Nutritional Implications of Coprophagy and Cecal Function in Two Microtine Rodents (*Microtus pennsylvanicus* and *Microtus pinetorum*)

by

Eric O. Johnson

Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Zoology

APPROVED:

Jack A. Cranford

Roy L. Kirkpatrick

Thomas A. Jenssen

October, 1982
Blacksburg, Virginia

ACKNOWLEDGEMENTS

I would like to thank the members of my committee, Dr. Jack Cranford, Dr. Roy Kirkpatrick, and Dr. Tom Jenssen for their time and guidance during this research project. I extend special thanks to the chairman, Jack Cranford, for his friendship and scientific dedication, without which this thesis could not have been completed.

I am especially grateful to Ms. Terry L. Derting for technical assistance with many phases of this project, and for providing pine voles, helpful suggestions, and moral support. I would also like to thank Mr. Jim Harrison for providing meadow voles; Mrs. Maureen Crumpton for technical support; and Mr. Christopher A. Aas for assisting with surgical procedures.

I would like to thank the Department of Biology and the Graduate School for providing financial assistance necessary for continuing my studies at VPI&SU. Research for this project was partially funded by a grant from the U.S. Dept. of Interior, Fish and Wildlife Service, (230-11-110-040-3729491) awarded to Dr. Jack A. Cranford.

Finally, I most deeply appreciate the enduring love, encouragement and support of my mother, father, and wife, Nancy, that leads me through each of my academic endeavors.

TABLE OF CONTENTS

LIST OF FIGURES

Figure

page

LIST OF TABLES

Table

INTRODUCTION

Digestion and assimilation strategies of herbivorous mammals are diverse but fall into two principle categories (Moir, 1968; Gartner and Pfaff, 1979; Hume and Warner, 1980). Ruminants ferment forage with a pregastric process that requires modification of the stomach and associated structures. Nonruminants employ postgastric fermentation requiring the development of special absorptive characteristics of the lower digestive tract and/or a recycling behavior (coprophagy) to return fermented material and products to the upper digestive tract for assimilation (Kenagy and Hoyt, 1980). These two processes are widely distributed through mammalian groups since the relative efficiency of each is determined by many factors including body size, forage type, and foraging technique (Moir, 1968; Janis, 1976; Hume and Warner, 1980).

The most complex combination of postgastric fermentation and coprophagic behavior utilized by a nonruminant occurs in the lagomorphs. Extensive observations confirm that these strict herbivores characteristically produce two types of feces - soft, mucous

covered pellets which are reingested and hard, dry pellets which are eliminated and ignored (Morot, 1882; Eden, 1940; Southern, 1940, 1942; Taylor, 1940; Hamilton, 1955; Kirkpatrick, 1956). The behavior occurs with regularity and a clear temporal organization (Lechleitner, 1957; Heisinger,1965; Jilge, 1974, 1976, 1978, 1979; Jilge and Meyer, 1975) which has facilitated research on its mechanism and nutritional implications (Thacker and Brandt, 1955; Hoover and Heitmann, 1975; Hornicke and Batsch, 1977; Knutson et al., 1977).

In contrast to Lagomorpha, Rodentia exhibts great variability in diet and nutritional biology (Landry, 1970; Baker, 1971; Kenagy and Hoyt,1980). Many early reports of coprophagy by rodents were incidental or descriptive, and indicated that the incidence of coprophagy in rodents was much less than in lagomorphs (Howell and Gersh, 1935; Harder, 1949; Ingles, 1961; Hamilton, 1962; Wilkes, 1962; Davis, 1969; Rood, 1970; Hoover et al., 1969; Jarvis, 1981). Much of the research on coprophagy in rodents has focused on the general nutritional consequences of the behavior in growing rats. Rats mechanically prevented from reingesting feces showed reduced growth rates (Barnes et al., 1963). Presumably, some nutritive elements unavailable in the diet (such as B-complex vitamins and amino acids) are supplied

through ingestion of products synthesized by endoflora harbored in the cecum of the lower digestive tract (Fridericia et al., 1927; Harder, 1949; Mickelsen, 1956; Daft et al., 1963; Fitzgerald et al., 1964). Unfortunately, these micronutrients have not been determined with precision (Barnes, 1962; Barnes et al., 1963), but the nutritional benefits of corophagy are probably quite similar for both rodents and lagomorphs (Kenagy and Hoyt, 1980).

Coprophagy in rodents is considered to be nutritionally beneficial, yet only a small amount of work has attempted to indicate the extent to which it occurs or the accompanying digestive mechanism in a rodent species (Kenagy and Hoyt, 1980). A recent investigation does indicate that reingestion behavior appears to be most frequent in herbivorous species, such as the microtines (Kenagy and Hoyt, 1980). Microtine rodents subsist primarily on forbs and grasses (Batzli and Cole, 1979) but do eat a wide variety of forage types (Zimmerman, 1965; Fleharty and Olson, 1969; Gill, 1977). Although few studies have closely examined the nutritional value of microtine forages, some dietary components appear to fluctuate with growing season (Cole and Batzli, 1979; Servello, 1981). Additionally, the nutritional quality of available forages may be affected by habitat manipulation (Cengel et al., 1978).

Few researchers have considered a relationship between changes in diet quality and the use of the cecum-coprophagy system found by rodents. Since free ranging animals may have a highly unpredictable diet which varies in quality over time, a mechanism compensating for dietary changes may involve coprophagy and postgastric fermentation processes. This study examined coprophagic behavior and cecal function in response to diet quality for the meadow vole (Microtus pennsylvanicus) and the pine vole (Microtus pinetorum). The nutritional response of these animals to high and low quality diets was assessed after the coprophagic or cecal component was eliminated from the digestive process. The nature of the nutritional response was determined by measuring food consumption, fecal production, diet digestibility, energy intake, and body weight dynamics.

LITERATURE REVIEW

The survival of an individual is often dependent upon its ability to efficiently obtain and process food. A host of dietary and nutritional adaptations -- ecological, behavioral, physiological, and morphological -- have been determined for a number of interrelated species (Golley, 1960; Voronstov, 1962; Morton, 1967; Janis, 1976). Of all organ systems, the gut shows the greatest variability among members of a taxonomic classification (Morton, 1967). The distinctive forms of the digestive system are due not so much to phylogenic affinity as to special adaptations to deal with unique food types utilized by the various species (Voronstov, 1962; Morton, 1967; Hume and Warner, 1980).

In this review, mammalian strategies for digesting plant food are examined, with special consideration given to small mammalian herbivores that utilize coprophagy or related mechanisms to recycle digesta.

Adaptations for Fermentation of Forage

Some of the most interesting and diverse adaptations of the mammalian gut are found in mammalian fibrivores, the herbivores that ingest massive amounts of plant food containing cellulose (Morton, 1967). This group of herbivores typically possesses a generalized digestive system that provides for a wide range of digestive capabilities (Golley, 1960; Morton, 1967; Landry, 1977). Storage and fermentation chambers often are prominent anatomical components, arising either from an expanded stomach, cecum, or colon. These areas harbor an important flora of obligate and facultative anaerobic bacteria (Morton, 1976; Janis, 1976).

Why fibrivores have evolved fermentation chambers requires explanation. Unlike hervbivorous types which subsist on the reproductive parts of plants (fruit, nuts, berries), fibrivores obtain a substantial portion of their nutritional requirements from the fermentation of plant parts containing large amounts of cellulose, the structural polysaccharide incorporated into plant cell walls. Although cellulose is probably the most common organic compound on earth, no mammal is known to produce a cellulase enzyme capable of degrading this carbohydarate, depriving animals not only of the nutritional value of the cellulose itself,

but also of the digestible compounds bound by the cell wall (Hume and Warner, 1980). Therefore, any animal subsisting on a fibrous diet must coevolve a symbiotic relationship with cellulase producing microoorganisms (Janis, 1976; Hume and Warner, 1980).

For a host animal to gain substantial energy from the end products of microbial metabolism, the metabolism must be anaerobic and include carbohydrate fermentation to volatile fatty acids (acetate, butyrate, propionate, and methane), proteolysis and deamination, vitamin synthesis, and lipid hydrolysis and hydrogenation (Hotzel and Barnes, 1966; Hume and Warner, 1980). A prerequisite for this activity is a region in the digestive tract where the retention of digesta enables adequate microbial growth to occur. Such areas are termed fermentation chambers (Morton, 1967; Janis, 1976; Hume and Warner, 1980).

Fermentation chambers are found either before or after the gastric-small intestine complex where foodstuffs are degraded and most nutrients are absorbed (Hoover et al., 1969). As a result, fibrivores generally fall into two main classes: foregut and hindgut fermenters (Hume and Warner, 1980).

Foregut fermenters most commonmly utilize rumination, a pregastric fermentation process where all food is

necessarily subjected to microbial fermentation before it passes to the gastric region of the gut (Kenagy and Hoyt, 1980). The advantages of this process include easy absorption of the fermentation products in the stomach and small intestine, and a highly efficient nitrogen recycling mechanism (Moir, 1968; Hume and Warner, 1980). Anatomically, rumination requires elaboration and modification of the esophagus and stomach of the foregut (Stevens et al., 1980). Hindgut fermenters employ postgastric fermentation that occurs after the degradative and absorptive area of the digestive system. This requires the development of special absorptive characteristics of the lower digestive tract and/or a recycling mechanism to return fermented products to the upper digestive tract for assimilation (Kenagy and Hoyt, 1980).

The evolutionary advantages of these two systems are not clearly understood. Moir (1968) asserted that foregut fermentation must be superior to hingut fermentation on the basis of the greater efficiency of fiber utilization by ruminants than by equids or other herbivores, and by the greater species diversity and higher numbers of ruminants as compared to other large non-ruminating herbivores. However, Janis (1976) pointed out that wild equids are able to use less digestible high fiber diets than can ruminants, since

the hindgut fermenting perissodactyls lack the reticulo-omasal orifice which restricts particle passage rate through the ruminant digestive tract. The perissodactyl system maintains an adequate nutrient procurement via a high forage intake and high digesta passage rate to compensate for food of lower digestibility (Janis, 1976; Hume and Warner, 1980).

Due to energetic considerations, absolute body size may also determine the fiber:protein ratio that can be tolerated in the ruminant diet. For ruminants below a certain size the fiber/protein ratio of the diet that they would have to select is so low that there would by no advantage gained from fermenting the food. Van Soest (in Janis, 1976) calculated a minimum possible weight of 5 kg for an animal in which a complete rumination process would be viable.

It appears that the relative efficiencies of foregut and hindgut fermentation processes are determined by a variety of factors including body size, forage type, and foraging pattern. As a result, all small mammalian fibrivores are non-ruminating hindgut fermenters (Hume and Warner, 1980).

Hindgut Fermentation Strategies

To accommodate hindgut fermentation, anatomical adaptations involve the colon, the cecum, or both. Within the hindgut, two alternative systems appear to have been adopted (Hume and Wanner, 1980). Colon fermentation employs the colon and cecum as one functional unit for fermentation. Cecum fermentation uses the cecum as the primary site for fermentation of small particulate and solute digesta separated from the remaining digesta bulk. These hindgut systems are somewhat correlated with the body size of the animal. All large hindgut fermenters (over 50 kg) are colon fermenters, while all small ones (under 5 kg) are cecum fermenters (Hume and Warner, 1980). At intermediate weights either system may occur, depending upon the process employed by evolutionary predecessors.

Colon Fermentation

Within the digestive tract of hindgut colon fermenters, the primary area of expansion is the proximal colon (Hume and Warner, 1980). In most cases the cecum is also enlarged, yet in a few systems it is entirely absent. If the cecum is present, it acts only as a colonic extension and there is thorough mixing of the digesta within the two organs (Bjornhag, 1972). As with most fermentations,

volatile fatty acids (VFA), B-complex vitamins, and microbial protein are all produced. Although uptake of VFA occurs in this area of the digestive tract (Gonzalez-Jimenez, 1977) and some vitamins appear to be absorbed here (Sorrel, et al., 1971), there does not appear to be much hydrolysis of microbial protein or amino acid absorption from this lower portion of the gut (Barnes et al., 1963; Gonzalez-Jimenez, 1977). The only apparent means of recovering such nitrogenous products is through reingestion (coprophagy) to return them to the upper gastro-intestinal tract (Hoover and Heitmann, 1975; Knutson, et al., 1977; Kenagy and Hoyt, 1980). Under normal circumstances large size colon fermenters are non-coprophagous (Schrug et al., 1977), indicating that body size is another important factor in determining the fiber:protein ratio that can be tolerated in the diet (Parra, 1978). Larger animals have the ability to tolerate higher fiber diets due to lower energy and protein requirements per unit body weight and to a longer exposure time of digesta to gut processes in their more extensive digestive tracts. Small non-coprophagous colon fermenters must necessarily select lower fiber:protein ratio diets than their larger counterparts (Janis, 1976; Hume and Warner, 1980).

