The Effects of a Whole Hemp Seed Based Diet and Housing Type on the Gut Microbiota in Mice

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Abstract

This study aims to observe changes in the gut microbiota of weanling female Swiss Webster Mice (CFW) fed a whole hemp seed-based diet, in combination with 3 different housing types. Over the span of 8 weeks, 45 mice were housed in 3 different housing types, conventional rack caging (CRC), static microisolators, and Blue Line Tecniplast cages (IVC). Mice in each housing group were either provided with a standard rodent chow (LabDiet 5P07 - Prolab® RMH 1000), or a custom whole hemp seed-based diet that contained 9.65% whole hemp seed (LabDiet 5ZT8 – Mod LabDiet® 5P07 w/ 9.65% Whole Hemp Seed). Fecal samples were collected weekly to quantify microbiota (GM) changes at a later date, while 2 necropsies were conducted to observe gross changes to gastrointestinal tract. Additionally, body weights were taken weekly to ensure growth of weanling mice, and as a measure of GM changes.

Introduction

Husbandry practices largely influence the development and maintenance of the GM throughout and animal's lifetime. With mice being one of the most widely used species in laboratory research, the importance surrounding how husbandry influences the GM becomes greater, as proper development and maintenance of the mouse GM aids in overall health status and welfare by supporting other physiologic functions within the body. Diet and housing type are husbandry factors that can be utilized to manipulate the mouse GM, while simultaneously evaluating welfare implications associated with changes to either factor.

In this pilot study, the Swiss Webster mouse was selected as the animal model for its versatile nature and timeline of GM development. As development and maintenance of the GM can be assessed in context to the conditions created through varying housing and diet combinations to determine if GM changes affect welfare.

This pilot study manipulated the GM through varying degrees of cage ventilation associated with CRC, static microisolators, and IVC in combination with a whole hemp seed-based diet. Ventilation associated with each cage type allows for varying degrees of interaction between mice, and macroenvironment and microenvironment microbes, encouraging a diverse GM. The addition of whole hemp seeds, derived from *Cannabis Sativa*, acts as a prebiotic for the GM by encouraging optimal performance of certain microbes within the mouse GM. Two diets, the standard Lab Diet 5P07 diet and Lab Diet 5ZT8 with 9.65% whole hemp seed, were utilized to encourage GM changes during the study.

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Methods

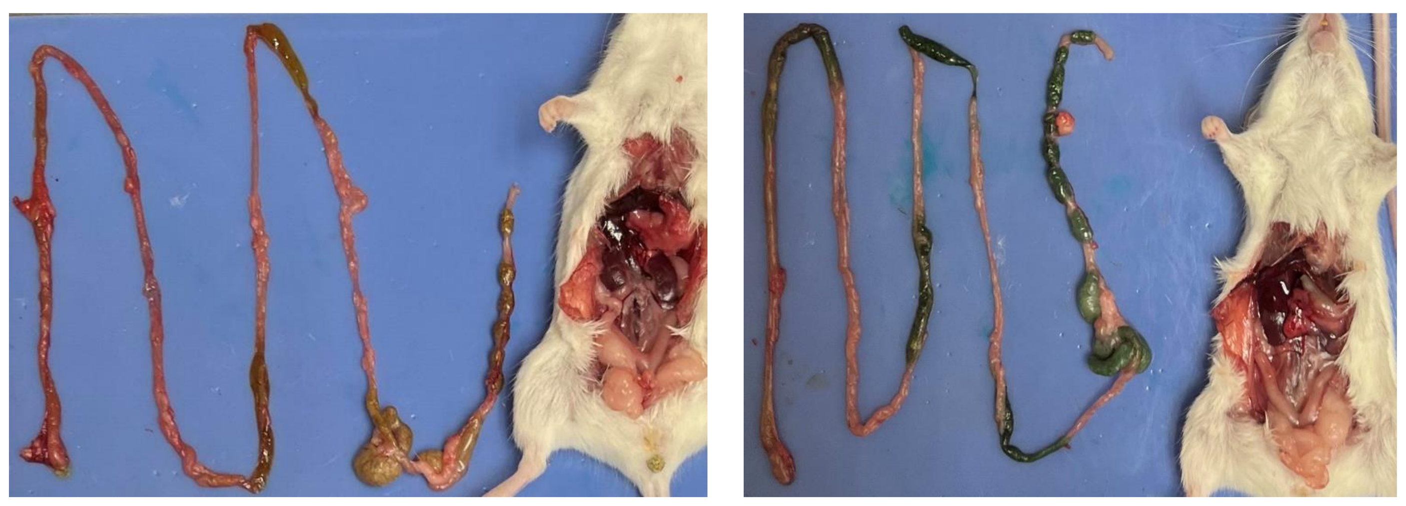
Mice were randomly assigned to one of three cage types upon arrival from Hilltop Labs. All cages were fed 5P07 for a five-day period, to allow for GM composition among all mice to adjust to control conditions of the current environment. Cages were then randomly selected to receive Lab Diet 5P07 or Lab Diet 5ZT8, depicted in figure 1. Cages were cleaned out weekly and individual weights of each mouse were measured for each cage, then all individual weights were averaged for an average cage body weight for each week to ensure growth. Additionally, average cage body weights were utilized as a qualitative measure to track if the GM composition was changing. All mice in each cage were then placed together in a clean empty cage for fifteen minutes for stool sample collection. Any bowel movements that occurred in the clean cage during this time were collected and frozen for later real-time qPCR analysis for a quantitative measure of GM composition changes. Two necropsies were also performed to provide a gross observation of the gastrointestinal (GI) tract and any gross changes that may have occurred.

