 630 nm using a spectrophotometer and an ELISA value was then calculated.

After blood samples were collected, they were microcentrifuged at 3500 rpm, the plasma removed and stored in a -20°C freezer for future analysis of MG antibody concentrations. MG-specific antibody concentrations were examined by separating plasma from blood samples.

Serum antibodies were quantified using the IDEXX MG antibody enzyme-linked immunosorbent assay test kit (IDEXX, Cat#99-06729). A blocking step was added to the original assay

Pathogen Load

We determined pathogen load using quantitative PCR following the procedure outlined by (Grodio et al. 2008) that targets the mgc2 gene of MG. Sterile cotton swabs were dipped in tryptose phosphate broth and used to swab conjunctiva in both eyes of birds for five seconds each. The tips of the swabs were then cut off and placed in 300 µl of the tryptose phosphate broth and frozen in a -20˚C freezer. Qiagen DNeasy 96 Blood and Tissue kits (Qiagen, Valencia, CA) and primers and probe that target mgc2 were used to extract genomic DNA. The total liquid volume of 15 µl included 7.5 µl of Primetime Master Mix (Bio-Rad Laboratories, Hercules, CA), 3.525 µl DNase-free water, 3 µl of DNA sample, 0.375 µl of forward and reverse primers and 0.225 µl of 10 um MG probe. A BioRad CFX-96 machine was used for cycling at 95˚C for three minutes, followed by 40 cycles of 95˚C for three seconds, and then 60˚C for 3 seconds. The ramp rate of the machine was set to 0.5/second. The mgc2 values were a summed total of both conjunctiva of each individual bird and sample day. Final concentrations were calculated by multiplying 3 µl, the amount of DNA sample used, by 66.666, to be comparable to the 200 µl that was produced from the elution step. Determined values for mgc2 were then log transformed to reduce the large outputs that were generated.

Statistical Analyses

Prior to all statistical analyses, data were checked for normality and homoscedasticity. Food intake, body mass, fat score, white blood cell counts, and MG antibody concentrations of

birds were compared across treatments using seven separate repeated measures ANOVAs (SAS proc mixed). In the first experiment each statistical model contained diet (protein or lipid diet), infection status (control or MG-infected), time and their interactions. We ran repeated measure ANOVAs on eye score and pathogen load without the control treatments to better evaluate whether diet had significant effects on these disease pathology endpoints and because controls did not exhibit conjunctival swelling or have MG pathogen loads. Including controls in the models did not change the statistical outcomes of these endpoints. Controls are presented in the figures for these endpoints. Similar models were used to assess feeding and MG endpoints measured in the second experiment, which contained the same independent variables as the first experiment, with the exclusion of diet. In all models, interactive terms that were strongly non-significant were removed from statistical models. In both experiments, we analyzed food consumption two ways, by day and by week. Daily feeding patterns provide higher resolution of feeding behavior during infection. Weekly feeding patterns were easier to compare visually, and capture feeding behavior at distinct phases of infection: pre-infection, peak infection, early recovery, and late recovery. Models were run using PROC MIXED in SAS 9.4M7 (SAS Institute Inc., Cary, NC, USA) and RStudio Desktop 1.4.1106 (Rstudio: Integrated Development for R. RStudio, PBC, Boston, MA, USA)

Results

Experiment One

Throughout the experiment birds fed the high protein diet on average consumed more food than birds fed the high lipid diet (Figure 1, Table 1, Diet: $F_{1,32}=1.68$; $p=0.2035$). Regardless of diet, infected birds reduced food consumption by 17-25% relative to uninfected birds after inoculation with MG but returned to pre-infection food consumption levels a week after infection

(Table 1, Week*Infection: $F_{3,96}$=14.17; p <0.0001). Although, all infected birds reduced food intake the week following infection, birds fed the high protein diet reduced their food intake significantly more than birds on the lipid diet during the first week post infection (Table 1, Diet*Week*Infection: $F_{3,96}$=2.62; p=0.0553. There were no other significant main or interactive effects on total grams of food consumed (in all cases $F \leq 1.68$; $p \geq 0.2035$).

Regardless of treatment, all birds were similarly sized and had similar furcular fat scores prior to MG infection; however, following MG infection and concomitant with the reduction in food intake infected birds exhibited a significant decrease in body mass (Figure 2, Table 1, Day*Infection: $F_{5,173}$=6.37; p<0.0001; Day: $F_{5,173}$=31.13; p<0.0001) and fat score (Figure 2, Table 1, Day*Infection: $F_{5,168}$=6.14; p<0.0001; Day: $F_{5,168}$=4.84; p=0.0004). On average, body mass for infected birds was 9.13-11.79% lower than control birds at seven days post infection and fat scores had decreased by 21.9-55.5% seven days post infection. Infected birds regained weight and fat, such that body mass and fat scores were similar to controls by 21-36 days post infection. There were no other significant main or two- and three-way interactive effects on body mass or fat scores (in all cases $F \leq 0.01$; $p \geq 0.9575$).

There was a significant effect of time on eye swelling, in which birds from both diets exhibited more severe swelling during the first 10 days of infection and swelling diminished in the days after peak infection (Day: 7-10). Macronutrient composition of the diet also significantly affected eye score, with generally more severe swelling in the birds fed the high lipid diet as compared to birds fed the high protein diet (Table 1, Diet: $F_{1,16}$=6.67 p=0.0.0201). On average the time to recovery of conjunctiva swelling of birds was 16 days in MG infected birds fed the high protein diet and 25 days for MG infected birds fed the high lipid diet (Diet: $F_{1,15}$ = 7.11 p=0.0176).

Infected birds in both diet treatments experienced a significant increase in MG specific antibody production as compared to control birds (Figure 2, Table 1, Infection * Day: $F_{3,93} = 9.26$ $p<0.0001$; Infection: $F_{1,30}=15.53$ $p=0.0004$), which did not differ among diets (Table 1, Diet: $F_{1,16}=0.7$ $p=0.4149$; Diet X Day: $F_{3,48} = 0.32$ $p=0.8123$.; Diet x Infection: $F_{1,30} = 0.00$ $p=0.989$). Similarly, infected birds in both diet treatments exhibited high pathogen loads in the first week of infection, which decreased by 14 and 21 days post infection (Table 3, Time: $F_{4,48}=80.17$ $p<0.0001$). Diet treatment did not have a significant effect on pathogen load (Table 3, Diet: $F_{1,17}=0.22$ $p=0.6421$; Diet X Day: $F_{4,48}=0.94$ $p=0.4512$).

The series of repeated measures ANOVAs revealed significant effects of infection and diet on production of different white blood cell types. Although there was a marginal Infection X Diet X Day effect on lymphocytes ($F_{3,84}=2.53$ $p=0.062$), there is no clear pattern that emerges, rather the various groups exhibited slightly different patterns over time. However, on average birds fed the high protein diet experienced significantly increased numbers of lymphocytes (Table 2, Diet: $F_{1,34}=8.61$ $p=0.006$) as compared to birds fed the high lipid diet. Finally, lymphocytes generally decreased in abundance over time (Table 2, Day: $F_{3,34}=9.21$ $p < 0.001$), but there were no other significant main or interactive effects on lymphocyte production (in all cases $p > 0.27$). Birds fed the high lipid diet produced significantly more eosinophils than birds fed the high protein diet (Table 2, Diet: $F_{1,34}=6.71$ $p=0.014$). In all groups, eosinophils increased from the day of inoculations to day 7 post inoculation and remained elevated to day 21 post inoculation (Table 2, Day: $F_{3,34}=7.42$, $p < 0.001$). There were no other significant main or interactive effects on eosinophil production (in all cases $p >0.44$). Heterophil production significantly decreased on day 7 post inoculation, but this was not a result of either infection or diet treatment (Table 2, Day: $F_{3,84}=3.66$, $p = 0.0155$, Infection: $F_{1,34}=0.01$, $p = 0.9405$, Diet:

$F_{1,34}$=2.91, p = 0.969). The heterophil:lymphocyte ratio was not affected by infection status of birds but did seem to have a possible interaction with the protein diet to reduce the ratio (Day: $F_{1,34}$=3.36, p =0.0755). significantly increased in infected birds in both diet treatments during the first week of infection (Table 9, Infection*Day: $F_{3,84}$=9.84, p < 0.0034). There were no other significant main or interactive effects on monocyte production (in all cases p >0.42).

In general, basophils remained low throughout the entire experiment, with individuals exhibiting 0-1 basophils at most treatments. However, because one individual in the MG protein treatment had five basophils on day 14 (all other birds in this treatment on day 14 had 0-2 basophils) post inoculation this generated a significant Infection X Diet X Day interaction on basophil production (Table 2, Infection*Diet*Day: $F_{3,84}$=3.8 p=0.0132; data not shown).