For example, among the colon fermenters, the equids appear to display the ability to greatly increase forage intake as the fiber content in food increases, so that they are able to occupy a niche at the extreme end of the range of food fiber content. On the other hand, the non-equid perissodactyls (e.g. tapirs, rhinos) ingest food of quite low fiber content, conditions in which a foregut fermentation with its necessary waste of soluble carbohydrates and good quality protein would be at a disadvantage (Janis, 1976).

Cecum Fermentation

In some hindgut fermenters, among which the rabbit is best studied, the enlarged cecum has been used not simply as an extension of the proximal colon, but as a fermentation chamber selectively retaining solutes and small particles from the coarser, high fiber and high lignin portions of the diet (Bjornhag, 1972). All mammals utilizing cecum fermentation are small, the largest being the koala (Hydrochorus hydrochaeris) (Gonzalez-Jimenez, 1977; Hume and Warner, 1980), and all engage in some form of reingestion behavior.

Combining selective fermentation and coprophagy is advantagous for small mammals due to body size effects on

food requirements. It enables the use of high fiber diets without the encumbrance of an oversized gut, and provides efficient vitamin and protein recovery from the fermentation products that are produced primarily within the cecum (Daft et al., 1963; Hotzel and Barnes, 1963; Coates et al., 1968). However, the combination of these two processes apparently varies in complexity with species since the anatomy, behavior, and foraging pattern of cecum fermenters are not always similar.

Digesta Recycling

Numerous descriptions and studies involving the recycling of digesta have coined various terms to differentiate often subtle or poorly understood differences in the behavior and physiology of the process. For example, some authors maintain that two distinct forms of recycling occur: coprophagy and cecotrophy (Hornicke and Bjornhag, 1980; Kenagy and Hoyt, 1980). For the sake of clarity, coprophagy is used here to describe the reingestion of feces without any indication of the cause, circumstances, or nature of the fecal material consumed. Reingestion is an alternative term also used to describe the consumption by an animal of its own feces.

Other terms imply special circumstances which require more precise experimental confirmation (Kenagy and Hoyt, 1980). For example, cecotrophy is often used to describe the ingestion of special feces which originate only from the cecum. A strict distinction between cecotrophy and coprophagy is difficult to establish since in nature many different or intermediate types of feces may be produced and ingested with differing frequency in response to different dietary conditions or physiological states (Hume and Warner, 1980; Kenagy and Hoyt, 1980). Another term frequently encountered in the literature is refection, originally defined as the production of bulky, gas occluded, whitish feces by rats on a vitamin deficient diet of uncooked starch; under these conditions the rats maintained a healthy condition due to intestinal synthesis of deficient nutritional elements which were either directly absorbed in the lower digestive tract or recycled to the small intestine by coprophagy (Fredericia et al., 1927). This is an experimentally induced state and a condition separate from coprophagy itself.

Any prolonged or repetitive exposure of digesta to the mechanical, chemical or absorptive processes of the digestive tract favors maximum utilization of nutrients. At the same time it also interferes with the intake and

processing of additional foods (Morton, 1967). Differing strategies have developed to cope with this passage rate dilemma (Moir, 1968), and the solution employed depends upon which nutritional parameters become limiting during particular situations. As a result, animals reingest feces for different reasons and at different times. Reingestion may be essential for one particular nutrient (McCuistion, 1966), or may provide several (Barnes, 1962; Barnes, et al., 1963). Which factors have priority is often difficult to discern, especially since laboratory nutrition often deviates greatly from the natural feeding habits of an animal (Hornicke and Bjornhag, 1980).

Lagomorpha

Extensive observations confirm that cecal fermentation and coprophagic behavior are essential to lagomorph nutrition. Several authors have reported that domestic rabbits produce two types of feces -- soft, mucous covered pellets during one or two periods in the day (for a total of 8-10 h/d; Jilge, 1979) which are swallowed whole after being taken from the anus; and hard, drier pellets during the night which are not consumed (Morot, 1882; Madsen, 1939; Eden, 1940; Southern, 1940, 1942). This pattern has since been confirmed in many other lagomorphs (Hamilton, 1955;

Watson, 1955; Kirkpatrick, 1956; Lechleitner, 1957; Haga, 1960). Due primarily to the highly periodic nature of this phenomenon, the process and its nutritional value have been extensively studied.

Detailed physiological studies have been made only on the domestic rabbit in which both types of feces originate from the cecal contents. All of the ileal digesta enters the cecum where it is thoroughly mixed (Bjornhag, 1972). The elaboration of hard feces occurs after this point as a result of both mechanical and absorptive processes in the colon. As the digesta passes through the proximal colon the concentrations of nitrogen, microorganisms, water soluble substances, and fine dry matter particles decrease to very low values (Bjornhag and Sperber, 1977). Bjornhag (1972) demonstrated that this compositional change is not due to absorption alone, but also to a separation between different components in the colon by two principle mechanisms. Anti-peristaltic movements of the proximal colon act to squeeze fluid and fine particles toward the cecum as larger, more undigestible particles proceed caudally. These movements are are facilitated by a second mechanism which involves the net secretion of fluid into the proximal colon and the net absorption of fluid from the cecum. This results in a threefold accumulation of the less absorbable ions, calcium,

magnesium, and potassium in the cecum as compared with the concentration in the ileal digesta.

The fecal pellets derived from these processes differ in chemical composition. The soft, reingested feces are composed essentially of cecal material that passes through the colon relatively unchanged (Hornicke and Bjornag, 1980), except that in many species they are encased in a strong mucous envelope (Jilge, 1974; Jilge and Meyer, 1975). Knutson et al., (1977) demonstrated that ammonia formed through hydrolysis of urea retained within the rabbit cecum contributes to microbial synthesis of essential amino acids. In addition to poorly absorbed ionic species and vitamin complexes, soft feces contain cecal microbial protein and amino acids which are subsequently digested and absorbed after reingestion. Therefore, the cecum and coprophagy play a central role in nitrogen metabolism of the rabbit (Knutson et al., 1977; Hoover and Heitmann, 1975).

The mechanisms triggering these periodic physiological changes in the gut are largely unknown. However, the secretion and absorption changes appear to be under the influence of several endogenous factors including the hormones aldosterone and vasopressin (Hornicke and Bjornhag, 1980). The periodicity of coprophgagic behavior has been observed and commented on for many years (Southern, 1942),

but only recently has it been described as a circadian rhythm, especially as observed in the rabbit (Heisinger, 1965; Jilge, 1976, 1978, 1979; Hornicke and Batsch, 1977). Entrainment of the rhythm to the onset of the light period, and a free run period (T=24.75 h) in the absence of a zeitgeber have been demonstrated for rabbits (Jilge, 1977,1979). This pattern varies within the lagomorphs, as pikas (family Ochotonidae) have no distinct reingestion period, but instead produce hard and soft feces in multiple phases randomly throughout the day and night (Haga, 1960). Additionally, the reingested material is not encased in a mucous envelope (Hornicke and Bjornhag, 1980).

Rodent Coprophagy

Coprophagic behavior has been described for a variety of other mammalian orders including insectivores (Crowcroft, 1952; Booth, 1956; Loxton et al., 1975), marsupials (Tyndale-Briscoe, 1973), primates (Charles-Dominique and Hladik, 1971 in Kenagy and Hoyt, 1980), carnivores (McCuistion, 1966; Kronfeld, 1974), and perissodactyles (Schurg et al., 1977). However, since rodents exhibt great variability in diet and nutritional biology (Landry, 1970; Baker, 1971; Barry, 1976; Damuth, 1981), coprophagy is probably better developed in this order than in any other

except for lagomorphs (Kenagy and Hoyt, 1980). Furthermore, recent investigations indicate that the process as it occurs in rodents may be qualitatively and quantitatively different from that employed by rabbits (Kenagy and Hoyt, 1980; Ouellette and Heisinger, 1980).

Evolution

Considering the evolutionary development of rodent fibrivores, Voronstov (1962) postulated that many myomorph rodents moved to the grassy steppe land as they developed during the Miocene. At the same time, their diets changed from high energy hard-to-get foods such as fruit and seeds to lower energy easy-to-get foods such as the vegetative parts of plants. Along with these dietary changes, certain morphological developments occured, including a general reduction in the organs of movement, changes in jaw musculature, increased size of the digestive tract, increase in the large:small intestinal ratio, and increased size and complexity of the cecum and colon.

These morphological adaptations to fibrivory were also accompanied by certain behavioral adaptations. Food hoarding habits declined in importance and disappeared, while activity and feeding patterns shifted from strictly nocturnal to diurnal or to ultradian patterns (Voronstov,

1962; Baker, 1971). Coprophagic behavior was probably associated with these dietary changes, as careful observation indicates that reingestion behavior is strongly developed in highly fibrivorous species (Kenagy and Hoyt, 1980).

Descriptions

Kenagy and Hoyt (1980) reported that early studies with rats, guinea pigs, and hamsters indicated that the occurrence of coprophagy appeared to be much less marked than in lagomorphs, as whole fecal pellets were not found in rodent stomachs as they were in rabbits (Madsen, 1939). Others questioned the adaptive value of rodent coprophagy by suggesting that it occured only randomly or associated with diet restriction or unusual artificial diets (Fridericia et al., 1927; Howell and Gersh, 1935). Other special explanations for the behavior included maternal nest cleaning or water conservation mechanisms (Howell and Gersh, 1935; Baverstock and Green, 1975).

Within the last few years, increasing numbers of references to coprophagy have appeared for a variety of rodent types including Aplodontia (Ingles, 1961), Dipodomys(Kenagy and Hoyt, 1980), Geomys (Wilkes, 1962), Heterocephalus (Jarvis, 1981), Microtus (Kenagy and Hoyt,

1980; Ouellette and Heisinger, 1980), and Kerodon (personal observation). The following patterns of coprophagic behavior have been described for different species:

1. All fecal pellets are taken directly from the anus, examined, and either discarded or consumed (pocket gopher) (Wilkes, 1962).
2. All fecal pellets are dropped to the ground except during certain reingestion phases when they are taken directly from the anus for consumption (rat, guinea pig, hamster, vole, cavy) (Kenagy and Hoyt, 1980; Ouellette and Heisinger, 1980).
3. All fecal pellets are dropped to the ground and some are later reingested from the litter as they are encountered. This may lead to ingestion of pellets produced by other individuals if their home ranges overlap (mice) (Hornicke and Bjornhag, 1980).

Most rodents which perform coprophagy exhibt a high degree of temporal organization of the behavior. Timing of coprophagy involves interaction with external rhythmic events, and with foraging and ingestion of food (Kenagy and Hoyt, 1980; Ouellette and Heisinger, 1980). This timing also probably involves internal temporal coordination with ongoing physiological processes in the gut (Kenagy and Hoyt, 1980).

Nutritional Significance

Much of the attention given to reingestion in rodents has focused on the general nutritional consequences of the behavior in growing rats. Rats prevented from coprophagy had reduced growth rates relative to controls (Barnes et al., 1963). Nutritional elements, such as B-vitamins and amino acids which were unavailable in the diet, were produced by endofloral synthesis in the lower digestive tract and returned for assimilation in the small intestine by coprophagy. Not all of the micro-nutrients involved have been specifically determined (Barnes, 1962). However, it is evident that coprophagy permits the absorption of certain vitamins and vitamin complexes (Hotzel and Barnes, 1963; Hornicke and Batsch, 1977).

Similar research on other rodents is conspicuously lacking (Hume and Warner, 1980; Kenagy and Hoyt, 1980). Work with rats and guinea pigs have produced conflicting evidence on the effects of coprophagy on digestive efficiency, protein utilization, and mineral balance (Stillings and Hackler, 1966; Hintz, 1969). It is evident that coprophagy is an important mechanism for nutrient acquisition in some rodents and should be considered an important factor when examining their digestive mechanisms (Barnes, 1962; Hotzel and Barnes, 1963; Hintz, 1969).

Evidence of digesta separation and delay mechanisms similar to those in the rabbit are suggested in at least one rodent fibrivore. The anatomy of the lemming (Lemmus lemmus) colon includes a longitudinal groove within the proximal and spiral portions which exclusively contains microorganisms (Sperber, 1968). The main lumen of the anterior spiral colon contains both food residues and microbes. Proceeding caudally, fewer and fewer microbes are found among the food residues of the distal colon. Presumably, the microbes and food residues are separated along the spiral colon groove in which microbes move anteriorly toward the cecum. As a result, the fecal nitrogen content is only a third that of the cecal digesta.

Most of the nutritional benefits of coprophagy are probably qualitatively similar for rodents and lagomorphs (Kenagy and Hoyt, 1980). This may be due to the similar fermentation mechanisms in each group. However, it has been suggested that the rodent system represents an intermediate or integrated strategy between colon and cecal fermentation (Hornicke and Bjornhag, 1980; Hume and Warner, 1980). There may be only limited selective retention of small particles and solutes in the cecum, or perhaps only special cecal contents mix partially with the remaining digesta of the proximal colon (Hume and Warner, 1980). The limited

information about rodent digesta separation and delay mechanisims suggest that they are not as efficient as those of the rabbit (Bjornhag, 1972). Additionally, morphological and chemical distinction between reingested and nonreingested feces of rodents is not as sharp as it is in lagomorphs. Pellets are usually uniform in size and shape, and are not encased with a mucous envelope. Perhaps a continuum exists between the refined cecotrophy in the strictly herbivorous and larger lagomorphs, and a simple coprophagy in smaller, omnivorous species where no differential selection of pellets occurs (Hume and Warner, 1980).