Figure 1: Experimental Design of Diet & Housing

	Control	Hemp
Conventional Rack Caging (CRC)	MS04	MS05 MS06
Static Microisolator	MS02	MS01 MS03
Blue Line Tecniplast Caging (IVC)	MS08 (A+B)	MS07 (A+B) MS09 (A+B)

Figure 2: Average Cage Body Weights in CRC Static Microisolators, and IVC

Figure 3: Gross Necropsy of CRC control (L) and hemp diet (R)



Results

Weekly average cage body weights were graphed to compare changes in body weight in each cage type (Figure 2). Overall, mice housed in IVC had some of the highest weekly average cage body weights, with static microisolators following. In relation to diet type, data indicated that in cages fed a whole hemp seed-based diet possessed greater weekly average cage body weights than cages fed the normal rodent chow diet across all three housing types. Mice in IVC and fed the whole hemp seed-based diet displayed the greatest weekly average cage body weights. While mice housed in IVC and fed the normal rodent chow diet had the greatest weekly average cage body weight among all cages fed the normal rodent chow diet. Both mice fed normal rodent chow, and mice fed the whole hemp seed diet in rack caging possessed some of the lowest average weekly cage body weights throughout the study. Gross necropsy of two mice from MS04 and MS05 revealed no significant findings in relation to the GI tract, other than the color of GI contents in the mouse from MS05, due to the green dye added into 5ZT8 (Figure 3). .

Discussion & Application

From the data gathered thus far, mice housed in IVC have a less diverse GM composition compared to CRC and static microisolators. Limited exposure to macroenvironment microbes can suppress or exaggerate certain microbe populations in the GM. Limited exposure to macroenvironment microbes in tandem with 5ZT8, may encourage microbe populations that contribute to increased growth through either greater energy use or fat production, indicated by the average cage body weights. 5P07 in combination with IVC, displayed similar results in terms of housing and GM composition, yet showed that 5P07 did not encourage GM compositional changes as much as 5ZT8 based upon average cage body weights. Static microisolator cages displayed similar results in terms of diet, where cages fed 5ZT8 appear to support microbes that contribute to growth in some facets more than 5P07. Despite this, static microisolator cages have lower average cage body weights than IVC, possibly due to a more heterogenous GM composition in which a greater array of microbes direct utilization of nutritional intake toward more physiological functions. CRC also displayed that mice fed 5ZT8 appear to support microbes contributing to growth more than 5P07. Overall CRC had some of the lowest average cage body weights, as ventilation allowed for the greatest exchange between macro and microenvironment microbes among the three cage types. Such exchange can lead to mice housed in CRC to possess the most diverse GM, in which nutritional intake will support and be utilized by a multitude of microbes, that may support numerous physiological functions, weight gain, or even become pathogenic in nature. Work continued in the Spring semester will further investigate this using qPCR and histology.

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