Characterization of Fecal Bacterial Populations in Canines: Effects of Age, Breed and Dietary Fiber

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Abstract

The effects of age, breed, and diet on fecal chemistry, enzyme activity, and bacterial populations of dogs were studied. Eighteen dogs from two age groups (young: 2.5 ± 0.5 years, old: 10.9 ± 0.7 years) and three different breeds (German shepherds, miniature schnauzers, and English setters) were rotated through a Latin Square design such that every dog was fed each of the diets. The test diets included a low-fiber (control) diet and a 10% fiber diet which contained 5% soybean hulls and 5% beet pulp. Inclusion of 10% fiber in the diet decreased the fecal concentration of ammonia, sulfide, and indole. Fiber inclusion significantly increased acetic, propionic, and butyric acid concentrations, while fecal pH decreased by 0.4 units. Fresh fecal samples were plated on selected aerobic and anaerobic culture media and DNA extracted for denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S ribosomal DNA fragments. Plate counts showed significant effects of breed (p ≤ 0.05) and age (p ≤ 0.01) on selected aerobic and anaerobic bacterial counts, while no significant effect of diet was found. Analysis of PCR-DGGE banding patterns showed there was a tendency for individual dogs to cluster together according to age (young or old dogs) and also for size (large or small dogs). However, the outstanding conclusion obtained from the DGGE analysis of fecal bacterial profiles was that individual dogs had their own characteristic banding pattern which was unique and stable. The relative stability and individuality of the patterns indicates that each individual harbored a characteristic fecal bacterial community which was independent of diet.

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Introduction

The microbial community inhabiting the gastrointestinal tract is characterized by its high population density and wide diversity, as well as by the complexity of interactions that take place within the ecosystem. Large populations of microorganisms inhabit the gut and form a closely integrated ecological unit with the host. This complex mixed microbial culture can be considered as the most metabolically adaptable and rapidly renewable organ of the body, which plays a vital role in the normal nutritional, physiological, immunological, and protective functions of the host animal [23].

Over the past 40 years, a wealth of information relating to the numbers, types and metabolic activities of bacteria that can be cultivated from the gut has been published, much of it based on the classic anaerobic techniques developed initially by Hungate and refined by Bryant [10]. However, few papers have been published on the normal intestinal biota of canines [5, 7, 11, 15, 22, 38, 40], unlike the large amount of information available for other animals (ruminants, rodents, and pigs) and humans. Most of the previously reported studies on canine fecal bacteria have been carried out with beagles, since they are widely used as canine research animals, and therefore even less is known about other canine breeds. While microbial activity (fermentation and digestion) in the colon, as indicated by the production of organic acids [6], appears to have been soundly established, little is known about the fluctuations in normal fecal bacterial populations and alterations caused by dietary changes, as well as breed and age differences.

The two major problems faced by microbial ecologists studying the gastrointestinal community are the inevitable bias introduced by culture-based enumeration and characterization techniques, and the lack of a phylogenetically based classification scheme (reviewed by Raskin et al. [31]). Indeed, most of our knowledge of gut bacterial communities has been derived using indirect microbiological techniques such as selective plate counts, selective enrichment, pure culture isolation, and most probable number estimates. It is now recognized that a genotypically based classification scheme, which also reflects natural evolutionary relationships, is desirable when describing the bacterial community inhabiting the intestinal tract and feces. The application of nucleic acid (DNA and RNA)-based techniques can be used to detect, identify, and quantify bacterial populations in the gut and overcome detection and classification problems.

Inclusion of dietary fiber in canine diets has been studied for a number of years and has been shown to have beneficial effects on energy metabolism, fecal characteristics, and transit times [14, 16, 17, 18, 22, 28, 33, 37]. Beet pulp is a commonly used fiber source in canine diets and, more recently, soybean hulls have been investigated for their potential as an alternative fiber source [14, 16, 17, 18]. Previous research has shown that an equal proportion of soybean hulls had a more pronounced effect on nutrient digestibility than did other fiber sources [14] and that large differences in nutrient digestibility could occur with the same levels of beet-pulp inclusion [16, 18]. Therefore, in the current study a smaller proportion of each type of fiber was chosen (5% each of beet pulp and soybean hulls), while the total dietary fiber content was increased to a level of 10%.

In this paper, we discuss the application of both conventional and molecular techniques to evaluate bacterial activity and diversity in canine fecal samples from animals fed diets containing two different fiber levels. The main experimental hypothesis under investigation was that a change from a low-fiber (Control) diet to a high-fiber (Fiber) diet, or vice versa causes a shift in fecal chemistry, enzyme activity, and bacterial populations in dogs. The experimental design also allowed the investigation of the effect of age and breed on fecal parameters.

Materials and Methods

Experimental Test Diets

The two diets used in this study were nutritionally complete, dry extruded diets that were identical except that the Fiber diet contained 5% soybean hulls plus 5% beet pulp to replace 10% corn starch in the Control diet. Both diets were manufactured in the Pet Products Pilot Plant atRalston Purina Company, St. Louis, MO. The nutrient profiles of the diets are comparable except for their fiber content (Table 1). Moisture, crude protein, fat, and fiber content were analyzed according to the official methods described by Association of Official Analytical Chemists (AOAC; 3) and American Association of Cereal Chemists (AACC; 1). Carbohydrate (nitrogen free extract) content was calculated by difference. Energy contents were determined as described by Association of American Feed Control Officials (AAFCO; 2).

Test Animals and Experimental Design

Eighteen dogs equally representing three breeds (German shepherds, English setters, and miniature schnauzers) and two age groups (2.5 ± 0.5 and 10.9 ± 0.7 yr) were used. Dogs were housed in individual cages at the Pet Care Center (Ralston Purina, St. Louis). All dogs were fed a common diet (Dog Chow