Microtine Rodents

After examining rodent reingestion in a variety of species, Kenagy and Hoyt (1980) asserted that the extent of coprophagy varies among rodents in relation to diet, being more important for the fibrivorous species. Microtine rodents are herbivorous animals which possess anatomical adaptations for a high bulk, fibrous diet including an extensive lower digestive tract (Golley, 1960). Coprophagy has been shown to be a normal, adaptive behavior in a few species of this group (Kenagy and Hoyt, 1980; Ouellette and Heisinger, 1980).

Few studies have examined the nutritional qualities of microtine forages (Batzli and Cole, 1979; Servello, 1981), but microtines do utilize a wide variety of food types including forbs, grasses, and seeds (Zimmerman, 1965; Batzli and Pitelka, 1969; Fleharty and Olson, 1969; Gill, 1977; Cengel et al., 1978). The type and amount of forage selected by microtines is known to depend upon seasonal availability (Batzli and Pitelka, 1969) and nutritional quality (Batzli and Cole, 1979). Batzli and Cole (1979) demonstrated that microtines probably do not regulate diet intake solely on the basis of energetic considerations, but also use other nutritive cues. Growing season affects levels of such dietary components as calcium, phosphorous, fiber, and lignin content in some microtine diets (Cole and Batzli, 1979; Servello, 1981).

For the short-tailed vole (Microtus agrestis), digestibility of vegetation varies seasonally as a result of chemical changes in plants (Evans, 1973). Monocots become larger and more fibrous as growing season progresses (Arnold, 1963; Batzli et al., 1981) becoming less digestible and perhaps also less palatable. Lemmings (Lemmus sibericus) take more time to select, handle, and consume monocots compared to young plants (Batzli et al., 1981). Additionally, the nutritional quality of available forages

may be affected by habitat type or condition. For example, the intensity of apple orchard maintenance is thought to influence levels of available food resources for the pine vole (Microtus pinetorum) (Cengel et al., 1978; Lochmiller, 1980).

Little research appears to have considered a relationship between diet quality and the cecum-coprophagy system found in cecum fermenters such as the microtines. Since free ranging animals may have unpredictable diets varying in quality or quantity over certain time periods, it may be reasonable to expect the function of the fermentation and reingestion mechanisms to compensate for such changes. As digestibility decreases, food intake increases (Batzli and Cole, 1979; Servello, 1981) and the amount of fecal reingestion could be expected to remain constant to supply nutrients at a sufficient level, to increase to allow for longer retention of selected forage in the digestive tract, or to decrease to allow greater food intake and passage rate.

Along with the structural adaptations of the gut for a high fiber diet, the microtine foraging pattern is characterized by short term (2-3 h) cycles of activity and feeding so that food is consumed both day and night (Pearson, 1960; Weigart, 1961; Seabloom, 1965; Ambrose,

1973; Kenagy and Hoyt, 1980). Correlated with this pattern is a multiphasic alteration of feeding and reingestion of feces causing the two to be generally exclusive of each other (Ouellette and Heisinger, 1980). This could suggest that short term temporal changes occur within the gut that result in qualitatively different products occuring concomitant with reingestion (Kenagy and Hoty, 1980). Such a complicated physiology would appear to be highly adaptive for a small mammalian cecum fermenter for which coprophagy is critical to its nutrient economy.

MATERIALS AND METHODS

Experimental Animals

The microtines used in this study, meadow voles (*Microtus pennsylvanicus*) and pine voles (*Microtus pinetorum*), were obtained from outbred laboratory colonies at Virginia Polytechnic Institute and State University (Montgomery Co., VA). Experimental adult male meadow voles (weighing 40-60 g) and pine voles (weighing 20-30 g) were kept singly caged in Wahmann hanging cages (180x225x150 mm). When not in experimentally controlled diet studies, meadow voles were suppplied with rabbit chow (Roanoke City Mills) and water *ad libitum*, while pine voles were supplied with rodent chow (Wayne Lab-Blox) and water *ad libitum*. Animal rooms were illuminated by fluorescent lights automatically controlled to provide a long photoperiod of 18L:6D and ambient air temperature was maintained at 20±1 C.

Experimental Diets

Since other investigators (Servello, 1981) have reported difficulty maintaining microtines on hand picked forage, commercially produced feeds were used as experimental diets during feed trials. Commercial feeds also insure consistent nutrient levels for all animals during consecutive feed trials.

Two feeds were selected, one high quality (HQ) diet and one low quality (LQ) diet. The Wayne Lab-Blox rodent chow was used as the HQ diet; it contained a minimum of 24.0% crude protein, 4.0% crude fat, and a maximum of 4.5% crude fiber. Purina Horse Chow 100 was used as the LQ diet; it contained a minimum of 10.0% crude protein, 2.0% crude fat, and a maximum of 30.0% crude fiber. These chows were ground with a Wiley mill (1 mm screen) to eliminate differences in particle size between the two diets and to facilitate accurate weighing.

A preliminary investigation indicated that pine voles maintained on rodent chow adjusted very slowly to changes in the consistency and nutritional quality of their diet. Therefore, to avoid unnecessary mortality caused by "diet shock", two procedures were followed when manipulating pine vole diets. Ground diets were mixed with 2% corn oil by weight (20ml oil/980g chow) to reduce the amount of fine

dust particles produced by milling. Secondly, graded steps in diet quality were made to adjust the animals to the LQ diet. Graded steps were accomplished by mixing high protein, low fiber feeds (rabbit and rodent chows) with the horse chow. The horse chow fraction was gradually increased to 100% over a 10 day period. These procedures were not necessary when altering the diet quality of meadow voles as they were maintained on an intermediate quality rabbit chow.

Coprophagy Prevention

Materials

Several approaches to the complete prevention of coprophagy in rats have been reported in the literature, often involving special cages or devices that were complicated and cumbersome (Hotzel and Barnes, 1966). The most successful method was devised by Barnes and coworkers (Barnes et al., 1963) who attached a cup to the tail of rats to cover the anus and collect the fecal pellets. This method seemed unacceptable for voles considering their small size, short tails, and good ability to gnaw and chew. Instead, a collar was devised to fit around the neck of a vole, preventing the animal from reaching the anus with its mouth or forefeet. Wire bottom cages permitted feces to fall through the floor upon elimination so that they were inaccessable to the animals.

The coprophagy prevention collars were made of two parts (Fig. 1).

For meadow voles, the first part was a soft, pliable plastic circle cut 60 mm in diameter with a central hole 15-20 mm in diameter to fit around the neck. Attached to the outer circumference with plastic glue was the second member, the lip of a light weight metal jar cap cut to form a semicircle. Collars were oriented around the neck of an animal so that the metal semicircle extended anteriorly about 10 mm below the neck, between the forefeet and head. The collar was fitted around the neck through a slit in the plastic ring above the head. Once in place and adjusted correctly for the size of each animal, the slit was glued together so that it remained correctly in place. The snug fit prevented the animal from pushing the collar off the neck. Excess plastic on the top side was removed to minimize weight. The resulting assembly was pie-shaped with the arc situated below the neck. Collars weighed from 4-7 % of the animal's body weight.

Pine vole collars were identical except for smaller dimensions to accommodate their smaller size and weight. The circular diameter was up to 45 mm, and the metal lip extended forward less than 10 mm.

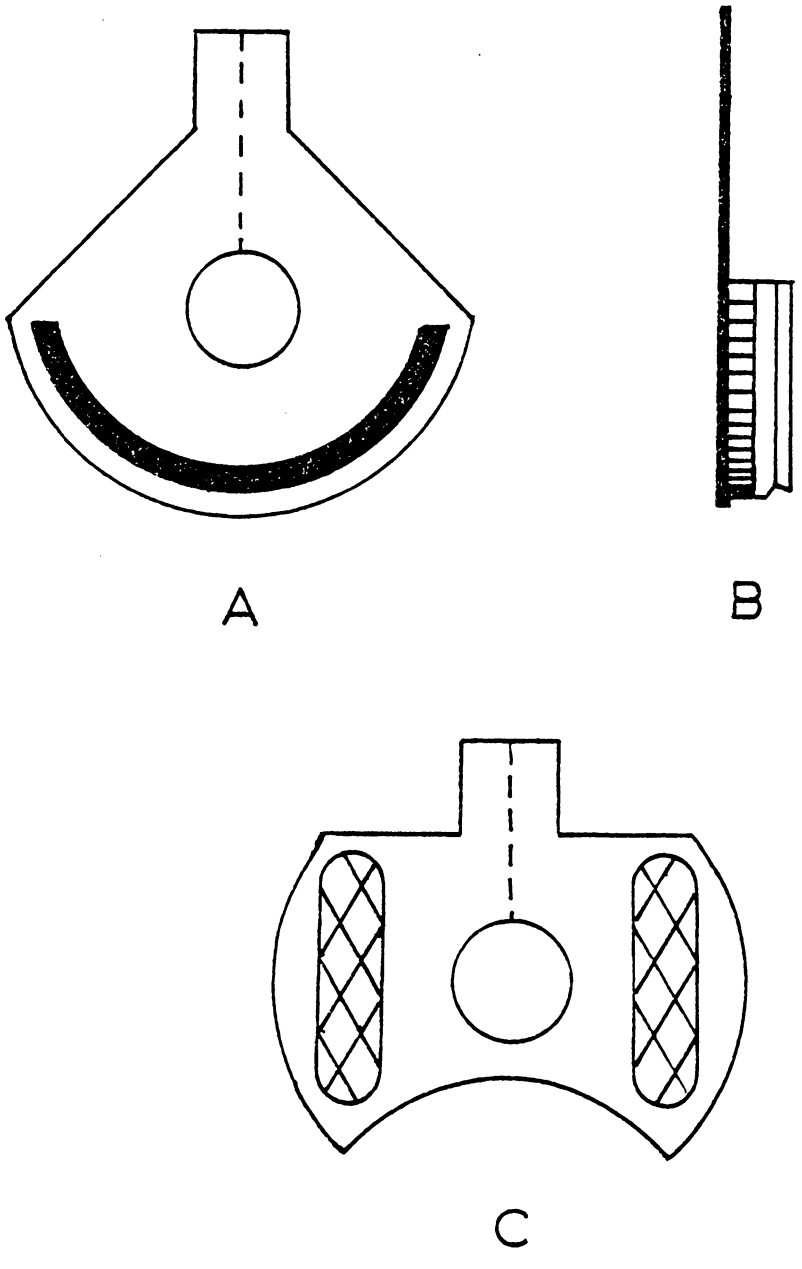

Figure 1: Diagram of a coprophagy-prevention collar from the front (A) and side (B), and of a control collar from the front (C). See text for a complete description.

To control for possible physiological stress of wearing a collar, a control collar was designed to mimic the size and bulk of the coprophagy-prevention collars. The semicrcular metal lip was flattened and divided so that one half could be attached to each side of the plastic ring surrounding the neck. Removing the excess plastic below the neck provided access to the anus. Behavioral observations confirmed that normal coprophagy occured with the control collar in place.

Experimental Design

Coprophagy-prevention effects were examined for both species using a 2x2 factorial design with diet quality and coprophagic ability as factors. Data were analyzed by analysis of variance (ANOVA) procedures, and mean values were compared with Duncans Multiple Range test, both applied using the SAS computer package (Helwig and Council, 1979). Eighteen animals randomly assigned to each diet class were divided into equal experimental and control groups. Experimental animals wore coprophagy prevention collars and control animals wore control collars, producing four treatment groups: noncoprophagous/HQ, noncoprophagous/LQ, coprophagous/HQ, and coprophagous/LQ.

The experimental protocol consisted of a five day pretrial period for collar and diet adjustment followed in order by a five day digestion trial and a six day post-trial period. Individual animals were weighed on day one of the pretrial period with an American Scientific Products model B1240-1 electronic balance (accurate to 0.1 g) and were provided with either the LQ or HQ diet ad libitum for the first five days. The digestion trial began on day six, when each animal was initially supplied with 35g of the LQ or HQ diet. Feed cups were replenished with premeasured 5g or 10g feed packets. The total amount given each day of the trial was recorded. This feeding procedure provided a constant supply of food in excess of daily needs even if large amounts were spilled or kicked from the feed cup. The true dry matter weight of each feed was determined after weighing replicate 10g samples which were oven dried at 60 C for 48 h. All packets to be used in one digestion trail were weighed at that same time as temporal changes in relative humidity would alter food packet weight. Following the digestion trial, all animals continued diet consumption ad libitum for six days and were weighed on the last day of the post-trial period.

