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The Effects of Host Diet on *Eimeria falciformis* in Mice

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ABSTRACT

Thirty-five six-week-old ICR® female mice were randomly placed in five groups. Each group consisted of seven mice with nearly equal body weights housed in one cage. The mice were given experimental diets of Lab Diet®, Puppy Formula®, Whole Corn, Suet Plus®, and Timothy Grass Pellets® one week prior to inoculation of 760 sporulated oocysts of *Eimeria falciformis* and continued until 12 days after parasite inoculation. The food rations had different levels of crude protein, fat, and fiber. Oocyst production was measured from Day 8 to Day 12 postinoculation.

The results demostrated that diet affects oocyst output. A diet high in protein (Lab Diet® and Puppy Formula®) resulted in the greatest oocyst production while a diet high in carbohydrates (corn) and fat (Suet Plus®) resulted in lower oocyst production.

INTRODUCTION

Coccidian parasites in the genera *Isospora* and *Eimeria* are common in vertebrate hosts. They have a direct life cycle and are mostly host specific. The effects of host diet on coccidian infections has been extentively studied in chickens and other poultry because of the parasite's economic importance to the poultry industry (Willis and Baker, 1981). However, there are few reports of the effects of host diet on coccidian infections in mammalian hosts. Although grain and seeds constitute much of their normal diet, mice are omnivores. Living among human habitation, their diet can vary widely from grain and plant material to dairy products, fruits, and even meat. *Eimeria falciformis* is host specific in mice and is highly pathogenic causing morbitity and mortality with the ingestion of only 1000 sporulated oocysts (Mesfin and Bellamy 1978). This study was designed to determine the effects of a high fat, high protein, or high fiber diet on oocyst production of *E. falciformis* infections in outbred mice.

MATERIALS AND METHODS

The Parasite

A pure isolate of *Eimeria falciformis* was diluted with distilled water to a concentration of 1000 sporulated oocysts per 1 ml of distilled water. Ten 5 week-old female ICR mice were each given 0.5 ml of the inoculum *per os* (500 oocysts/mouse). The mice feces were collected on Days 8-12 postinoculation (PI) and mixed with 2.5% (w/v) $K_2Cr_2O_7$. Air was pumped through the fecal- $K_2Cr_2O_7$ solutions for five days to allow the oocysts to

sporulate. The fecal- $K_2Cr_2O_7$ solutions were pooled and this solution served as the stock inoculum for the experiment conducted two weeks later.

Preparation of Parasite Inoculum

The stock $K_2Cr_2O_7$ solution containing sporulated oocysts of *E. falciformis* was centrifuged (450g for 10 min) to remove the $K_2Cr_2O_7$ and diluted with distilled water to obtain 760 oocysts/0.5 ml. One half milliliter was inoculated *per os* into each of 35 six-week-old female, ICR®, pathogen free, outbred mice (Harlan Laboratories, Inc., Indianapolis, IN) using a one cc tuberculin syringe fitted with a two inch, curved feeding needle with a 2.25 mm ball diameter (Popper & Sons, Inc., New Hyde Park, NY).

Experimental Design

Thirty-five six-week-old ICR® female mice were randomly placed in five groups. Each group consisted of seven mice with nearly equal group body weights and housed in one cage. The mice were housed in a room maintained on a 12-hour day-night cycle (8:00 AM-8:00 PM), 22°C ambient temperature and 50% humidity. Each group was given an experimental diet and water ad libitum one week prior to parasite inoculation and continued on the regimen until the completion of the experiment. One group was given Timothy Grass Pellets® (Standlee Hay Co., Inc., Eden, ID), another group was given Suet Plus® (Wildlife Sciences, LLC, Chaska, MN), a third group received Puppy Formula® (Diamond Pet Foods, Meta, MO), the fourth group received Lab Diet® (PMI Nutrition International, LLC, Brentwood, MO), and the fifth group was given Whole Corn (Agrimaster®, Janesville, WI). The relative amounts of protein, fat, and fiber are listed in Table 1.