Experiment Two

When birds had the option of feeding from both the high protein and high lipid diet, infected birds exhibited a decrease in total caloric intake after infection with MG as compared to control birds (Figure 6, Table 4, Infection*Week: $F_{3,57}$=5.59 p=0.002). Similar to infected birds in the first experiment, infected birds in experiment two reduced caloric intake compared to control birds during the first week post infection, then returned to pre-infection levels of caloric intake the following week. MG-infected birds also showed macronutrient specific changes in feeding during infection relative to control birds; MG-infected birds maintained intake of lipids relative to controls in the first week post infection (Table 4, Infection*Week: $F_{3,57}$=0.8158 p=0.8158).

Birds infected with MG experienced decreases in body mass (Figure 7 Table 4, Day*Infection: $F_{4,77}$=5.19 p=0.0009) and fat score (Table 4, Day*Infection: $F_{4,74}$=2.7 p=0.0372)

relative to control birds. Infected birds also exhibited swollen eye conjunctiva during infection, whereas control birds did not experience swelling (Table 4, Day: $F_{2,38}$=1.36 p=0.2696). Aside from one bird that exhibited swollen conjunctiva past the end of the second experiment, infected birds recovered by day 16 post infection.

Infected birds experienced a significant increase in MG specific antibody production as compared to control birds (Table 4, Infection: $F_{1,19}$=10.46 p=0.0044) and antibodies remained elevated 21 days post infection. Similarly, infected birds had significantly elevated , pathogen loads relative to controls, which were highest at 7 days post infection then significantly decreased throughout the remainder of the experiment (Figure 6, Table 8, Day*Infection: $F_{4,56}$=15.73 p<0.001)Changes in relative abundance of white blood cells of control and MG-infected birds that had the choice of feeding on both diets indicated an increase in monocytes in control birds on day 14 post inoculation compared to MG-infected birds (Figure 9, Table 5, Day*Infection: $F_{3,57}$=2.5 p=0.0683). There was not a significant effect of infection on eosinophil production (Table 5, Infection: $F_{1,21}$=0.67 p=0.4229), but the production of eosinophils was lower on day 21 of the experiment in both treatments as compared to the other days (Table 5, Day: $F_{3,57}$=3.02 p=0.0373). There was no other significant difference detected in relative abundance of any other white blood cell between control and MG-infected birds (in all cases $F_{3,57}$ >0.84; p >0.125. No significant relationships were found between time or infection status on the H:L ratio of birds in the experiment (Day: $F_{1,21}$=1.02 p=0.3230; Infection: $F_{3,57}$=0.16 p=0.9238). Similar to the first experiment, the presence of basophils was rare and only one was detected throughout the course of this experiment.

Discussion

Macronutrients in an organism's diet are crucial resources that play different roles in shaping an individual's immune processes. The importance of macronutrients in the diet to host immunity becomes increasingly complicated to consider when the host is infected with a pathogen, because both the host and the pathogen are dependent on the host's acquisition of resources. Despite this, few studies in vertebrate host pathogen systems have parsed out the individual effects of macronutrients on host disease recovery and how those effects are reflected in dietary preference during an infection. This study was conducted to investigate how specific macronutrients would affect disease pathology during a pathogenic challenge in a vertebrate host and whether hosts exhibit macronutrient-specific shifts in feeding during infection. Our results indicate that a diet rich in lipids but poor in proteins result in greater disease pathology after infection with MG, but infected birds, when given the choice reduce protein intake, but not lipid intake immediately following infection.

Experiment 1: Does Diet Affect Host Disease Outcomes?

In the first experiment, we found that birds fed a high protein diet consumed more calories than birds fed a high lipid diet, regardless of infection status. When we quantified the total grams of protein and the total grams of lipid that birds in each diet treatment consumed, we found that on average, birds assigned the protein diet were consuming 77.33% more protein per day (2.97+/-0.07 vs. 0.674 grams +/- 0.01) than birds on the lipid diet and birds assigned the lipid diet were consuming 68.7% (0.687 +/- 0.02 vs. 0.313 grams +/- 0.007) more lipids than birds on the protein diet. During the first week of infection, infected birds in both diet treatments reduced their food intake, a behavioral pattern referred to as illness-induced anorexia, which is commonly observed across all vertebrates in response to pathogenic infection (Murray and

Murray 1979). Although infected birds on both diets reduced food intake following infection, this reduction was greater in the birds fed the high protein diet (39.11% vs 17.34%). These results are consistent with prior research in our lab, in which zebra finches significantly reduced protein consumption, but not lipid consumption during a non-pathogenic immune challenge (Love et al. 2013). Feeding rates in infected birds from both diet treatments returned to pre-infection levels a week after MG inoculations.

Although birds fed both high lipid and high protein diets exhibited illness-induced anorexia, birds fed the high protein diet had less severe disease pathology (swollen eye conjunctiva; a measure of disease severity; Hawley et al. 2011) over the course of infection than birds fed the high lipid diet, though both groups exhibited similar peak pathology. Although the mechanism behind the reduced pathology is unclear, this result could suggest that a certain threshold of protein intake is required prior to and during infection for optimal recovery. Dietary protein is well established as an essential component for the regulation and activation of both T and B lymphocytes, both of which are important for recovery from an infection (Li et al. 2007). Survival during infection in relation to increases in immune cells from dietary protein have been demonstrated in both invertebrates and vertebrates (Jahanian 2009, Lee et al. 2008). Further, diet studies using invertebrate systems, such as caterpillars infected with *Spodoptera exempta*, have found that individuals shift diet consumption to obtain increased levels of proteins as a possible way to increase antimicrobial responses (Povey et al. 2009).

In house finches, conjunctival swelling correlates positively with conjunctival pathogen load and the likelihood of transmitting MG (citations). However, in our system pathogen load did not differ between birds fed the two diets, even though birds fed the high fat diet had greater pathology than birds fed the high protein diet. This finding has several important implications.

First, high protein diet birds may be capable of transmitting MG even though the conjunctiva is not heavily swollen. This could alter typical transmission dynamics in a population because birds can detect illness in conspecifics and engage in avoidance behaviors to prevent illness, but they may not detect illness in high protein birds even though they are still capable of transmitting the pathogen. Second, high protein birds appear to tolerate infection better than high lipid birds because they had less severe pathology but similar pathogen load as high lipid birds. This indicates that diet plays an important role in whether individuals tolerate or resist an infection and suggests that studies exploring how resource availability shapes host-pathogen evolutionary dynamics are needed. These findings provide crucial support for the importance of understanding the role of dietary macronutrients in the investigation of disease dynamics in vertebrate host-pathogen systems.

Although infected birds fed the high protein diet exhibited less conjunctival swelling than birds fed the high fat diet, birds fed the high protein diet lost significantly more furcular fat in the first week of infection than birds fed the high fat diet and fat stores remained diminished five weeks post infection. Considering that changes in body mass after infection were similar across diet groups, the significant reduction in furcular fat in infected birds fed the high protein diet suggest that these birds relied heavily on fat stores to meet the energy demands of fighting the infection and may be an important cost for clearing the pathogen. Studies focused on how dietary shifts affect body composition and metabolic routing of nutrients in birds have found evidence that animals fed foods with higher lipid content have increased circulating lipids and body fat percentage (McWilliams and Podlesak 2006, McWilliams and Smith 2009), but insight on how diet composition and infection interact to alter body composition remains an area not well understood. Considering the results we obtained from this experiment, there may be optimal

thresholds for both macronutrients that must be met to both maintain body condition and tolerate infection. Reduced fat stores have important implications for an individual's ability to survive important and costly life history events. For instance, studies illustrate that having low fat scores can be detrimental to successful migration (Ramenofsky 1990, Lindstrom and Piersma 1993) and effectiveness in combating harsh weather conditions (Cuthill and Witter 1993, Reed and Rogers 2003, Rogers 2015).

White blood cell composition and levels are important to how an organism will respond to an infection. White blood cell levels were affected by diet and infection and varied throughout infection. Birds that were assigned the high protein diet experienced significantly increased levels of lymphocytes compared to birds from the other three treatments. Further, analyses indicated that heterophils did not differ among treatment groups, which can lead to differences in heterophil/lymphocyte ratios in birds. Poultry with low heterophil/lymphocyte ratios have been observed to be in a lower stress state than those with a higher ratio, who become more prone to having weaker infection responses (Al-Murrani et al. 1997) Although we did not detect effects of infection on Heterophil:lymphocyte ratios, birds on the protein diet had a marginally lower heterophil/lymphocyte ratio than birds on the lipid diet. Birds fed the lipid diet produced significantly more eosinophils than birds fed the protein diet. Considering that eosinophils have been observed to be involved in anti-parasitic processes that cause inflammation and tissue damage (Behm et al. 2000), it appears that they are working to clear the MG pathogen but may be heightening the overall inflammation of the conjunctiva caused by the infection. Birds in both diet treatments that were infected with MG experienced significantly elevated levels of monocyte production seven days post-infection. The similar increase of levels in monocytes and the important role they play in phagocytosis of pathogens likely plays an important part in the

similar pathogen loads seen throughout infection for both diet treatments (Dale et al. 2008). With pathogen load also reaching the greatest recorded numbers on the seventh day of infection, it appears that the immune response to the infection is not dependent on any one particular macronutrient.