Dropped feed and feces were collected daily during the digestion trial from trays beneath each cage. Feces and

uneaten food were oven dried (60 C for 48 h), carefully sorted, and weighed on a Metler H31AR balance (accurate to 0.001g). Food consumption equaled the difference between dry weight of the total amount of food available and food remaining. Fecal production was determined on a dry weight basis. The apparent dry matter digestiblity (ADMD) of each diet equaled 100 minus the percentage of consumed diet that was egested as feces. Individual body weight dynamics were determined for the entire experiment from initial and final body weights. An average body weight value was calculated for use in expressing food consumption and fecal production on a body weight basis. Finally, digestible energy (DE) was determined after samples of the food and feces were ground with a Wiley mill (mesh size 40), pelleted, and combusted in a Parr adiabatic bomb calorimeter. Corrections were made for both fuse wire and nitric acid formation. DE intake (DEI) was calculated for each individual and expressed on a metabolic body weight basis ($DEI/g^{\bullet .75}$).

Cecum Removal

Materials

To examine the nutritional implications of the cecum in microtines, the structure was surgically removed from the digestive system. Little information on such a procedure

for rodents existed, so surgical methods were developed after a certain amount of trial and error and professional consultation. Batzli and Cole (1979) reported that lemmings (Lemmus sibericus) had relatively empty digestive tracts after a three hour starvation, and Bowden and coworkers (Bowden et al., unpub., 1981) demonstrated that pine voles pass at least 50% of a fecal marker in six hours. Since food passage rate through the microtine digestive system is relatively fast, animals were taken off food 2-4 h prior to surgery. Preliminary investigation showed that the cecum and proximal colon were not greatly distended with food residues after this short starvation period, which simplified the following ligation and removal procedure.

Animals were anesthesized with an intramuscular injection of ketamine hydrochloride (Ketacet; Bristol Labs), using a dose rate of 50 mg/kg body weight. Hair was clipped from the abdomen and the incision area washed with 70% alcohol. A small (2 cm) incision into the peritoneal cavity was made slightly saggital to the ventral midline, starting about one cm caudal to the last rib. The cecum is a prominant structure filled with food residues that crosses the ventral midline of the abdomen. The structure was extruded through the incision with foreceps until the junction of the ileum and colon was observed. A double 0

silk ligature was placed just distal to the ileal-cecal junction around the cecum. The cecum was removed with a lateral cut 5 mm from the ligature. The cut end of the remaining tissue was flushed of food residues with distilled water. This end was then tied with double 0 silk adjacent to the initial ligature. The colon and small intestine were returned to their original position, and the skin and musculature were clamped together with surgical wound clips.

A sham procedure was performed on a second group of animals to control for trauma and discomfort of anesthesia and surgery. Following anesthesia, the abdomen was clipped, washed, and opened as described above. The cecum was located and extruded, but then returned to the abdominal cavity. Surgical wound clips were used to close the incision.

After surgery, animals were placed in shoebox cages containing wood shavings as bedding. They were returned to hanging cages after three days. Experimental treatments manipulating diet quality were not initiated until after a minimum recovery period of 10 days.

Experimental Design

As in the previous experiment, the effects of cecum removal were examined in both species using a 2x2 factorial design with diet quality and cecal condition as factors. The combination of these factors produced four treatment groups: cecectomized/HQ, cecectomized/LQ, sham/HQ, and sham/LQ. ANOVA procedures were applied to data using the SAS computer package.

Due to a high post-operative mortality (65%) from the cecectomy procedure in meadow voles, only 10 cecectomized (CX) animals were available for the diet studies, which were conducted in two phases. Each phase consisted of a five day pretrail period followed by a five day digestion trial and a six day post-trial period. In phase one, the 10 CX voles were weighed on day one and fed the HQ diet for five days ad libitum. Eighteen sham operated (S) voles were also weighed on day one, but were randomly divided equally between the HQ and LQ diet regimes and fed ad libitum for five days. Beginning on day six, the digestion trial was conducted for each group as described above. Final weights of all individuals were taken following the post-trial period of ad libitum diet consumption Body weight dynamics, food consumption , fecal production, ADMD, and DE were determined as previously described.

After a gradual 10 day transition period to the LQ diet, the CX meadow voles entered the second phase of the design. Six shams from the HQ diet group in phase one remained on the HQ diet and also completed the second phase protocol. The parameters determined for these two groups were the same as those determined in phase one. The sham voles served as a control to insure that the results from the second phase were comparable to those gathered in the phase one.

This complicated design was not used for the pine vole CX experiment since post-operative survival in pine voles was superior to that in meadow voles. All four pine vole treatment groups were initiated into the experiment at the same time.

Following all experiments with cececomized voles, surviving animals were sacrificed in order to examine the condition of the digestive tract and the surgical area. The entire tract was excised, photographed, and preserved. The residual portion of the cecum, and the proximal and spiral portions of the colon were measured and recorded.

Behavioral Observations

Materials

The most effective way to characterize the frequency and pattern of coprophagic behavior in rodents is by direct observation. Jilge (1979) devised an automatic recording device which operated on fecal pellet weight differences to record soft and hard fecal production periods of the rabbit. Due to the similar morphology of reingested and nonreingested fecal pellets of rodents, his system could not be used for these animals. My observational techniques were modeled after those of Kenagy and Hoyt (1980). Since the typical coprophagic routine in rodents involves bending the head down to the anus while sitting on the hindfeet, the best observational vantage for this behavior is from underneath the animal. A four compartment observational chamber was constructed with plexiglas sides and a 5 mm wire mesh floor (Figure 2). The overall interior dimensions of the chamber were 61x61 cm and each interior comprtment was 30.5x30.5 cm. Two mirrors (60x40 cm) were hung at 45° angles from the floor midline below the chamber floor in order to observe the underside of the voles.

With the observer sitting quietly in front of the chamber, two animals in separate compartments were simultaneously observed. Animals were maintained on a LD

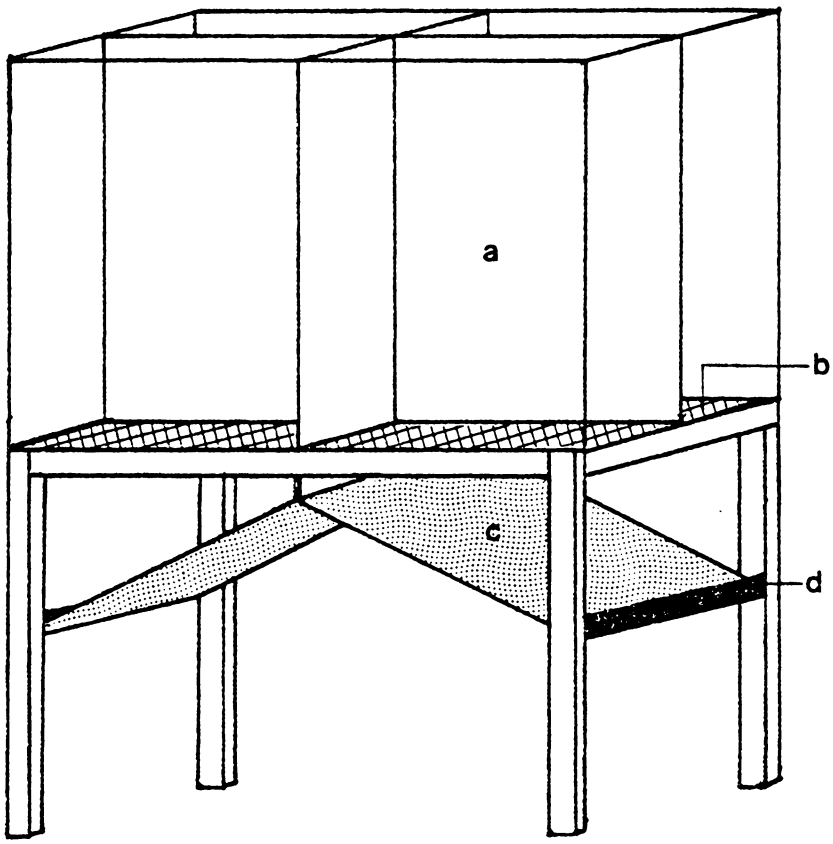

Figure 2: Diagram of chamber used for coprophagy observation; a) single compartment, b) wire mesh floor, c) suspended observation mirror, d) fecal collection trough.

16:8 and behavioral observations in the dark were made under red light. The wire mesh permitted spilled feed, feces, and urine to drop from the enclosure so that the view from underneath was not occluded by accumulating material. Fecal pellets eliminated during an observation were collected in a trough on the lower edge of the mirror. Food and water were available ad libitum in the observation chamber.

Experimental Design

The character, timing, and frequency of coprophagy as performed by intact and cecectomized animals maintained on HQ and LQ diets were determined by direct observation of both species. The intact animals had not participated in digestion trials, but cecectomized animals had participated in the trials previously described. A minimum of six individuals within each diet class and cecal condition were observed during five to eight separate periods. The length of the observaton periods varied from 30-60 min, depending upon the activity of the voles. Previous research has demonstrated that microtine rodents alternate frequently throughtout the day and night between reingestion and non-reingestion of feces, so that coprophagy occurs with near equal frequency both day and night (Kenagy and Hoyt, 1980; Ouellette and Heisinger, 1980). Preliminary observations

were made at various points in the 24 h cycle to confirm this pattern for meadow and pine voles, and to establish that observations could be made at any time period selected. Since microtines reingest feces with regular frequency in synchrony with activity periods and feeding (Kenagy and Hoyt, 1980; Ouellette and Heisinger, 1980), it was necessary to include both activity and rest periods during observations.

During observation periods, eliminated fecal pellets were collected in the trough and counted, and each coprophagic event was recorded. The percentage of fecal pellets reingested from the total number produced was determined for each observation. From replicate observations, the mean percentage of fecal pellets reingested by each individual was calculated. The mean values were compared using the Wilcoxon rank sum test (Hollander and Wolfe, 1973). Differences in the amount of coprophagy were examined for intact animals on both diets and for cecectomized animals on both diets.

RESULTS

Coprophagy Prevention

Meadow Voles

Meadow vole body weight changes were significantly influenced by diet quality (g, $p \leq .001$; %, $p \leq .01$; ANOVA) and by coprophagic ability (g, $p \leq .01$; %, $p \leq .05$; ANOVA) (Table 1). Coprophagous and noncoprophagous voles gained weight on the HQ diet, but lost weight on the LQ diet (Table 2). Within the HQ diet regime, coprophagous voles gained a significantly greater amount of weight than did noncoprophagous voles ($p \leq .05$, Duncans Multiple Range test) (Table 2). Similarily, within the LQ diet regime, coprophagous voles lost less weight than did noncoprophagous voles, although the differences were not significant (Table 2).

Food consumption by meadow voles was significantly influenced by diet quality ($p \leq .0001$, ANOVA) (Table 1), as mean consumption of the LQ diet by coprophagous and noncoprophagous voles was nearly 60% greater than HQ diet consumption (Table 2). Coprophagic ability also influenced food consumption when expressed on a body weight basis

(g/day/g vole; $p \leq .05$, ANOVA) (Table 1). Within each diet class, noncoprophagous voles consumed slightly higher amounts than did coprophagous voles (Table 2).

Fecal production was similarily affected by diet quality and coprophagic ability (Table 1). Fecal production by coprophagic and noncoprophagic voles on the LQ diet was more than three times that of comparable voles on the HQ diet (Table 2). Additionally, noncoprophagous animals produced slightly greater amounts of feces than did coprophagous animals within each diet class (Table 2).

Diet quality and coprophagic ability also affected both indices of diet digestibility. Apparent dry matter digestibility (ADMD) was influenced by diet quality ($p \leq .0001$, ANOVA) and coprophagic ability ($p \leq .05$, ANOVA) (Table 1). Similarily, digestible energy (DE) was influenced by diet quality and coprophagic ability ($p \leq .0001$, ANOVA) (Table 1). Within each coprophagic class, the mean ADMD and DE values for voles on the LQ diet were 40% and 45% lower, respectively, than HQ diet values (Table 2). Within each diet class, mean ADMD and DE values for noncoprophagous voles were slightly lower than those for coprophagous voles (Table 2). Digestible energy intake was significantly affected only by diet quality ($p \leq .001$, ANOVA) (Table 1). Mean DE intake of the LQ diet was nearly 15% lower than that of the HQ diet, regardless of coprophagic ability (Table 2).

During this experiment, four voles prevented from coprophagy died, one on the HQ diet and three on the LQ diet. Two coprophagous voles died while on the LQ diet.

Pine Voles

Body weight changes were significantly affected by diet quality (g, $p \leq .01$; %, $p \leq .001$; ANOVA) and by coprophagic ability (g and %, $p \leq .05$; ANOVA) (Table 3). Coprophagous and noncoprophagous animals displayed positive body weight dynamics on the LQ diet (Table 4). Within the HQ diet regime, coprophagous voles had higher mean weight gains than did noncoprophagous voles, although the differences were not significant (Table 4). Similarily, within the LQ diet regime, coprophagous voles lost less weight than did noncoprophagous voles (Table 4).