Pine wood shavings were used for bedding material for the mice from the time they arrived until Day 7 postinoculation (PI). On Day 7 PI all mice were placed in clean cages fitted with wire screen floors, 7 mm square and raised approximately 25 mm above the bottom of the cage to collect the feces. A small amount of water was added to the bottom of the cage to keep the feces moist and the oocyst viable. Feces collection took place the same time each day from Day 8 PI to Day 12 PI. Each day during feces collection, the mice were placed in a new, clean cage with a clean wire screened floor. The feces from each cage were mixed with 2.5% $K_2Cr_2O_7$, homogenized in a Waring blender for 10 seconds, strained through 40 and 60 mesh sieves, and the volume measured in a graduated cylinder and recorded. Approximately 50 ml of the fecal- $K_2Cr_2O_7$ solution was retained to determine oocyst concentration later using a McMaster counting chamber. The average oocyst count for each mouse in each dietary group each day after parasite inoculation was calculated using the following equation:

$$\frac{\left\lceil\left(\frac{C_1+C_2}{0.3}\right)\times 10\right\rceil \times Y}{7}$$

where C_1 is the total oocyst count on the left side of the counting chamber, C_2 is the total oocyst count on the right side of the counting chamber, 0.3 is the total volume in ml from each side of the McMaster counting chamber, 10 is the total ml of the dilution factor, Y is the total volume in ml of fecal- $K_2Cr_2O_7$ suspension, and 7 is the number of mice in each dietary group.

RESULTS

Host Diet vs. Oocyst Production

Oocyst production in the mice was compared from Day 8 PI to Day 12 PI among the five experimental diets (Table 1). Mice infected with *E. falciformis* and given Lab Diet produced the most oocysts of any experimental group reaching a peak oocyst production on Day 9 PI of 6.1 million oocysts and resulting in a total oocyst production of the 5 day patent period of 12.9 million oocysts (Fig. 1). Mice given Puppy Formula showed the second highest oocyst output with a peak oocyst production on Day 9 PI of 4 million oocysts and a total production during the patent period of 8.5 million oocysts. Mice with a Suet diet had a peak oocyst production on Day 9 PI of 2.4 million oocysts and a total oocyst output of 4.9 million oocysts. Mice given Whole Corn showed a peak oocyst production on Day 9 PI of 1.4 million oocysts and a total oocyst output of 2.8 million oocysts. The mice given only Timothy Grass had a high mortality rate. Two mice on the Timothy Grass diet died before given the parasite and four additional mice died during the prepatent period of the parasite. Only one mouse lived to produce oocysts during the patent period (10,000 oocysts on Day 9 PI and 80,000 oocysts on Day 10 PI). The remaining mouse in the Timothy Grass group died on Day 10 PI.

A two-way analysis of variance without replication was used to compare total oocyst counts for each dietary group and Day 8 PI to Day 12 PI. Because of the large mortality among the Timothy Grass group, it was excluded from the statistical analysis; the df = 3.

There was a trend towards significance ($F = 2.86$, $P = 0.08$) when comparing food rations to total oocyst production and there was a significant difference when comparing oocyst production between groups by individual days postinoculation ($F = 4.94$, $P = 0.013$).

Body Weights

Body weights were compared before and after the experiment for each group except the Timothy Grass group because of its 86% mortality (Fig. 2). The initial individual body weights for each group ranged between 22-30 grams with average group body weights ranging from 24.8 to 28.4.

Based on a T-test assuming equal variance, the Suet group showed an average body weight loss, although not significant, from 24.8 to 22.8 gms. The Whole Corn group had significant average body weight loss, 28.4 to 24.2 gms ($t = 2.93$, $P = 0.01$). Conversely, mice fed the Puppy Formula ($t = 10.10$, $P < 0.01$) and Lab Diet ($t = 5.71$, $p < 0.01$) gained weight significantly, 25 to 32.7gms and 25.4 to 33.1 gms respectively.