We found that infected birds in either dietary treatment did not produce significantly different amounts of the MG-specific antibody. This result appears to indicate that macronutrient composition in the diet does not play a significant role in these specific antibodies production rate. Given that the antibodies function to neutralize the infection and signal their presence to the complement system and appear in similar quantities in all infected birds, there could be a few ways to interpret any role that dietary macronutrients could play in their production. Hosts immune system may function to prioritize certain pathways, dietary resources, and stored recourses from other functions during an infection as an adaptation to reduce disease severity when dietary resources are limited. Alternatively, the production of the antibodies may not be energetically costly and macronutrient dependent enough to be altered by drastically different macronutrient ratios of the diet. Our result reveals that this specific host developed a relatively standardized level of MG-specific antibody production it will invest in regardless of drastically different macronutrient ratios in the diet.

Experiment 2: Selective Macronutrient Intake During Infection

In the second experiment, birds experienced similar handling and infection as birds in the first experiment but had access to both diets throughout the study rather than access to only the high fat or the high lipid diet. Like the first experiment, illness-induced anorexia occurred in the first week post-infection and later returned to pre-infection levels of dietary consumption. By offering both diets, we could determine whether birds exhibit macronutrient-selective feeding

during infection. When birds had access to both diets, intake of protein in the first week post-infection was lower as compared to controls, whereas lipid intake remained similar to controls. Birds may selectively reduce protein intake during an infection because of its importance to the pathogen, particularly when they have had ample access to dietary proteins prior to infection (Lin et al. 2020).

Having access to both diets allowed infected birds to mitigate the more severe disease outcomes that resulted from consuming only one of the macronutrient specific diets. Specifically, infected birds were able to maintain fat stores similarly to birds in experiment one that were fed the high lipid diet and their conjunctiva recovery closely resembled the recovery time of experiment one birds that were fed the high protein diet. The reduction of body fat noted in the high protein birds in experiment one during infection appears to reveal a strong demand for lipids in the host during infection and maintaining lipid consumption could allow the host to fight off the infection and maintain fat stores. It is likely crucial to maintain or return to a healthy body condition quickly to effectively reproduce and survive post recovery. Whereas lipids seem important to maintaining and recovering body condition during and after infection, access to proteins either before or after peak infection appear to be more important in minimizing inflammation during infection.

Meeting a particular level of dietary protein intake in both experiments reveals a close relationship with the reduction in swelling of the eye conjunctiva produced by MG. Considering that no connections were revealed between particular macronutrients in the diet that altered the pathogen load or antibody production, it seems possible that dietary protein may be affecting other immunological pathways that were not investigated. One possibility for the difference in eye swelling could have been a difference in the levels and types of cytokines being produced.

Other studies have revealed that increased levels of interleukin-1 act as pro-inflammatory agents (Dinarello 1997), while other cytokines, such as interleukin-5 and interleukin-10, have been shown to play an anti-inflammatory role during inflammatory infections (Ferraz and Pirola 2017). Considering that one of the primary producers of these cytokines, lymphocytes, did not differ in levels in infected individuals, makes it difficult to begin to predict which cytokines could be changing in abundance.

This study broadens our understanding of the dynamics between diet and a host's response to infection with a replicating pathogen. Our findings suggest that consuming higher levels of dietary protein is important for clearing the physical symptoms of MG infection, though this may deplete fat stores. Interestingly, while tolerance to MG was higher in birds fed the protein diet, pathogen load data suggests that resistance to the pathogen was not significantly different between infected groups consuming either diet. An increase in tolerance to the pathogen found in our study suggests that macronutrient availability, from both anthropogenic and natural sources, may play an important role in individual disease pathology and pathogen transmission. In addition, when considering the significant decrease in fat stores of birds consuming the high protein diet, proper balance of lipid and protein consumption needs to be achieved to avoid negative effects on reduced body condition on survival and reproduction.

Table 1

Results from individual repeated measures ANOVAs used to compare feeding behavior, body condition, disease pathology, and MG antibody responses of canaries fed either a high lipid or high protein diet, then inoculated with Frey's media or *Mycoplasma gallisepticum*.

Variable	Effect	Num df	Den df	*F*	*p*
Diet Consumption	**Infection**	1	32	0.01	0.9212
	Diet	1	32	1.68	0.2035
	Week	3	96	18.21	<.0001
	Diet*Infection	1	32	0.15	0.7021
	Week*Infection	3	96	14.17	<.0001
	Diet*Week	3	96	4.47	0.0056
	Diet*Week*Infection	3	96	2.62	0.0553
Body Mass	**Infection**	1	35	0.66	0.4233
	Diet	1	35	0.59	0.4477
	Day	5	173	31.13	<.0001
	Diet*Infection	1	35	0.01	0.9406
	Day*Infection	5	173	6.37	<.0001
	Diet*Day	5	173	0.60	0.6983
Fat Score	**Infection**	1	35	1.04	0.3157
	Diet	1	35	1.48	0.2316
	Day	5	168	4.84	0.0004
	Diet*Infection	1	35	0.82	0.3705
	Day*Infection	5	168	6.14	<.0001
	Diet*Day	5	168	0.56	0.7313
	Diet*Day*Infection	5	168	0.51	0.7665
Eye Score	**Diet**	1	16	6.67	0.0201
	Day	14	213	33.91	<.0001
	Diet*Day	14	213	1.17	0.3003
Antibodies	**Infection**	1	30	15.53	0.0004
	Diet	1	30	0.85	0.3631
	Day	3	93	8.15	<.0001
	Diet*Infection	1	30	0.00	0.9893
	Day*Infection	3	93	9.26	<.0001
	Diet*Day	3	93	0.24	0.8655

Table 2

Results from individual repeated measures ANOVAs used to compare white blood cell production in canaries.

Variable	Effect	Num df	Den df	*F*	*p*
Heterophils	**Infection**	1	34	0.01	0.9405
	Diet	1	34	2.91	0.0969
	Day	3	84	3.66	0.0155
	Infection*Diet	1	34	0.57	0.4570
	Infection*Day	3	84	0.30	0.8276
	Diet*Day	3	84	0.81	0.4933
	Infection*Diet*Day	3	84	1.64	0.1867
Lymphocytes	**Infection**	1	34	0.01	0.9354
	Diet	1	34	8.61	0.0060
	Day	3	84	9.21	<.0001
	Infection*Diet	1	34	1.24	0.2724
	Infection*Day	3	84	1.21	0.3109
	Diet*Day	3	84	0.64	0.5907
	Infection*Diet*Day	3	84	2.53	0.0624
Eosinophils	**Infection**	1	34	0.20	0.6591
	Diet	1	34	6.71	0.0141
	Day	3	84	7.42	0.0002
	Infection*Diet	1	34	0.09	0.7640
	Infection*Day	3	84	0.91	0.4409
	Diet*Day	3	84	0.76	0.5186
	Infection*Diet*Day	3	84	0.60	0.6152
Monocytes	**Infection**	1	34	0.44	0.5098
	Diet	1	34	0.01	0.9082
	Day	3	84	9.84	<.0001
	Infection*Diet	1	34	0.66	0.4211
	Infection*Day	3	84	4.90	0.0034
	Diet*Day	3	84	0.20	0.8961
	Infection*Diet*Day	3	84	0.52	0.6664
Basophils	**Infection**	1	34	0.93	0.3427
	Diet	1	34	2.23	0.1448
	Day	3	84	1.66	0.1816
	Infection*Diet	1	34	0.07	0.7913
	Infection*Day	3	84	0.84	0.4749
	Diet*Day	3	84	0.83	0.4804
	Infection*Diet*Day	3	84	3.80	0.0132

Table 3

Results from individual repeated measures ANOVAs results for pathogen load throughout infection in canaries provided either a high lipid or high protein die and inoculated with MG.

Variable	Effect	Num df	Den df	*F*	*p*
Pathogen Load	**Diet**	1	17	0.22	0.6421
	Day	4	48	80.17	<.0001
	Diet*Day	4	48	0.94	0.4512

Table 4

Results from individual repeated measures ANOVAs used to compare feeding behavior, body condition, disease pathology, and MG antibody responses of canaries fed both a high lipid or high protein diet, then inoculated with Frey's media or *Mycoplasma gallisepticum*.

Variable	Effect	Num df	Den df	*F*	*p*
Diet Consumption	**Infection**	1	19	0.01	0.9265
	Week	3	57	9.72	<.0001
	Infection*Week	3	57	5.59	0.0020
Lipid Consumption	**Infection**	1	19	2.82	0.1093
	Week	3	57	8.83	<.0001
	Infection*Week	3	57	0.31	0.8158
Protein Consumption	**Infection**	1	19	10.38	0.0045
	Week	3	57	7.20	0.0004
	Infection*Week	3	57	1.75	0.1667
Body Mass	**Infection**	1	23	5.17	0.0326
	Day	4	77	5.54	0.0006
	Infection*Day	4	77	5.19	0.0009
Fat Score	**Infection**	1	22	1.31	0.2646
	Day	4	74	3.32	0.0147
	Infection*Day	4	74	2.70	0.0372
Antibodies	**Infection**	1	33	17.36	0.0002
	Day	3	99	8.97	<.0001
	Infection*Day	3	99	10.12	<.0001

Table 5

Results from individual repeated measures ANOVAs used to compare white blood cell production in MG-inoculated canaries, provided both a high lipid and high protein diet.