The remaining parameters -- diet consumption, fecal production, ADMD, DE, and DE intake -- were significantly influenced only by diet quality (Table 3). Regardless of coprophagic ability, pine voles on the LQ diet consumed nearly 15% more food per day and 30% more per gram body weight per day than did voles on the HQ diet (Table 4). Mean fecal production on the LQ diet was nearly three times that on the HQ diet (Table 4). Mean ADMD and DE values for the LQ diet were respectively 40% and 45% lower than HQ diet

values (Table 4). Mean DE intake of the LQ diet was more than 30% lower than that of the HQ diet (Table 4). One coprophagous and one noncoprophagous pine vole died when on the LQ diet during this experiment.

Cecum Removal

Meadow Voles

Of the four groups tested, sham-operated (S) voles maintained on the HQ diet had the greatest mean increase in body weight (2.34g) which was also the greatest mean percent gain (3.5%) (Table 5). The mean percent loss between CX and S voles on the LQ diet were nearly identical (-5.1% and -5.0%, respectively). As a result, only diet quality significantly influenced body weight changes ($p \leq .001$, ANOVA) (Table 6), as CX and S groups both displayed average weight gain on the HQ diet and average weight loss on the LQ diet (Table 5).

Food consumption by meadow voles (g day^{-1} and g $day^{-1}g^{-1}$) was significantly affected by diet quality ($p \leq .0001$, ANOVA), and by cecal function when consumption is expressed on a body weight basis (g $day^{-1}g^{-1}$; $p \leq .001$, ANOVA) (Table 6). Mean consumption of the HQ diet was significantly lower than LQ diet consumption, regardless of cecal condition. Within the HQ diet regime, mean

consumption between S and CX voles was not significantly different, although CX voles ate slightly more on a body weight basis than did S voles (Table 5). Within the LQ diet regime, mean consumption between the two groups was significantly different ($p \leq .05$, Duncans MR test) as CX voles consumed over 20% more of the diet per day (g) and over 30% more per gram body weight per day than did S voles (Table 5).

Both diet quality and cecal function had significant affects on fecal production, as reported in Table 6. The influence of diet quality ($p \leq .0001$, ANOVA) was evident since mean fecal production on the LQ diet was nearly three times greater than on the HQ diet, regardless of cecal condition (Table 6). The effect of cecal condition was significant ($p \leq .001$, ANOVA) and was especially evident within the LQ diet group, where mean fecal production by CX animals was nearly 50% greater than for S voles (Table 5). Within the HQ diet group, CX voles produced more feces on the average than did S voles, but the difference was not significant. Aside from these main effects, there was a significant interaction ($g\ day^{-1}$, $p \leq .01$; $g\ day^{-1}g^{-1}$, $p \leq .001$), indicating that the effects of diet quality and cecum condition were not totally independent of each other (Table 6).

Diet digestibility was similarily affected by the two factors. The ANOVA indicated that both ADMD and DE were significantly affected by diet quality and cecal condition ($p \leq .0001$), and by the interaction of the two factors ($p \leq .05$) (Table 6). As a result, all treatment means for ADMD and DE were significantly different from each other ($p \leq .05$, Duncans MR test). The mean ADMD value for CX voles on the HQ diet was 6% lower than for S voles. On the LQ diet, the mean ADMD of CX voles was 10% lower than for S voles. The mean DE for CX voles on the HQ diet was 4% lower than for S voles. On the LQ diet, mean DE of CX voles was 9% lower than for S voles. DE intake was significantly affected only by diet quality ($p \leq .0001$, ANOVA) (Table 6). As reported in Table 5, mean DE intake of the LQ diet was nearly 25% lower than that of the HQ diet.

During this experiment, three cecectomized voles died (one on the HQ diet, two on the LQ diet), as did one sham-operated vole on the LQ diet.

Pine Voles

Of the four experimental treatment groups, positive weight dynamics were observed only in the S/HQ group (Table 7). The weight losses observed in the remaining three groups were not statistically different from each other,

however, the largest mean weight loss values was for the S/LQ group (-3.84g and -15.9%). Only diet quality significantly influenced body weight dynamics ($p \leq .01$, ANOVA) (Table 8) since, within each cecal class, voles on the LQ diet lost weight compared to those on the HQ diet (Table 7).

Food consumption was significantly influenced by diet quality (g day^{-1} and g $day^{-1}g^{-1}$, $p \leq .0001$), cecal condition (g day^{-1}, $p \leq .01$; g $day^{-1}g^{-1}$, $p \leq .05$), and by the interaction of the two factors (g day^{-1},$p \leq .01$; g $day^{-1}g^{-1}$, $p \leq .05$) (Table 8). Mean consumption of the HQ diet was significantly lower than LQ diet consumption, regardless of cecal condition (Table 7). Within the HQ diet regime, mean consumption values of S and CX voles were not different, although CX values were slightly higher. Within the LQ diet regime, mean consumption values of the two groups were significantly different ($p \leq .05$, Duncans MR test) as CX voles consumed nearly 40% more of the diet than did S voles.

Fecal production (g day^{-1} and g $day^{-1}g^{-1}$) was similarily influenced by both main effects and their interaction (ANOVA) (Table 8). The effect of diet quality ($p \leq .0001$) was apparent as mean fecal production on the LQ diet was over four times that on the HQ diet, regardless of cecal condition (Table 7). The effect of cecal function on fecal production (g day^{-1}, $p \leq .0001$; g $day^{-1}g^{-1}$, $p \leq .01$) was

evident since mean values for CX voles were 40% greater than for S voles within the LQ diet regime, and 30% greater than S voles within the HQ diet regime (Table 7).

Diet digestibility (ADMD and DE) was significantly affected only by diet quality ($p \leq .0001$, ANOVA) (Table 8). Mean ADMD and DE values on the HQ diet were nearly 50% greater than on the LQ diet, regardless of cecal condition (Table 7). Within the HQ diet regime, mean ADMD and DE values for CX voles were nearly 7% lower than for S voles, but these differences were not significant (Table 7). DE intake was significantly influenced only by diet quality ($p \leq .05$, ANOVA) (Table 8). DE intake by voles on the LQ diet was lower than that of voles on the HQ diet, even though the two CX voles on the LQ diet displayed unusually high DE intake values (Table 7). However, DE intake for S voles on the LQ diet was 30% lower than for S voles on the HQ diet (Table 7).

Behavioral Observations

Preliminary observations made at random time periods confirmed that meadow and pine voles reingest feces in multiple phases throughout a 24 hour day. Observational data were primarily collected shortly after onset of the light phase or shortly before the onset of the dark phase.

Although it was possible to observe behavior during the dark phase under low intensity red light, accurate counts of individual coprophagic events were difficult to make under the low light conditions.

Food was supplied ad libitum in the chamber, and feeding bouts occured during a high percentage of observational periods. A typical 30 min observational period usually included at least one active feeding bout. When not actively feeding or exploring the enclosure, voles usually remained in one specific corner and either slept or groomed. Reingestion behavior typically occured before or after feeding bouts, during the resting phase. Since many animals selected a second specific corner in which to eliminate nonreingested fecal pellets, reingestion often became somewhat predictable, in that the behavior was often associated with a specific area within the enclosure. Exceptions occured when animals seemed to employ a fecal testing behavior at less predictable times and areas. Fecal pellets were often received from the anus with the incisors and then were either dropped or consumed, suggesting that the animal assessed the quality of the fecal pellet by taste, smell, and/or texture.

The results from the meadow vole observations indicate that only diet quality had a significant influence on the

frequency of fecal reingestion (Table 9). The mean percentage of fecal pellets reingested by intact meadow voles on the HQ diet (12.1%) was significantly lower than the mean reingestion percentage for intact voles on the LQ diet (18.9%; $p \leq .01$, Wilcoxon Rank Sum test). The same difference was found for CX meadow voles, as CX/HQ voles reingested on the average 12.2% of feces produced which was significantly lower than the mean reingestion value of 17.0% for CX/LQ voles ($p \leq .05$, Wilcoxon RS test).

Although intact pine voles maintained on the LQ diet reingested a higher mean percentage of fecal pellets than did intact voles on the HQ diet (Table 9), the difference was not significant ($p \geq .05$, Wilcoxon RS test). Mean reingestion percentages for CX pine voles were slightly lower than for intact voles. The difference between reingestion values of voles on the LQ and HQ diets were again not significant (Table 9).

DISCUSSION

Weight loss caused by the prevention of coprophagy was significant for both meadow and pine voles (Tables 2 and 4). The similar response in body weight dynamics due to coprophagy prevention demonstrates that the behavior is important in the maintenance of the nutritional status of these microtine rodents. Diet quality was also a conspicuous nutritional factor influencing weight changes (Tables 2 and 4). The four possible combinations of control and experimental treatments produced graded body weight changes, indicating that the individual factor effects were additive. Coprophagous animals on the HQ diet gained the most weight, noncoprophagous animals on the LQ diet lost the most weight, and intermediate weight changes occurred in the remaining two combinations of coprophagic ability and diet quality. Although the effects of diet quality had higher levels of statistical significance than those for coprophagic ability, coprophagy prevention influenced body weight changes, irrespective of diet type.

The nutritional importance of coprophagy was readily demonstrated for both rodents; however, the nutritional

response was not identical for the two species. The differences in food consumption, fecal production, and diet digestibility between coprophagous and noncoprophagous voles within each diet class were slight, but consistent (Table 2). Noncoprophagous meadow voles had higher mean food consumption and fecal production values, and lower mean digestibility values than did coprophagous voles. In general, noncoprophagous meadow voles consumed more food and processed it less efficiently than did coprophagous voles. Similar effects attributable to coprophagy prevention were not observed in pine voles, for which food consumption and processing efficiency were affected only by diet quality (Table 4).

The body weight changes observed in this study are consistent with previous reports of growth depression caused by coprophagy-prevention in rats (Barnes et al., 1963; Stillings and Hackler, 1966) and guinea pigs (Hintz, 1969). Barnes et al. (1963) found that growth depression was accompanied by lower food consumption; consequently, growth reduction was not attributed to lower food utilization efficency, but to voluntary reduction in food consumption and to inaccessibility of growth stimulating factors present in the feces. However, Stillings and Hackler (1966) reported that noncoprophagous rats generally increased food

consumption even though growth was depressed. In the present study, depression in food consumption by noncoprophagic pine voles was not observed (Table 3), and noncoprophagic meadow voles actually consumed significantly greater amounts of food per gram body weight than did coprophagous voles (Table 2). These results were important in establishing that food consumption by the voles was not physically restricted by the prevention collars. The prevention of coprophagy in meadow voles also led to lower diet digestibility (ADMD and DE, Table 1).

Although coprophagy influenced diet digestibility in meadow voles, digestible energy intake per gram metabolic body weight (DE intake) by meadow and pine voles was not significantly affected by coprophagy prevention (Tables 2 and 4). Increased food consumption by noncoprophagic meadow voles compensated for lower diet digestibility and maintained DE intake at levels similar to those of coprophagic voles within each diet class. Recycling feces would not be expected to automatically improve energy balance, since feces and food compete for intake, and feces contain less digestible energy than the original food source (Hornicke and Bjornhag, 1981). Reingestion could improve energy availability when the food supply is limited or of very low digestibility. Meadow voles in this study appeared

to maintain energy intake at normal levels despite digestibility perturbations introduced by coprophagy prevention, and pine voles did not appear to experience appreciable energy loss from coprophagy prevention. Since these animals had an abundant food supply, coprophagy is probably most important for the acquisition of specific dietary nutrients, perhaps even at the expense of maximal energy intake. Therefore, body weight loss in meadow and pine voles resulting from coprophagy-prevention must be due to the loss of specific dietary factors provided through fecal reingestion.

Fecal reingestion may provide or improve upon several different dietary components. For example, coprophagy may improve protein utilization for some rodents, but the evidence remains conflicting. The large intestine is undoubtably an important region for nitrogen metabolism. Although bacterial protein synthesis in the rabbit cecum and proximal colon is facilitated by urea influx from the small intestine and from diffusion from the blood (Knutson et al., 1977), only a portion of bacterially synthesized amino acids are absorbed from the large intestine (Hoover and Heitmann, 1975). The majority remains protein-bound and is available only upon reingestion. Barnes et al., (1958) reported that coprophagy in rats did not significantly influence protein

digestibility, however, Stillings and Hackler (1966) found reduced protein utilization by noncoprophagic rats, and Hintz (1969) observed lower apparent digestibility of protein by guinea pigs prevented from coprophagy. Specifically, Stillings and Hackler (1966) documented reduced nitrogen utilization in noncoprophagous animals, as nitrogen absorption decreased and urinary and fecal nitrogen excretion increased. These authors postulated that some factor in the feces may be required for optimal nitrogen utilization, but did not speculate on the identity of the component or its mechanism of action.