DISCUSSION

Development of a parasite may be affected by nutrition of its host both directly by the supply of growth factors and nutrients, and indirectly through altered environmental conditions in the host, such as the physiological state, ion concentration, immunological state, or bacterial fauna (Kretschmar 1968). Mice provide an excellent model to study the nutritional requirements of coccidia in a mammalian host because of their varied diet. Frandsen (1983, 1985) demonstrated that rats on a low protein diet and infected with concurrent infections of *Nippostrongylus brasiliensis* and *Eimeria nieschulzi* had less

weight gain and increased severity of the effects of the parasitic infections than infected rats with a high protein diet. Although anorexia was a primary symptom of the parasitic infections and a major factor in the reduction of dietary protein intake for the rat host, it was critical in the rats with a low protein diet. The mice in this study given Puppy Formula with a crude protein content greater than 31% demonstrated significantly greater numbers of oocysts than mice fed Lab Diet with less than 22% protein and both groups showed comparable weight grains. Becker and Morehouse (1936) tested multiple diets including various levels of protein using *Eimeria miyairii* in rats. Although they reported slightly higher oocyst production in rats given a high protein diet compared to a "normal" diet, their study used only 5 rats in each group and the higher protein diet was supplemented with casein and Steenbock's ration for protein.

Mice given the Suet ration with a fat content of greater than 35% produced significantly fewer oocysts than groups on Puppy Formula or Lab Diet that had low fat content of 3% and 4% respectively. However, the Suet fed mice also showed weight loss during the infection period. Yang et al. (2006) found the opposite results in chickens infected with coccidia. Chickens infected with *Eimeria tenella* and given diets supplemented with saturated fatty acids had higher mortality (and more oocyst production) than chickens fed diets without oil. The mice with the corn diet, high in carbohydrates and comparatively low in protein, fat, and fiber also lost weight during the experiment but also showed comparatively low oocyst production. The group given Timothy Grass Pellets apparently had too little nutritional value to sustain their metabolic rate because they began dying before the parasite challenge.

This study suggests a balanced or high protein diet that promotes growth and weight gain for the mammalian host also promotes the reproductive potential of the coccidian parasite.

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Table 1. Diets used in this study with contents of crude fat, crude protein, and crude fiber amounts.

	Timothy Grass Pellets®	Suet Plus®	Puppy Formula®	Lab Diet®	Whole Corn
Protein	<8%	<5%	>31%	<22%	<6.5%
Fat	<1.8%	>35%	<3%	<4%	<4%
Fiber	>35%	<8%	<10%	<8%	<5%

Figure 1. Average number of oocysts produced per mouse on experimental ration per day postinoculation with *Eimeria falciformis*.

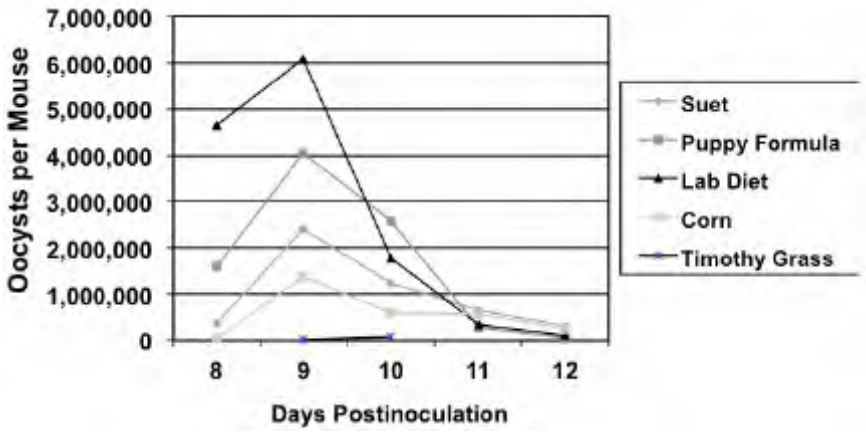

Figure 2. Comparison of average body weights of mice one week before inoculation of *Eimeria falciformis* (initial weight) and on Day 12 postinoculation of *Eimeria falciformis* (final weight).