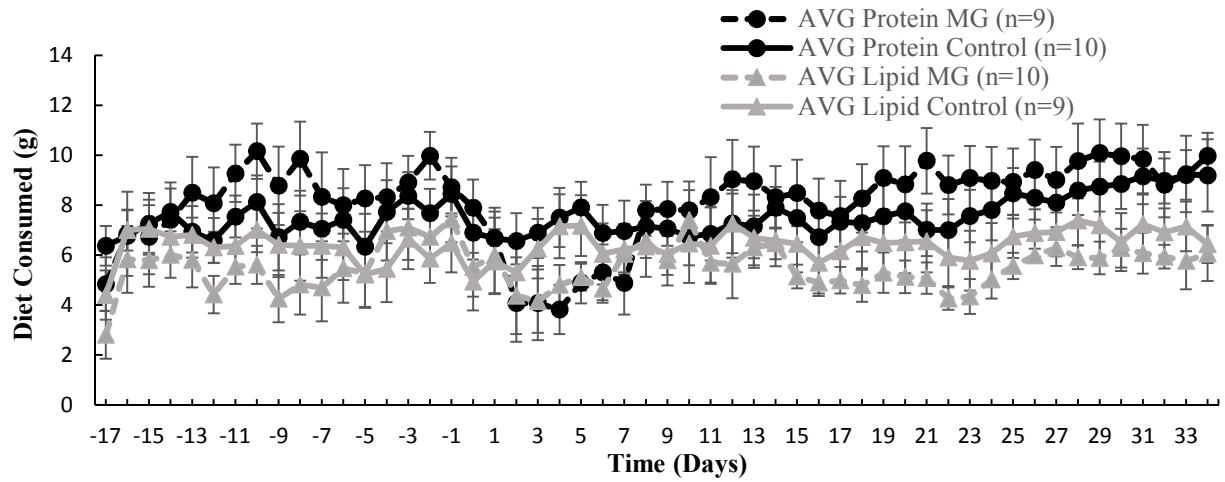
Variable	Effect	Num df	Den df	*F*	*p*
Heterophils	**Infection**	1	21	0.92	0.3495
	Day	3	57	1.27	0.2945
	Infection*Day	3	57	2.00	0.1245
Lymphocytes	**Infection**	1	21	0.1673	0.1673
	Day	3	57	0.2552	0.2552
	Infection*Day	3	57	0.1707	0.1707
Eosinophils	**Infection**	1	21	0.4229	0.4229
	Day	3	57	0.0373	0.0373
	Infection*Day	3	57	0.6341	0.6341
Monocytes	**Infection**	1	21	0.3060	0.3060
	Day	3	57	0.0136	0.0136
	Infection*Day	3	57	0.0683	0.0683
Basophils	**Infection**	1	21	0.3824	0.3824
	Day	3	57	0.4789	0.4789
	Infection*Day	3	57	0.4789	0.4789

Table 6

Results from individual repeated measures ANOVAs results for pathogen load throughout infection in canaries provided both a high lipid or high protein die and inoculated with MG.

Variable	Effect	Num df	Den df	*F*	*p*
Pathogen Load	**Infection**	1	22	42.19	<.001
	Day	5	56	12.59	<.001
	Infection*Day	4	56	15.73	<.001

A)



B)

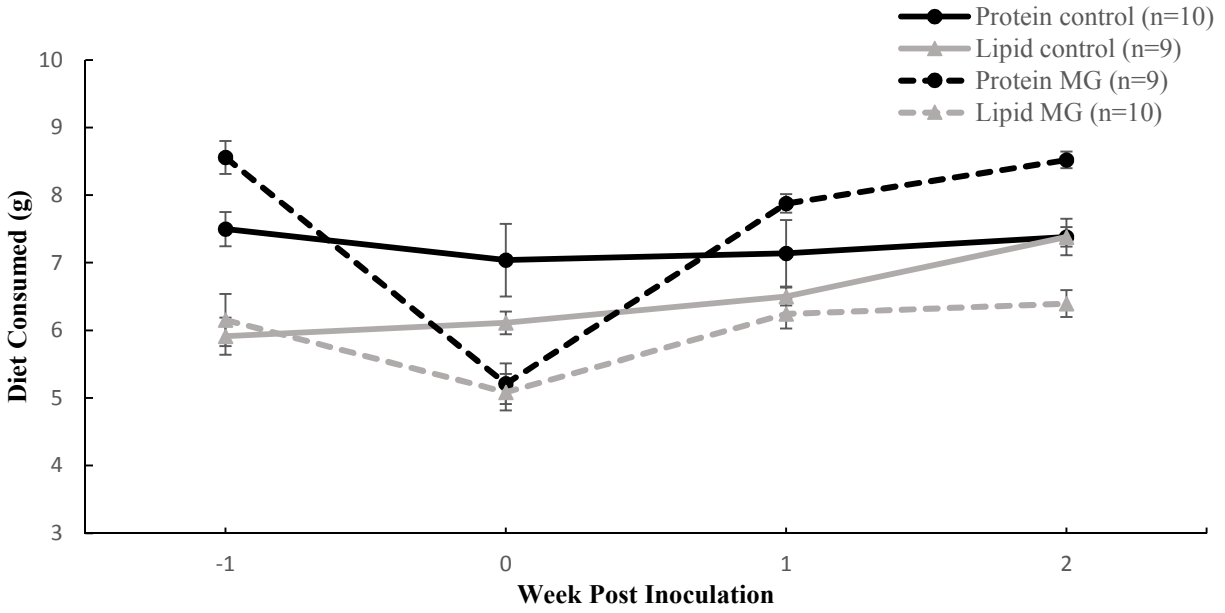


Figure 1. Diet consumed by day (A) and average daily food consumption by week (B) in canaries fed a high protein (20:80 lipid:protein) or high lipid (80:20 lipid:protein) diet and inoculated with either MG or FREY's media (controls) on day 0. Data are reported as means ± standard error.

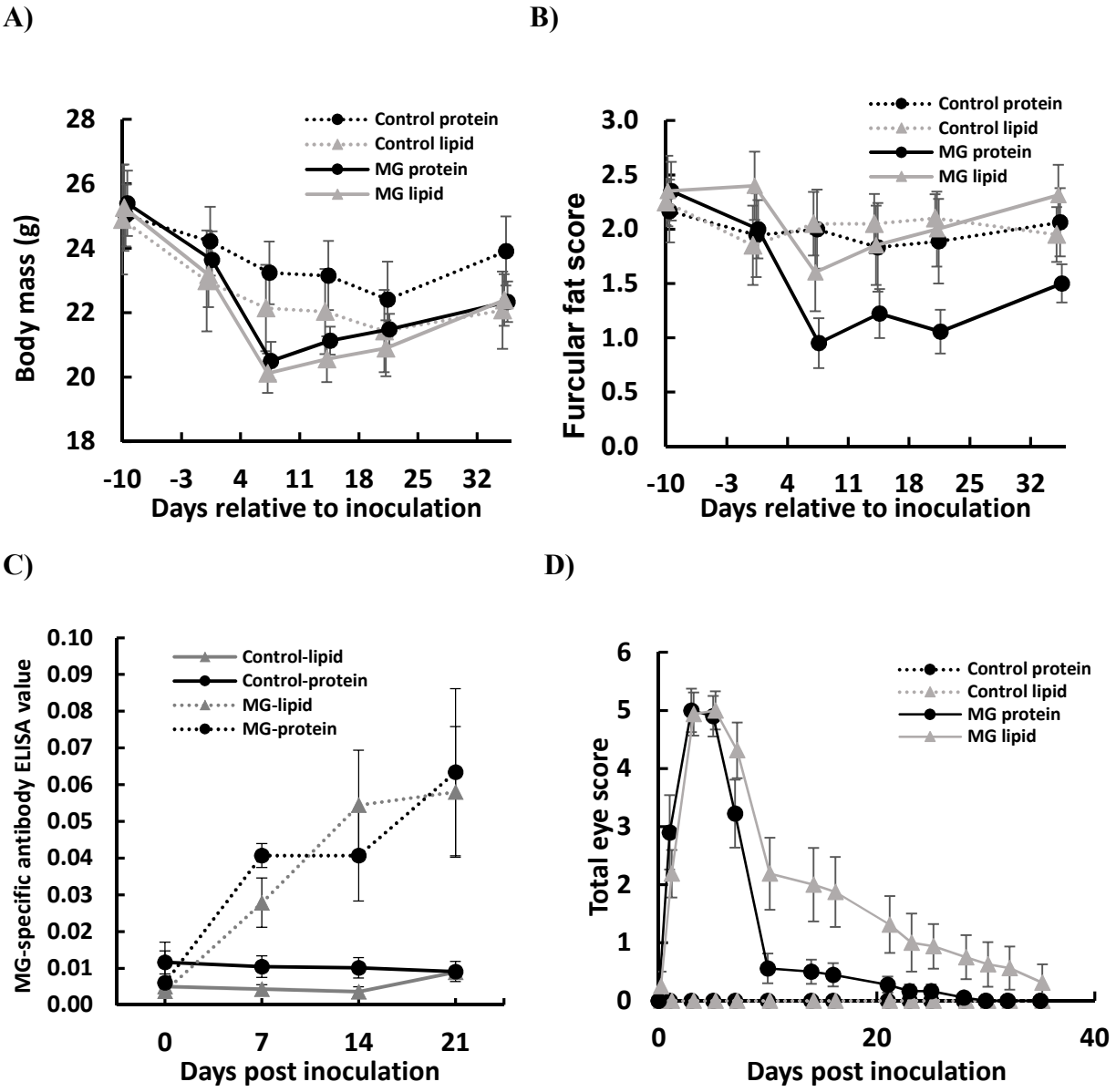


Figure 2. Body mass (A) fat score (B) MG specific antibody concentration (C) and total eye score (sum of swelling in the R and L eye conjunctiva) (D) in canaries fed a high protein (20:80 lipid:protein) or high lipid (80:20 lipid:protein) diet and inoculated with either MG or FREY's media (controls) on day 0. Sample sizes are as follows: Control lipid (n=9), control protein (n=10), MG lipid (n=10), MG protein (n=9). Data are reported as means ± standard error.