In the present study, accurate determination of protein digestibility was prohibited by the collection methods used in digestibility trials and by the dissimilar protein compostion of the diets. Standard metabolism cages were not used during the digestibility trials, so fecal and urinary wastes were not separated upon elimination, which did not permit their separate analysis. Furthermore, while the two experimental diets were selected to be markedly different in protein content, the sources of the protein fraction of each diet were also dissimilar. Stillings and Hackler (1966) found protien utilization as influenced by coprophagic ability to be different for diets containing proteins derived from different sources, even though the diets

contained equal amounts of crude protein. For these reasons, the influence of coprophagy on protien utilization by meadow and pine voles was not determined, but could be an important factor in microtine nutrition.

Coprophagy is known to enhance bacterial vitamin synthesis and utilization in many species (Barnes, et al., 1963; Hotzel and Barnes, 1963). Although bacterial vitamin synthesis in rats maintained on special artificial diets can provide adequate amounts of most B-complex vitamins through coprophagy, bacterial synthesis in rats on normal diets provides sufficient levels of only vitamin K (Hotzel and Barnes, 1963; Hornicke and Bjornhag, 1981). It has been suggested that the limited vitamin synthesis occurring in normal rats is due to their relatively small ceca. Bacterial synthesis in the considerably larger cecum of rabbits provides B-complex vitamins well in excess of daily needs (Hornicke and Bjornhag, 1981), and the dominant structure of the microtine digestive tract is also the cecum (Golley, 1960). Measurements made in conjunction with this study confirm previous descriptions (Golley, 1960) that the cecum of adult meadow voles averages 110 cm in length, varies in diameter, and weighs approximately 25% of the total wet weight of the intestinal tract. Consequently, vitamin synthesis within the microtine cecum may be more comparable to that of the rabbit than the rat.

Aside from vitamin supplementation, fecal reingestion may provide nutritive factors which have yet to be determined. Barnes et al. (1963) reported significant growth depression in noncoprophagic rats even when all necessary dietary nutrients were supplied. However, investigations on the nature of a growth stimulating factor obtained through coprophagy did not meet with success. Evidence for another unknown dietary factor provided by reingestion which improved nitrogen utilization in rats was reported by Stillings and Hackler (1966).

In the present study, the deleterious effects of coprophagy prevention on diet digestibility and body weight gain were similar in magnitude for both quality diets. This result is consistent with previous indications that coprophagy in these two microtines provides select dietary nutrients but is not critical for maximal energy or protein intake. Coprophagy may become more important in providing energy or protein for animals with dietary restrictions more severe than that of the LQ diet in this study. For example, deer mice (Peromyscus maniculatus) were able to withstand diets devoid of protein only when coprophagy was permitted (Karch, 1974).

Digestive capabilities for meadow and pine voles appear to be similar when fed either the low or high quality diets.

The HQ diet was digested (ADMD and DE) at nearly twice the efficiency as was the LQ diet (Tables 2 and 4). These differences in diet digestibility most likely correspond to differences in fiber content of the diets. Keys and Van Soest (1970) reported that forage digestibility by meadow voles decreased as fiber and lignin content in the diet increased. This relationship has been documented in other microtines, including pine voles (Servello, 1981; Shull et al., 1981). The LQ diet used in this study was a commercial horse feed composed primarily of a mixture of grasses (monocotyledons) and alfalfa (a dicotyledon), and contained nearly five times the crude fiber of the HQ diet. Monocots are generally less digestible than dicots (Batzli and Cole, 1979; Batzli et al., 1981) and the average digestible energy of monocots by microtine rodents ranges from 50-55% (Batzli and Cole, 1979). Except for cecectomized animals, meadow and pine voles had mean DE values for the LQ diet around 45% (Tables 2 and 4). This is lower than most values reported for microtines, especially for a diet composed of monocots and dicots (Batzli and Cole, 1979).

Batzli and Cole (1979) found that several microtine rodents were able to adjust forage intake to meet nutritional requirements. Lower diet digestibility was compensated by higher diet intake so that digestible

nutrient intake remained relatively constant. Specifically, digestible dry matter and digestible energy intake remained uniform over a wide range of diet digestibility. In the current study, mean DE intake of the HQ diet by meadow and pine voles was within the range of values predicted by Batzli and Cole (1979) for adult microtines (1.2-1.4 $kcal/g \cdot ^{.75}$). However, for both species, DE intake on the LQ diet was significantly lower than that on the HQ diet (Tables 2 and 4). Consumption of the LQ diet was consistently higher than HQ diet consumption, but was not sufficient to maintain a DE intake similar to that on the HQ diet.

The LQ diet apparently was of too poor quality to provide adequate energy for maintenance, as meadow and pine voles lost weight on the LQ diet, regardless of experimental treatment. Schull et al. (1982) found that pine voles increased intake to maintain a constant energy intake on diets as low as 55% DE. However, although not significant, these animals were beginning to lose weight, indicating that 55% DE may have been a boarderline maintenance diet. Batzli and Cole (1979) observed two cases with similar results. Brown lemmings (Lemmus sibericus) on a moss diet of 25.4% DE, and prairie voles (Microtus ochrogaster) on a bluegrass diet of 49.6% DE did not maintain intake at levels

sufficient to maintain normal DE intake, and either died or lost weight. In the lemming's case, they postulated that diet digestibility was so low that the animals were unable to process the food fast enough and did not maintain high levels of intake. Described by Ammann (1977) as digestive tract fill, this phenomenon is well known in ruminants, but does not explain the low intake of bluegrass by prairie voles. The authors suggested that bluegrass may contain certain chemicals that inhibit forage consumption. This has been demonstrated for some plant extracts containing secondary compounds including alkaloids, phenols, and terpenes that influence forage preferences of brown lemmings (Jung and Batzli, 1981). Either or both of these mechanisms could be involved in the inhibited DE intake of the LQ diet by meadow and pine voles. It is more likely, however, that the threshold point for digestive tract fill is being approached at the high consumption level necessary to compensate for the low DE of the diet.

In many respects the effects of cecum removal on the nutritional status of meadow and pine voles were unlike the effects attributed to coprophagy prevention. Unfortunately, high mortality in the cecectomy experiment with pine voles made meaningful statistical comparisons difficult, especially those involving cecectomized pine voles on the LQ

diet (n=2). An obvious contrast between the two experiments (cecum removal and coprophagy prevention) was that body weight dynamics in the cecectomy experiments were significantly influenced only by diet quality (Tables 6 and 8), whereas in the coprophagy prevention experiments body weight dynamics were affected by both factors. As reported in Tables 5 and 7, sham (S) and cecectomized (CX) voles of both species lost similar amounts of weight on the LQ diet, S and CX meadow voles and S pine voles gained weight on the HQ diet, and only CX pine voles lost weight on the HQ diet.

Diet quality effects during the cecectomy experiment were similar to those previously described. Additionally, many of the other nutritional characteristics were significantly affected by cecum removal. Cecum removal was associated with increased food passage rate, as food consumption and fecal production levels rose for both species (Tables 6 and 8). In meadow voles, cecum removal also significantly lowered diet digestibility (ADMD and DE; Table 6). However, the effects of cecum removal were more severe for animals on the LQ diet than on the HQ diet. Cecectomized voles consumed slightly higher amounts of the HQ diet than did S voles, but CX consumption of the LQ diet was significantly greater than that of S voles (Tables 5 and 7). Similarily, fecal production by CX pine and meadow

voles on the LQ diet was 40% and 50% greater, respectively, than that of S voles on the LQ diet. In meadow voles, ADMD and DE of both diets were significantly lowered by cecum removal, but the reduction in LQ diet digestibility (10%) was twice that for the HQ diet (5%) (Table 5). HQ diet digestibility by cecectomized pine voles also was reduced by 5%. However, LQ diet digestibility did not appear to be significantly affected by cecum removal, but this may be due to the small sample size (Table 7).

Post-mortem procedures were performed on five cecectomized meadow and five cecectomized pine voles in order to determine the relative sizes of residual ceca that remained in the gut. Residual ceca in pine voles ranged from 12-17 mm; in meadow voles they ranged from 9-43 mm. In almost every case, individuals which possessed a larger residual cecum had higher ADMD and DE values. This helps to explain some of the variation inherent in the data. It also partially explains why there was a significant interaction between the main effects of diet quality and cecal condition influencing diet consumption, fecal production, ADMD, and DE in meadow voles, and food consumption and fecal production in pine voles (Tables 6 and 8). With a significant interaction, the factors are not independent of one another; the simple effects of a factor differ, and the magnitude of

any simple effect depends upon the level of the other factor. Since neither of the main effects had an overriding influence on any of the nutritional characteristics, the interaction is probably due to differential digestion of the two diets as influenced by the size and function of the cecum.

The influence of cecum removal on diet digestibiltiy demonstrates the contribution the structure makes to microtine nutrition. Its presence becomes especially important when the animals are maintained on a fibrous feed of low digestibility. Vegetative forage contains substances that vary in digestibility (Keys and Van Soest, 1970) and rate of digestibility (Bjornhag and Sperber, 1977), and the thick walled cells and lignified materials that make up a high percentage of available carbohydrates in the structural parts of plants generally are more indigestible as compared to more parenchymatous portions of the plant (Keys and Van Soest, 1970). Animals which consume these relatively indigestible foods are obliged to eat large amounts and pass residues at a high rate. For example, microtines increase forage intake as diet digestibility decreases (Keys and Van Soest, 1970; Batzli and Cole, 1979; Servello, 1981), and pass fibrous forage through the digestive tract faster than they do a less fibrous seed diet (Kostelecka-Myrcha and

Myrcha, 1964). Rapid food passage rate does not favor the more leisurely process of microbial fermentation necessary to efficently process fibrous foods, further depressing diet digestibility (Janis, 1976). Small animals, such as the microtines used in this study, will be especially sensitive to this, as they must consume large amounts of food relative to their size and to the volume of their digestive tracts. It is advantagous, therefore, to pass relatively indigestible fractions of the diet as fast as possible, and to retain the more digestible particles in areas that allow for sufficient microbial digestion.

Mechanisms which serve to selectively separate fine and coarse digesta particles between the cecum and large intestine have been described in a variety of small herbivores (Bjornhag and Sperber, 1977). For example, the lemming (Lemmus lemmus) colon effects a separation by which fine digesta particles, including microorganisms, are retained in the cecum and proximal colon, and coarser residual food particles are passed immediatly down the colon. This helps to retain microorganisms in high concentrations, increasing the rate of carbohydrate fermentation in the cecum. As in rabbits, the cecal contents are periodically passed through the colon, after which they are reingested (Bjornhag and Sperber, 1977).

Additionally, Batzli and Cole (1979) found that brown lemmings were able to maintain energy intake at extremely low forage digestibilities, reflecting adaptations for utilizing forages of low nutrient concentration. Few studies have reported microtine forage digestibilities to be as low as those found for lemmings (ADMD = 33-37%, DE = 34-39%). This evidence suggests that separation mechanisms allowing for retention of small digesta particles in the cecum and proximal colon are essential for small herbivores utilizing diets of low digestibilities. In the present study, surgical removal of the microtine cecum must have severely disrupted these mechanisms. Consequently, the ability to digest forage was reduced, especially when the food was a high fiber forage of low digestibility.

Although cecum removal had a significant effect on food processing efficiency, DE intake was not significantly affected by cecal function (CX vs. S) in either meadow or pine voles (Tables 6 and 8). Table 5 shows that meadow voles on the HQ diet had lower mean DE intake values than those predicted by Batzli and Cole (1979) or those that were observed in the coprophagy prevention trial (Table 1). However, cecectomized meadow voles maintained DE intake at the same level as their controls. Pine voles on the HQ diet displayed energy intake values similar to those seen in

coprophagy prevention trials, and cecectomized pine voles also maintained DE intake at the same level as their controls (Table 7). The high DE intake observed for the two CX pine voles surviving the LQ diet are not consistent with the trends in the other three groups, and probably are not representative due to sample size. Since diet consumption increased in the other three groups as digestibility was lowered by cecum removal, both meadow and pine voles displayed an ability to compensate for lower diet digestibilities so that DE intake was maximized for a given diet.

Mammalian herbivores, including microtine rodents, have nutritional adaptations that differ among closely related species (Jung and Batzli, 1981). In the present study, pine and meadow voles responded differently to the two diets, and pine voles were especially difficult to maintain on the LQ diet. Both vole species digested the HQ diet with similar efficiency (ADMD and DE); however, to maintain a DE intake comparable to that of the meadow voles, pine voles consumed considerably more of the diet per gram body weight (Tables 1 and 3). Unlike meadow voles, pine voles required a gradual transition period to adjust to the LQ diet, since in a preliminary study 75% of pine voles switched suddenly to the LQ diet died.

Pine voles are fossorial animals that dig and burrow in their natural habitats; in the laboratory they tend to dig in food bowls, often spilling much of the available feed. During the coprophagy prevention experiments, food spillage was not a problem because collars restricted their ability to kick food out of the bowls. In the cecectomy experiments, cups were fitted with metal rings to restrict the top opening, and food was replenished twice a day. This insured a constant supply of food for pine voles even though food spillage continued for some voles on the LQ diet; voles that died on the LQ diet during the experiment always had an abundant food supply. Shull et al. (1982) also reported problems maintaining pine voles on a ground diet of similar low quality (DE = 44%), as voles spilled large quantities of food, and 1 of 5 (20%) died on the diet even after a device to control food spillage from bowls was used. Mortality in the present study was most severe for cecectomized pine voles, as 11 of 13 (85%) died on the LQ diet during the experiment. These deaths were associated with reduced digestive capabilities induced by cecum removal.

Although pine voles did not perform well on the low energy levels of the low quality diet, it is still difficult to explain why DE intake of the LQ diet by control meadow voles in the cecectomy experiment was depressed below DE

intake levels of the HQ diet. Apparently, cecectomized meadow voles did not experience digestive tract fill (where food passage rate is maximal due to low diet digestibility) at the same level of intake or diet digestibility that it appeared to occur in intact voles. The cecectomized voles on the LQ diet were able to compensate somewhat for the unusually low diet digestibility by drastically increasing food intake and fecal production, maintaining DE intake at a level similar to that of their controls (Table 5). However, the controls lost weight while consuming 20-30% less than did cecectomized voles, indicating that this DE intake level was not sufficient to meet maintenance requirements.

Why cecectomized meadow voles on the LQ diet increased passage rate above that observed for contols could be explained if cecum removal permits artificially high intake and food passage rates, since smaller amounts of food residues are being retained in the digestive tract. Additionally, the effects of secondary plant compounds on forage intake and palatability could be affected by alterations in microbial populations of the digestive tract induced by cecectomy. Secondary plant compounds have been demonstrated to influence microbial function in the rumen (Nagy et al., 1964). Perhaps the influence of these compounds on forage intake by small mammalian herbivores is

mediated through microbes in the gut. This influence could be reduced with the reduction of microbial populations caused by cecum removal.

The behavioral pattern of coprophagy observed for both meadow and pine voles was similar to that previously described for microtine rodents (Kenagy and Hoyt, 1980; Ouellette and Heisinger, 1980). Sitting back on their hind feet, voles turn their head down to receive fecal pellets directly from the anus with the incisors, so that coprophagy is unaffected when the animals are maintained on a raised wire screen. Many herbivorous rodents which employ coprophagy perform the behavior in an identical fashion, including mice and rats (Barnes, 1962; Kenagy and Hoyt, 1980). Direct reingestion provides a nearly continuous anaerobic environment for reingested fecal pellets, a condition which may be essential for some microbes or nutrients contained in feces that are important to the animal. Barnes et al. (1963) found that the growth stimulation effect of coprophagy was observed to its fullest extent only when fecal pellets were ingested directly on extrusion from the anus, and interpreted the need for a closed-circuit recycling mechanism as a requirement for maintenance of anaerobiosis. They were unable to stimulate growth in noncoprophagic rats with aerobic bacterial

cultures, and growth levels returned to normal only when cecal contents kept from air contamination were fed. These results imply that microbial function may continue in the upper portions of the digestive tract for some time after feces are reingested.

The stomach of many rodents has an anterior section used primarily for short term food storage (Gartner and Pfaff, 1979). This nonsecretory portion is known as the forestomach, and is lined with a dense layer of lactobacilli (Wilkins, 1981). Large numbers of fecal bacteria are also introduced by coprophagy into the stomach, but these bacteria are not retained for long periods in the gastric region. Jilge and Meyer (1975) demonstrated that in rabbits the high levels of coprophagy-induced anaerobic bacteria of the stomach were reduced to zero between sequential reingestion periods. Similarly, the prevention of coprophagy has been demonstrated to alter levels of microbial flora in the rodent digestive tract (Barnes, 1962). It has been suggested that the forestomach may provide rumen-like functions for some rodents due to the bacterial content; however, the evidence is unclear (Gartner and Pfaff, 1979). Gartner and Pfaff (1979) found no indications of microbial protein digestion in the rat forestomach, and surgical removal of the structure

demonstrated that it served primarily as a food storage area. In another study, surgical removal of the forestomach of hamsters (Mesocricetus auralus) did not effect the overall nutrition of the animals, as body weight dynamics and diet digestibilities were unaffected (Ehle and Warner, 1978).

Ouellette and Heisinger (1980) reported that meadow voles on alfalfa pellets performed 40-50 reingestion bouts in a 24 h period, one bout being a 10 min interval in which the behavior occurred. They found that feeding and reingestion acitivity occurred with equal frequency day and night (15L:9D) and that feeding and reingestion behaviors were often temporally distinct, but not mutually exclusive of each other. Calculating from these results, meadow voles should perform two reingestion bouts per hour on the average as the behavior occurs randomly throughout the 24 h day. In my study, coprophagic events occurred during approximately 90% of the observations on meadow and pine voles, in agreement with the above expectations.

As reported in Table 9, intact meadow and pine voles on the HQ diet reingested similar amounts of feces that they produced (12.1 and 15.2, respectively). Mean reingestion percentages were higher when intact voles were on the LQ diet, but the increase was significant only for meadow

voles. Coprophagy prevention in meadow voles was associated with lower energy and dry matter digestibility, and with body weight loss in both species. Perhaps the nutritionally important factors present in the feces are lower in LQ fecal material as compared to the HQ diet feces. This is a reasonable conclusion since food processing time necessarily decreases with increasing food passage rate, as occurs with low quality foods. A mechanism separating digesta components between the cecum and colon may produce a higher volume of reingestible cecal material in association with the increased food processing rate, ultimately resulting in higher amounts of reingestion of material that is processed faster but is lower in quality.

Secondary plant compounds were considered as possibly affecting forage intake. Although purely speculative, such compounds could have a similar influence on fecal intake, depending upon the resistance of the compounds to digestive processes, absorption and metabolism. The voles did employ what Kenagy and Hoyt (1980) described as a fecal testing behavior. A small percentage of the feces were taken from the anus, and due to taste and/or smell, were rejected and dropped. Kenagy and Hoyt found the behavior to be most obvious in the herbivorous kangaroo rat (Dipodomys microps), which displays a well organized daily alteration between

reingestion and nonreingestion, similar to rabbits. The behavior occurred prior to and immediately after the daily reingestion phase, when fecal quality was changing. Observations on fecal testing by meadow and pine voles were not quantified, but appeared primarily to follow the reingestion phase, which occurs with more lability than in the kangaroo rat. The apparent ability of the voles to assess fecal pellet quality suggests that a separation mechanism probably does exist in the gut, and that separated materials are periodically released down the colon. It is also possible that dietary factors (i.e. plant chemicals) could indeed affect fecal pellet palatability or quality as assessed by the vole.

Cecectomized voles of both species on the HQ diet reingested the same proportion of feces as did intact voles. On the LQ diet, reingestion by CX meadow voles increased nearly 5% over that of the HQ diet, but the increase was smaller in magnitude than it was for intact voles, which increased reingestion on the LQ diet by over 7% (Table 9). Cecectomized pine voles on the LQ diet actually reingested a smaller proportion of feces than they did on the HQ diet, but the difference was not significant and the sample size was small (n=3). For each species CX voles on the LQ diet reingested a smaller proportion of feces than did intact voles on the LQ diet.

The depression in fecal reingestion on the LQ diet would be consistent with the reduction in digesta separation ability caused by cecum removal. As food passage rate increases with low diet digestibility, the volume of reingestible cecal material produced would be lower than if the cecum were intact, depressing fecal reingestion below the level found in intact animals. In fact, for pine voles, fecal reingestion by CX voles on the LQ diet was lower than for voles on the HQ diet. However, fecal reingestion on the HQ diet was not influenced much by cecectomy, as would be predicted, since the diet is more highly digestible and not as dependent upon the functions of the cecum for processing.

The regulatory aspects of fecal reingestion and its adaptability to changing nutrient requirements are not well known. Several authors have predicted that an increase in digesta utilization should occur when food availability or the concentration of certain nutrients in the food drops, or when nutrient requirements for the animal rise (Stillings and Hackler, 1966; Hornicke and Bjornhag, 1980). For example, vitamin-deficient rats (Barnes, 1962) and protein-deficient mice (Karch, 1974) and horses (Schurg et al., 1977) may totally consume feces for short periods of time. In the current study, lower diet quality was associated with increased fecal consumption, and was significant for meadow

voles. Since commercially formulated diets were used, it was not possible to determine if only one factor or several were responsible for increased reingestion. Rabbits may respond to diet quality changes in a similar fashion. Proto et al. (1978, in Hornicke and Bjornhag, 1980) reported that rabbits did not reingest all of the soft fecal pellets produced when a commercial diet concentrate was fed, but reingested 100% of the pellets when fed green vegetation.

Three factors providing appropriate signals for reingestion have been suggested (Hornicke and Bjornhag, 1980): 1) mechanoreceptors in the rectum, 2) smell and taste, and 3) an internal drive modified by the nutritional state of the animal. The fecal testing behavior observed in this study suggests that voles may be able to determine fecal quality to some extent by smell, taste and/or texture. Kenagy and Hoyt (1980) reported a highly developed fecal testing behavior for the kangaroo rat. As mentioned before, these rodents alternate once daily between reingestion and non-reingestion, much like rabbits. Fecal testing behavior became more frequent for kangaroo rats during transitions between the two periods. Reingestion periods occur much more frequently in voles, which may have obscured distinct transition periods where testing behavior may be expected to occur. Additionally, higher levels of fecal reingestion

were associated with the low quality diet, which was nutritionally deficient in some respects, especially for pine voles.

In some cases, a compensatory augmentation of coprophagy may result from digestive processes that occur without regulatory intervention on the part of the animal itself (Hornicke and Bjornhag, 1980). Bjornhag (1972) found that coprophagy-prevented rabbits increased digesta separation efficiency so that smaller amounts of protein, micro-organisms, and water soluble substances were excreted. Additionally, Hornicke and Bjornhag (1980) reported that the ratio of nitrogen in soft feces to nitrogen in hard feces increased as crude protein in the diet decreased, and suggested that separation and retention of protein and micro-organisms was more efficient as dietary protein decreased.

If microtines possess a similar ability to improve digesta utilization in deficiency situations by changing the amount and/or composition of the ingested feces, cecum removal would be predicted to depress the benefits and amounts of feces reingested, especially in a low quality situation. Digesta separation efficiency would not improve as it could for intact voles. These expectations are consistant with the observed results for cecectomized meadow and pine voles.

In summary, some nutritional adaptations of meadow and pine voles differ, but coprophagy and cecal function have been demonstrated to be essential for the maintenance of a proper nutritional status. It is likely that coprophagy is required primarily for dietary supplementation of specific nutrients synthesized by micro-organisms in the lower gastrointestinal tract. Cecal function contributes significantly to diet digestibility, energy procurement, and protein ingestion, and becomes especially important as diet quality decreases. The regulatory mechanisms controlling coprophagy and cecal function appear to be physiological and behavioral. An internal digesta separation mechanism may determine the amount and/or composition of reingestible material; in turn, the voles seem to assess the quality of feces produced and reingest only suitable types.

LITERATURE CITED

Ambrose, H.W. 1973. An experimental study of some factors affecting the spatial and temporal activity of Microtus pennsylvanicus. J. Mamm., 54:79-110.

Ammann, A.D., R.L. Cowan, C.L. Mothershead, and B.R. Baumgardt. 1973. Dry matter and energy intake in relation to digestibility in white-tailed deer. J. Wildl. Manage., 37:195-201.

Arnold, G.W. 1964 Factors within plant associations affecting the behavior and performance of grazing animals. In: Crisp, D.J.,(ed.) Grazing in terrestrial and marine environments. Blackwell, Oxford. pp 133-154.

Baker, R.H. 1971. Nutritional strategies of myomorph rodents in North American grasslands. J. Mamm., 52:800-805.

Barnes, R.H. 1962. Nutritional implications of coprophagy. Nut. Rev., 20:289-291.

Barnes, R.H., E. Kwong, and G. Fiala. 1958. Effects of prevention of coprophagy in the rat. III. Digestibility of protein and fat. J. Nutr., 65:251.

Barnes, R.H., G. Fiala, E. Kwong. 1963. Decreased growth rate resulting from prevention of coprophagy. Fed. Proc., 22:125-128.

Barry, R.E. 1976. Mucosal surface areas and villous morphology of the small intestine of small mammals: functional interpretations. J. Mamm., 57:273-289.

Batzli, G.O. and F.R. Cole. 1979. Nutritional ecology of microtine rodents: digestibility of forage. J. Mamm., 60:740-750.

Batzli, G.O., H-J.G. Jung, G. Guntenspergen. 1981. Nutritional ecology of microtine rodents: Linear foraging rate curves for brown lemmings. Oikos, 37:112-116.

Batzli, G.O. and F.A. Pitelka. 1969. Condition and diet of cycling populations of the california vole, Microtus californicus. J. Mamm., 52:141-163.

Baverstock, P. and B. Green. 1975. Water recycling by lactation. Science, 187: 657-658.

Bjornhag, G. 1972. Separation and delay of contents in the rabbit colon. Swed. J. Agr. Res., 2:125-136.