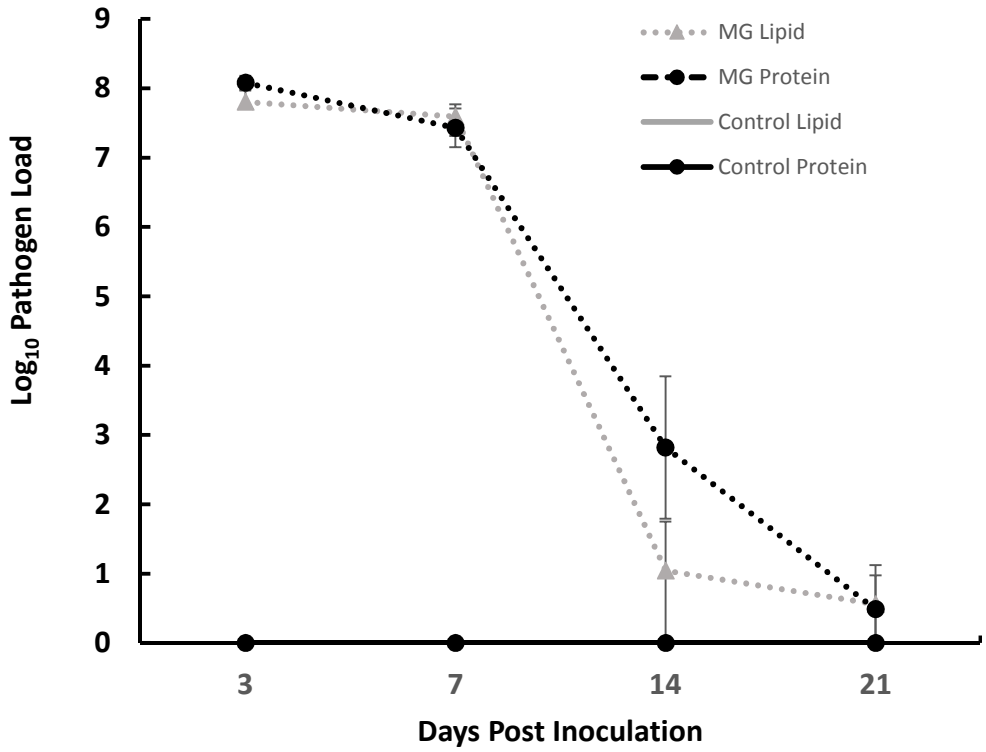


Figure 3. *Mycoplasma gallisepticum* (MG) pathogen load in the eye conjunctiva of canaries throughout infection. Sample sizes for canaries infected with MG are as follows: MG Lipid (n=8) and MG Protein (n=8) . Data are reported as means ± standard error.

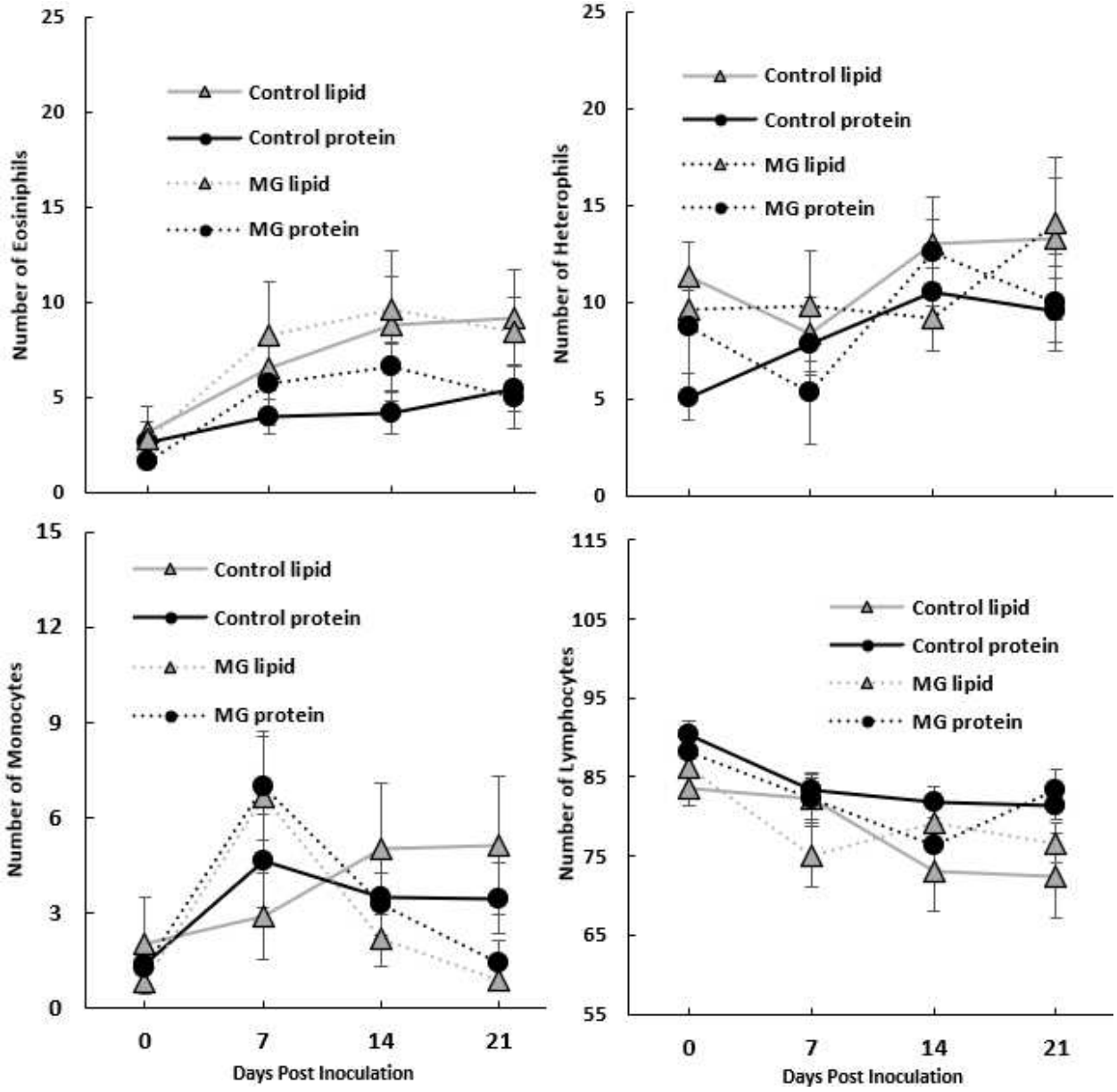


Figure 4. White blood cell counts throughout infection in the first experiment for eosinophils, heterophils, monocytes, and lymphocytes. Sample sizes are as follows: Control Lipid (n=7), Control Protein (n=10), MG Lipid (n=10), MG Protein (n=8). Data are reported as means ± standard error.

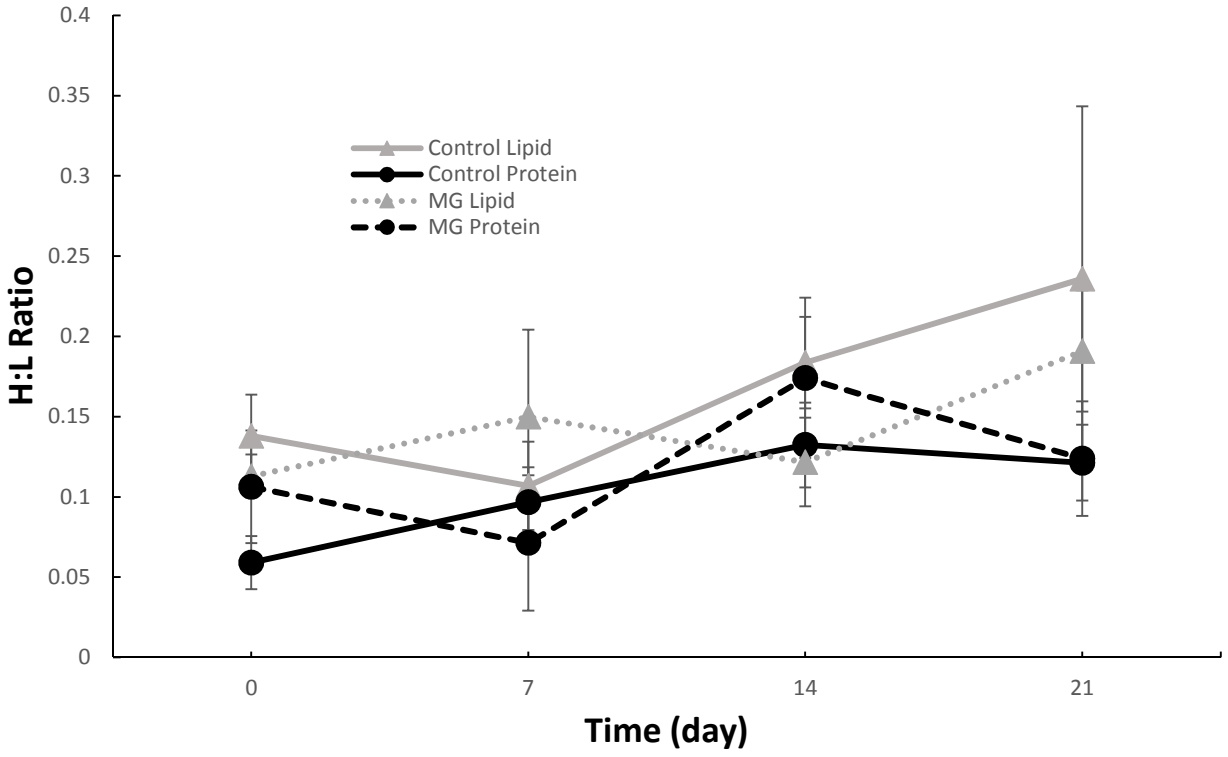
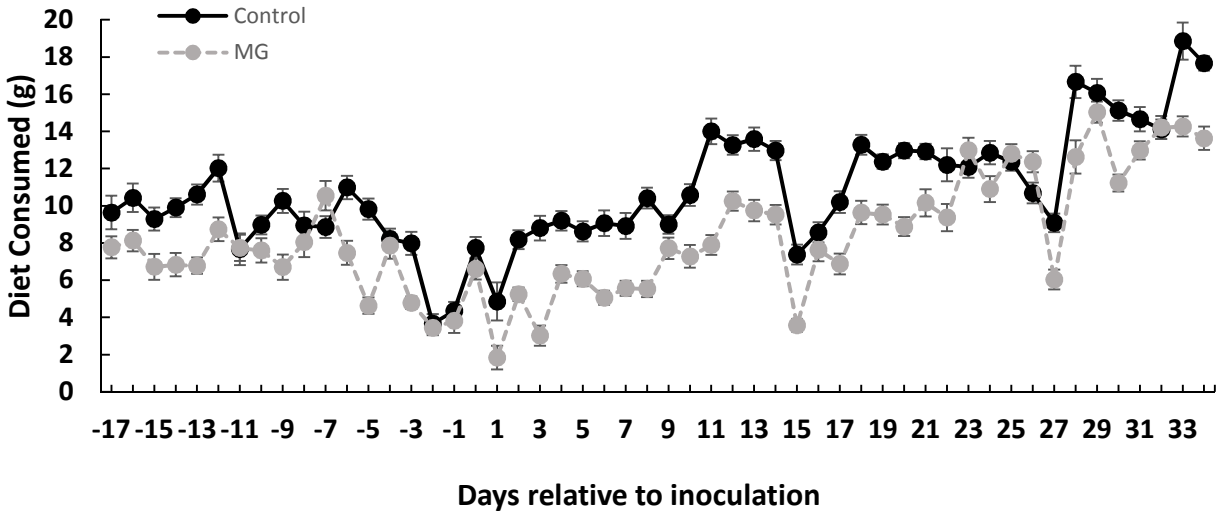
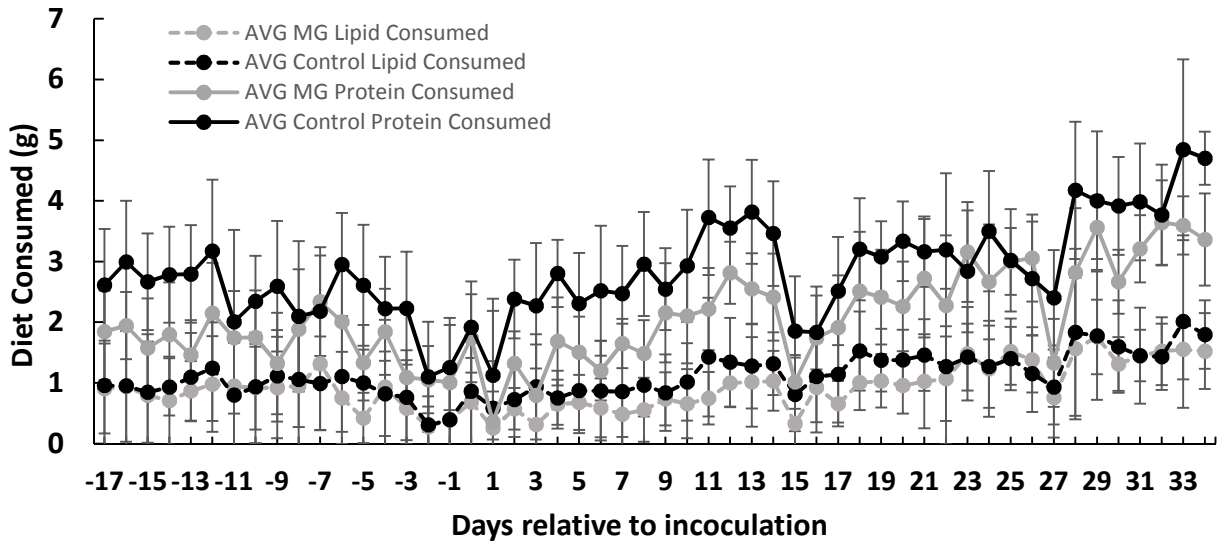


Figure 5. Heterophil:Lymphocyte ratio of canaries fed a high protein (20:80 lipid:protein) or high lipid (80:20 lipid:protein) diet and inoculated with either MG or FREY's media (controls). Sample sizes are as follows: Control Lipid (n=9), Control Protein (n=10), MG Lipid (n=10), and MG Protein (n=9).

A)


Control
MG
Diet Consumed (g)
0
2
4
6
8
10
12
14
16
18
20
-17 -15 -13 -11 -9 -7 -5 -3 -1 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33
Days relative to inoculation

B)


AVG MG Lipid Consumed
AVG Control Lipid Consumed
AVG MG Protein Consumed
AVG Control Protein Consumed
Diet Consumed (g)
0
1
2
3
4
5
6
7
-17 -15 -13 -11 -9 -7 -5 -3 -1 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33
Days relative to incoculation

C)

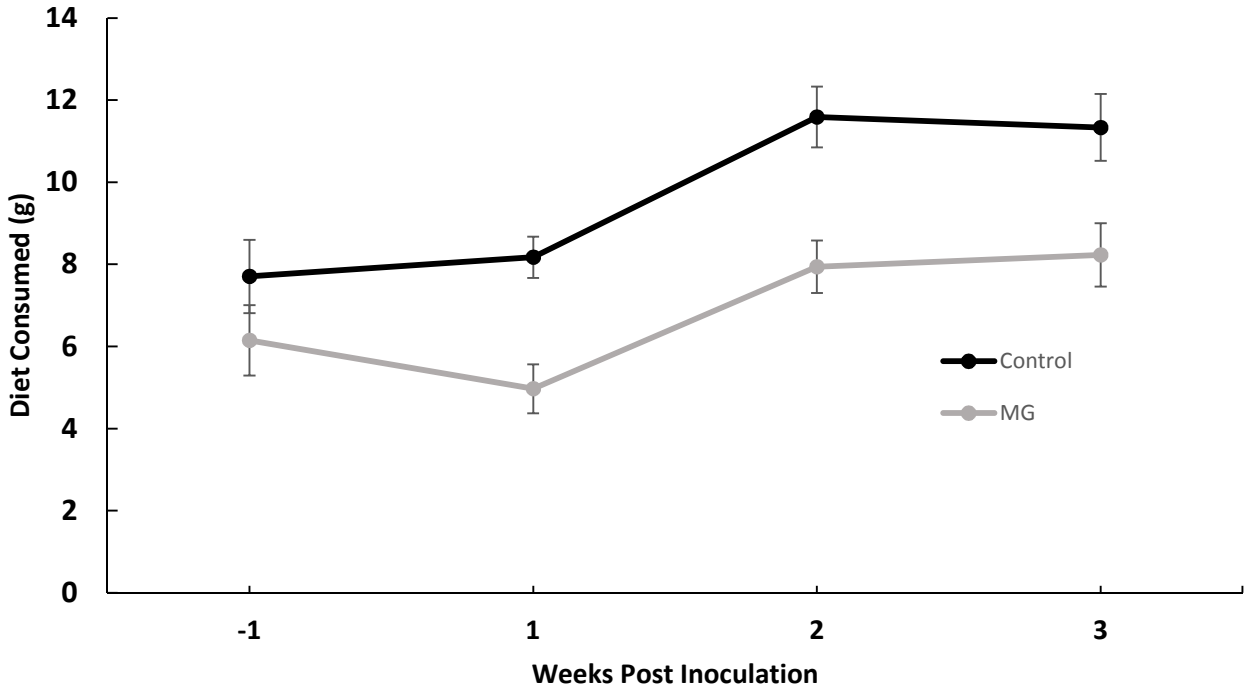


Figure 6. Average diet consumed by day (A), average grams of lipids and proteins consumed in canaries on average by week (B), and average daily diet consumption by week (C) in canaries given the choice of feeding on both a high protein (20:80 lipid:protein) and high lipid (80:20 lipid:protein) diet Sample sizes were as follows: Control (n=12) and MG (n=9). Canaries were inoculated on day 0. Data are reported as means ± standard error.

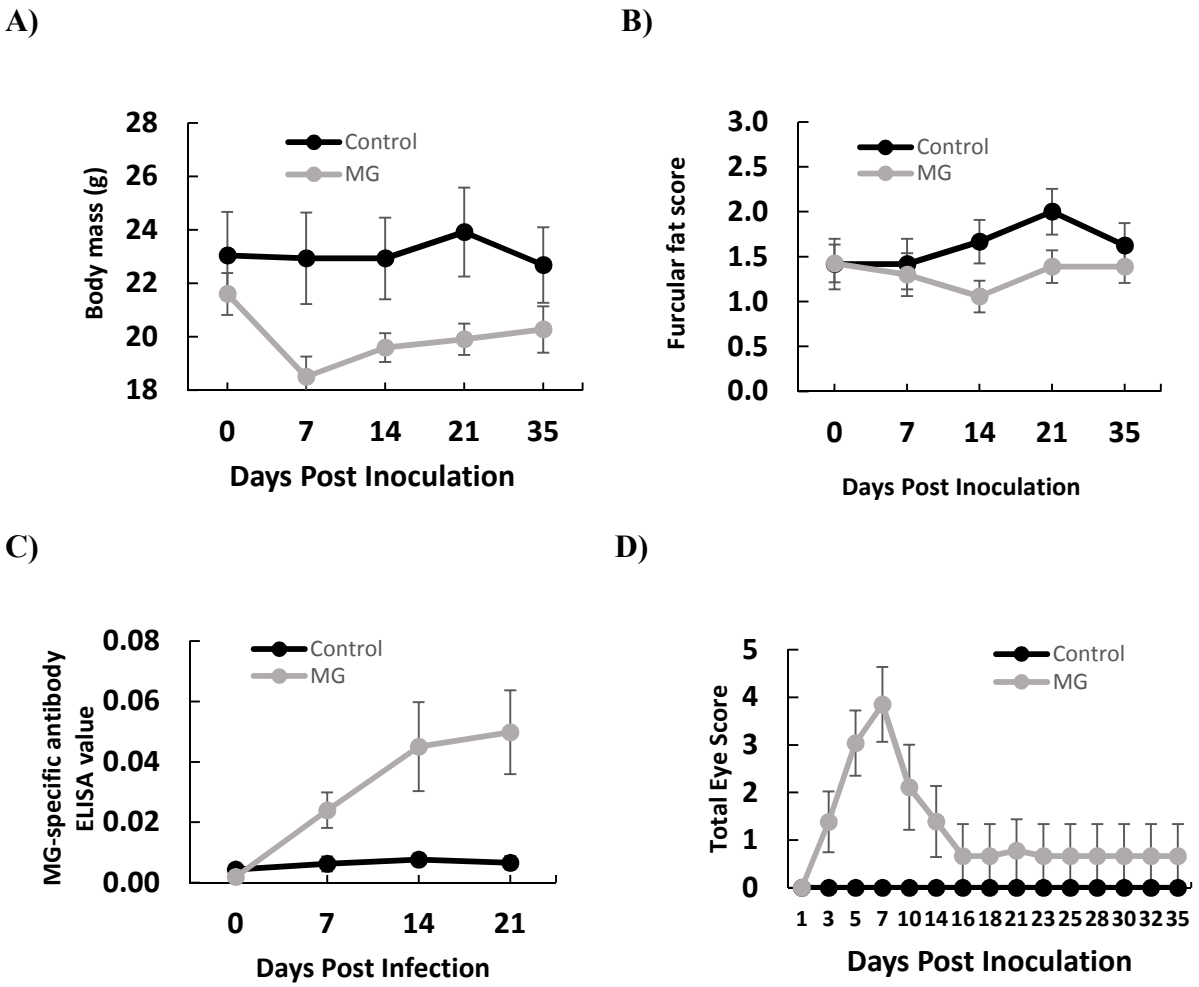


Figure 7. Body mass (A), furcular fat score (B), MG specific antibody concentration (C), and total eye score (D) (sum of swelling in the R and L eye conjunctiva) in birds provided the choice of a high protein (20:80 lipid:protein) and high lipid (80:20 lipid:protein) diet. Sample sizes are as follows: Control (n=12) and MG (n=9). Data are reported as means ± standard error.

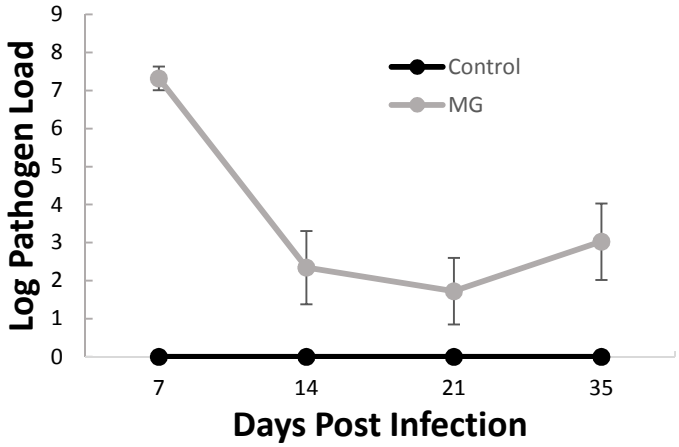


Figure 8. *Mycoplasma gallisepticum* (MG)Pathogen load throughout infection in canaries provided both a high protein (20:80 lipid:protein) or high lipid (80:20 lipid:protein) diet, and either inoculated with MG (n=9) or FREY's media (controls) (n=12). Data are reported as means ± standard error.

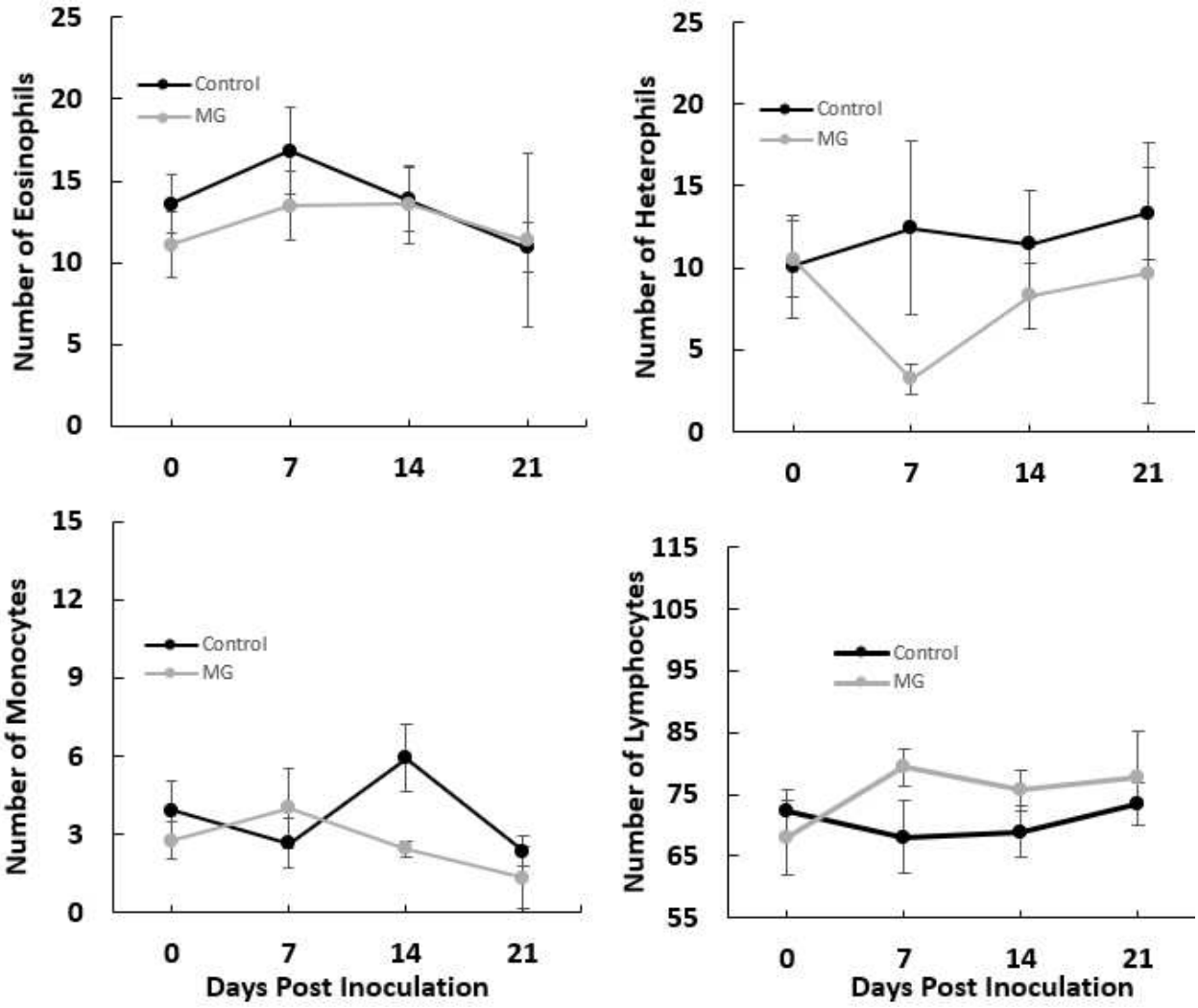


Figure 9. White blood cell counts throughout infection in canaries provided both a high protein (20:80 lipid:protein) or high lipid (80:20 lipid:protein) diet, and either inoculated with MG (n=8) or FREY's media (controls) (n=12). Data are reported as means ± standard error.

LITERATURE CITED

Adamo, S. A., et al. "Competition between immune function and lipid transport for the protein apolipophorin III leads to stress-induced immunosuppression in crickets." *Journal of Experimental Biology* 211.4 (2008): 531-538.

Al□Murrani, W. K., et al. "Heterophil/lymphocyte ratio as a selection criterion for heat resistance in domestic fowls." *British Poultry Science* 38.2 (1997): 159-163.

Amar, Salomon, et al. "Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge." *Proceedings of the National Academy of Sciences* 104.51 (2007): 20466-20471.

Behm, C. A., and K. S. Ovington. "The role of eosinophils in parasitic helminth infections: insights from genetically modified mice." *Parasitology Today* 16.5 (2000): 202-209.

Carvajal-Lago, Laura, et al. "Implications of diet on mosquito life history traits and pathogen transmission." *Environmental Research* (2021): 110893.

Cotter, S.C., Simpson, S.J., Raubenheimer, D., Wilson, K. 2011. Macronutrient balance mediates trade-offs between immune function and life history traits. Functional Ecology 25, 186-98.

Cunningham-Rundles, Susanna, David F. McNeeley, and Aeri Moon. "Mechanisms of nutrient modulation of the immune response." *Journal of Allergy and Clinical Immunology* 115.6 (2005): 1119-1128.

Dale, David C., Laurence Boxer, and W. Conrad Liles. "The phagocytes: neutrophils and monocytes." *Blood, The Journal of the American Society of Hematology* 112.4 (2008): 935-945.

Dinarello, C. A. "Role of pro-and anti-inflammatory cytokines during inflammation: experimental and clinical findings." *Journal of biological regulators and homeostatic agents* 11.3 (1997): 91-103.

Friedman, A and Sklan, D. 1995. Effect of dietary fatty acids on antibody production and fatty acid composition of lymphoid organs in broiler chicks. Poultry Science, 74: 1463–1469.

Grieger, T. A., and M. J. Kluger. "Fever and survival: the role of serum iron." *The Journal of physiology* 279.1 (1978): 187-196.

Griffioen-Roose, Sanne, et al. "Protein status elicits compensatory changes in food intake and food preferences." *The American journal of clinical nutrition* 95.1 (2012): 32-38.

Grodio, Jessica L., et al. "Detection and Quantification of Mycoplasma Gallisepticum genome Load in Conjunctival Samples of Experimentally Infected House Finches (Carpodacus Mexicanus) Using Real-Time Polymerase Chain Reaction." Avian Pathology, vol. 37, no. 4, 2008, pp. 385–391.

Hawley, Dana M., et al. “Experimental Infection of Domestic Canaries *(Serinus Canaria Domestica*) With Mycoplasma Gallisepticum: a New Model System for a Wildlife Disease.” Avian Pathology, vol. 40, no. 3, 2011, pp. 321–327.

Hite, Jessica & E. Cressler, Clayton. (2018). Resource-driven changes to host population stability alter the evolution of virulence and transmission. Philosophical Transactions of the Royal Society B: Biological Sciences. 373.

Jahanian, R. "Immunological responses as affected by dietary protein and arginine concentrations in starting broiler chicks." *Poultry Science* 88.9 (2009): 1818-1824.

Klasing, Kirk C. Comparative Avian Nutrition. CAB International, 1998.

Klasing, Kirk C. Nutrition and the immune system, British Poultry Science, 48:5, 525-537, 2007

Koch, R. E., Kavazis, A. N., Hasselquist, D., Hood, W. R., Zhang, Y., Toomey, M. B., & Hill, G. E. (2018). No evidence that carotenoid pigments boost either immune or antioxidant defenses in a songbird. Nature Communications, 9(1), 491.

Lee, K. P., S. J. Simpson, and Ken Wilson. "Dietary protein□quality influences melanization and immune function in an insect." *Functional Ecology* 22.6 (2008): 1052-1061.

Li, Peng, et al. "Amino acids and immune function." British Journal of Nutrition 98.2 (2007): 237-252.

Lin, Borong, et al. "Role of protein glycosylation in host-pathogen interaction." *Cells* 9.4 (2020): 1022.

Lindström, Åke, and Theunis Piersma. "Mass changes in migrating birds: the evidence for fat and protein storage re□examined." *Ibis* 135.1 (1993): 70-78.

Love, A.C. (2013). *PERCEPTION OF INFECTION: BEHAVIORAL AND PHYSIOLOGICAL RESPONSES TO ILLNESS-RELATED SOCIAL CUES*. Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Macpherson AJ, Hunziker L, McCoy K, Lamarre A. IgA responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. Microbes Infect. 2001; 3:1021–1035.

Moghimian, Negar, et al. "Impacts of changes in land use/cover on soil microbial and enzyme activities." *Catena* 157 (2017): 407-414.

Murray, M. J., and A. B. Murray. "Anorexia of infection as a mechanism of host defense." *The American journal of clinical nutrition* 32.3 (1979): 593-596.

Pirola, Luciano, and José Candido Ferraz. "Role of pro-and anti-inflammatory phenomena in the physiopathology of type 2 diabetes and obesity." *World journal of biological chemistry* 8.2 (2017): 120.

Plowright, Raina K., et al. "Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (Pteropus spp.)." *Proceedings of the Royal Society B: Biological Sciences* 278.1725 (2011): 3703-3712.

Podlesak, David W., and Scott R. McWilliams. "Metabolic routing of dietary nutrients in birds: effects of diet quality and macronutrient composition revealed using stable isotopes." *Physiological and Biochemical Zoology* 79.3 (2006): 534-549.

Povey, S., Cotter, S.C., Simpson, S.J., Lee, K.P. & Wilson, K. (2009) Can the protein costs of bacterial resistance be offset by altered feeding behaviour? Journal of Animal Ecology,78, 437–446.

Povey, Sonia, et al. "Dynamics of macronutrient self□medication and illness□induced anorexia in virally infected insects." *Journal of Animal Ecology* 83.1 (2014): 245-255.

Ramenofsky, M. "Fat storage and fat metabolism in relation to migration." *Bird migration*. Springer, Berlin, Heidelberg, 1990. 214-231.

Restani, M., J. M. Marzluff, and R. E. Yates. 2001. Effects of anthropogenic food sources on movements, survivorship, and sociality of common ravens in the Arctic. Condor 103:399-404

Rogers, Christopher M., and Amanda K. Reed. "Does avian winter fat storage integrate temperature and resource conditions? A long□term study." *Journal of Avian Biology* 34.1 (2003): 112-118.

Rogers, Christopher M. "Testing optimal body mass theory: Evidence for cost of fat in wintering birds." *Ecosphere* 6.4 (2015): 1-12.Sen, R., Nayak, L. & De, R.K. A review on host–pathogen interactions: classification and prediction. Eur J Clin Microbiol Infect Dis 35, 1581–1599 (2016).

Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. Philadelphia: Lea and Febiger, 1994.

Smith, Susan B., and Scott R. McWilliams. "Dietary macronutrients affect lipid metabolites and body composition of a migratory passerine, the white-throated sparrow (Zonotrichia albicollis)." *Physiological and Biochemical Zoology* 82.3 (2009): 258-269.

Witter, Mark S., and Innes C. Cuthill. "The ecological costs of avian fat storage." *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 340.1291 (1993): 73-92.