Bjornhag, G., and I. Sperber. 1977. Transport of various food components through the digestive tract of turkey, geese, and guinea fowl. Swed. J. Agric. Res., 7:57-66.

Booth, Y.S. 1956. Shrews (Crocidura cassiteridum) on the Scilly Isles. Zool. Soc. Lond. Proc., 126:167-170.

Bowden, K.W., F.A. Servello, R.L. Kirkpatrick. 1981. The rate of food passage in the pine vole. Virginia Polytechnic Institute and State University (unpublished manuscript). 9 pp.

Cengel, D.J., J.E. Estep, R.L. Kirkpatrick. 1978. Pine vole reproduction in relation to food habits and body fat. J. Wildl. Manage., 42:822-833.

Coates, M.E., J.E. Ford, G.F. Harrison. 1968. Intestinal synthesis of vitamins of the B complex in chicks. J. Nutrition, 22:493-500.

Cole, F.R. and G.O. Batzli. 1979. Nutrition and population dynamics of the priarie vole, Microtus ochrogaster, in central Illinois. J. An. Ecol., 48:455-470.

Crowcroft, P. 1952. Refection in the common shrew. Nature, 170:627.

Daft, F.S., E.G. McDaniel, L.G. Herman, M.K. Romine, J.R. Hegner. 1963. Role of coprophagy in utilization of B vitamins synthesized by intestinal bacteria. Fed. Proc., 22:129-133.

Damuth, J. 1981. Home range, home range overlap, and species energy use among herbivorous mammals. Biol. J. Human Soc., 15:185-193.

Davis, S.D. 1969. Hibernation: Instestinal protozoa populations in ground squirrels. Exp. Para., 26:156-165.

Eden, A. 1940. Coprophagy in the rabbit: origin of night feces. Nature. 145:628-629.

Ehle, F.R., and R.G. Warner. 1978. Nutritional implications of the hamster forestomach. J. Nutr., 108:1047-1053.

Evans, D.A. 1973. Seasonal variation in the body composition and nutrition of the vole, Microtus agrestis. J. An. Ecol., 42:1-16

Fitzgerald, R.J., B.E. Gustafson, E.G. McDaniel. 1964. Effects of coprophagy prevention on intestinal microflora in rats. J. Nutr., 84:155-159.

Fleharty, E.D. and L.E. Olson. 1969. Summer food habits of Microtus ochrogaster and Sigmodon hispidus. J. Mamm., 50:475-486.

Fridericia, L.S., P. Freudenthal, S. Gudjonsson, G. Johansen, N. Schoubije. 1927. Refection, a transmissable change in the intestinal content enabling rats to grow and thrive without vitamin B in the food. J. Hygiene, 27:70-102.

Gartner, K. and J. Pfaff. 1979. The forestomach of rats and mice, a food store without bacterial protein digestion. Zbl. Vet. Med., 26:530-541.

Gill, A.E. 1977. Food preferences of the california vole, Microtus californicus. J. Mamm., 58:229-232.

Golley, F.B. 1960. Anatomy of the digestive tract of Microtus. J. Mamm., 41:89-99.

Gonzalez-Jimenez, E. 1977. Digestive physiology and feed of capybaras. Pages 167-177 In: Rechcigl, M., (ed.) CRC handbook series in nutrition and food, section G. CRC press, Cleveland.

Haga, R. 1960. Observations on the ecology of the japanese pika. J Mamm., 41:200-212.

Hamilton, W.J. 1955. Coprophagy in the swamp rabbit. J. Mamm., 36:303-304.

_____ 1962. Reproductive adaptations of the red tree mouse. J. Mamm 43:486-504.

Heisinger, J.F. 1965. Analysis of the reingestion rhythm in confined cottontails Ecology, 46:197-201.

Helwig, J.T. and K.A. Council. 1979. SAS user's guide, 1979 ed. SAS Institute, Inc. Cory, NC.

Hintz, H.F. 1969. Effect of coprophagy on digestion and mineral excretion in the guinea pig. J. Nutr., 99:375-378.

Hollander, M. and D.A. Wolfe. 1973. Nonparametric statistical methods. J. Wiley and Sons, N.Y.

Hoover, W.H., and R.N. Heitmann. 1975. Cecal nitrogen metabolism and amino acid absorption in the rabbit. J. Nutr., 105:245-252.

Hoover, W.H., C.L. Manning, H.E. Sheerin. 1969. Observations on digestion in the golden hamster. J. An. Sci., 28:349-352.

Hornicke, H. and F. Batsch. 1977. Cecotrophy in rabbits - a circadian function. J. Mamm., 42:411-412.

Hornicke, E. and G. Bjornhag. 1979. Coprophagy and related strategies for digesta utilization. Pages 707-730 In: Ruckebush, Y., and P. Thweind (eds.) Digestive Physiology and Metabolism in Ruminants. Proceedings of the 5th Int. Symp. on Ruminant Physiology. AVI Publishing Co., Westport, Conn.

Hotzel, D. and R.H. Barnes. 1966. Contributions of the intestinal microflora to the nutrition of the host. Vit. Hormo., 24:115-171.

Howell, A.B. and I. Gersh. 1935. Conservation of water by the rodent Dipodomys. J. Mamm., 16:1-9.

Hume, I.D. and A.C.I. Warner. 1980. Evolution of microbial digestion in mammals. Pages 665-684 In: Y. Ruckebush and P. Thivend (eds.). Digestive Physiology and Metabolism in Ruminants. Proceedings of the 5th Int. Symp. on Ruminant Physiology. AVI Pub. Co., Westport, Conn.

Ingles, L.G. 1961. Reingestion in the mountain beaver. J. Mamm., 42:411-412.

Janis, C. 1976. The evolutionary strategy of the Equidae and the origins of rumen and cecal digestion. Evol., 30:757-774.

Jarvis, J.U.M. 1981. Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. Science, 212:571-573.

Jilge, B. 1974. Soft feces excretion and passage time in the laboratory rabbit. Lab. An., 8:337-346.

_____ 1976. The entrainment of circadian soft feces excretion in the rabbit. J. Int. Cycle Res., 7:229-235.

_____ 1978. Automatic recording of cecotrophy in the rabbit. Lab. An., 12:19-20.

_____ 1979. The entrainment of cecotrophy-rhythm of the rabbit followding 6- and 12-h phase shifts of the zeitgeber. Chronobiologia, 6:33-38

Jilge, B. and H. Meyer. 1975. Coprophagy-dependent changes of the anaerobic bacterial flora in stomach and small intestine of the rabbit. Z. Vers., 17:308-314.

Jung, H.-J.G., and G.O. Batzli. 1981. Nutritional ecology of microtine rodents: effects of plant extracts on the growth of arctic microtines. J. Mamm., 62:286-292.

Karch, P.B. 1974. The role of experience in the behavioral/physiological adaptation of Peromyscus maniculatus bairdii to extended periods of a protein free diet. Unpublished Ph.D. dissertation. Michigan State University. 149 pp.

Kenagy, G.J. and D.F. Hoyt. 1980. Reingestion of feces in rodents and its daily rhythmicity. Oecologia, 44:403-409.

Keys, J.E. and P.J. Van Soest. 1970. Digestibility of forage by the meadow vole (Microtus pennsylvanicus). J. Dairy Sci., 53:1502-1508.

Kirkpatrick, C.M. 1956. Coprophagy in the cottontail. J. Mamm., 37:300.

Knutson, R.S., R.S. Francis, J.L. Hall, B.H. Moore, J.F. Heisinger. 1977. Ammonia and urea distribution and urease activity in the GI tract of rabbits (Orycotolagus and Sylvilagus). Comp. Biochem. Physiol., 58:151-154.

Kostelecka-Myrcha, A. and A. Myrcha. 1964. The rate of passage of foodstuffs through the alimentary tracts of certain Microtidae under laboratory conditions. Acta Theriologia, 9:37-53.

Kronfeld, D.S. 1973. Diet and the performance of racing sled dogs. J. Am. Vet. Med. Assoc., 162:470-473.

Landry, S.O. 1970. The Rodentia as omnivores. Quart. Rev. Biol., 45:351-372.

Lechleitner, R.R. 1957. Reingestion in the blacktailed jackrabbit. J. Mamm., 38:481-485

Lochmiller, R.L. 1980. Bioenergetics and nutrition of the pine vole (Microtus pinetorum) in two Virginia apple orchards. M. S. Thesis. Virginia Polytechnic Institute and State University. 184 pp.

Loxton, R.G., D. Raffaelli, M. Begon. 1975. Coprophagy and the diurnal cycle of the common shrew, Sorex araneus. J. Zool., London. 177:449-453.

McCuistion, W.R. 1966. Coprophagy: a quest for digestive enzymes. Vet. Med. Sm. An. Clin., 61:445-447.

Madsen, H. 1939. Does the rabbit chew the cud? Nature, 143:981-982.

Moir, R.J. 1968. Ruminant digestion and evolution. In: Code, C.F. (ed.) Handbook of physiology, Sect. 6, vol. 5. American Physiol. Soc., Wash., D.C.

Morot, C. 1882. Des pelotes stomaeales etes leporides. Mem. Soc. Centr. Med. Vet. 12, ser.1 Paris.

Morton, J. 1967. Guts: the form and functon of the digestive system. The Inst. of Biol., studies in biology no. 7. London.

Nagy, J.G., H.W. Steinhoff, G.M. Word. 1964. Effects of essential oils of sagebrush on deer rumen microbial function. J. Wildlf. Mang., 28:785-790.

Ouellette, D.E. and J.F. Heisinger. 1980. Reingestion of feces by Microtus pennsylvanicus. J. Mamm. 61:366-368.

Parra, R. 1978. Comparison of fore and hind gut fermentation in herbivores. In: G.G. Montgomery (ed.) The ecology of arborial folivores. pp 205-229. Washington: Smith Inst. Press.

Pearson, O.P. 1960. Habits of Microtus californicus revealed by automatic photographic recorders. Ecol. Monog., 30:231-249.

Rood, J.P. 1970. Ecology and social behavior of the desert cavy (Microcavia australis). Amer. Mid. Nat., 83:415-454.

Schurg, W.A., D.L. Frei, P.R. Check, D.W. Holtan. 1977. Utilization of whole corn plant pellets by horses and rabbits. J. Anim. Sci., 45:1317-1321.

Seabloom, R.W. 1960. Daily motor activity and corticosterone secretion in the meadow vole. J. Mamm., 46:286-295.

Servello, F.A. 1981. Nutritional ecology of the pine vole in relation to apple tree root damage. M. S. Thesis. Virginia Polytechnic Institute and State University. 152 pp.

Shull, A.M., F.A. Servello, R.L. Kirkpatrick, A.P. Tipton. 1982. Digestible energy intake, body weight, and body fat changes in pine voles fed diets of different digestibilities. Virginia Polytechnic Institute and State University (unpublished manuscript). 6pp.

Sorrel, M.F., O. Frank, A.D. Thomson, H. Aquino, H. Baker. 1971. Absorption of vitamins from the large intestine in vivo. Nut. Rep. Int., 3:143-148.

Southern, H.N. 1940. Coprophagy in the wild rabbit. Nature, 145:262.

_____ 1942. Periodicity of refection in the wild rabbit. Nature, 149:553-554.

Sperber, I. 1967. Physiological mechanisms in herbivores for retention and utilization of nitrogenous compounds. In: Isotope studies on the nitrogen chain. pp 209-219. IAEA, Vienna.

Stevens, C.E., A.F. Sellers, F.A. Spurrell. 1960. Function of the bovine omassum in ingesta transfer. Am. J. Physiol., 198:449-455.

Stillings, B.R. and L.R. Hackler. 1966. Effect of coprophagy on protein utilization in the rat. J. Nutr., 90:19-24.

Thacker, E.J. and C.S. Brandt. 1955. Coprophagy in the rabbit. J. Nutr., 55:375-385.

Voronstov, N.W. 1962. The ways of food specialization and evolution of the alimentary system in muroidea. Symp. Theriol., 1960:360-377.

Watson, J.S. and R.H. Taylor. 1955. Reingestion in the hare Lepus europaeus Pal. Science, 121:314.

Wilkins, T.D. 1981. Microbiological considerations in interpretation of data obtained with experimental animals. Banbury Report 7: Gastrointestinal Cancer: endogenous factors. Cold Spring Harbor Laboratory.

Wilks, B.J. 1962. Reingestion in geomyid rodents. J. Mamm., 43:267.

Zimmerman, E.G. 1965. A comparison of habitat and food of two species of Microtus. J. Mamm., 46:605-612.

TABLES

Table 1: Mean square values from 2x2 ANOVA for parameters determined in the 16 day coprophagy prevention experiment with meadow voles. Significant ($p < .05$) and non-significant (NS) p values are reported below the appropriate mean square. ADMD = apparent dry matter digestibility, DE = digestible energy.

Source	BODY WT. CHG.		CONSUMPTION		FECAL PROD.		DIGESTIBILITY		DE INTAKE
	(g)	(%)	g/day	g/day/g	g/day	g/day/g	ADMD	DE	
Collar (CL)	79.81	352.59	0.32	0.01	4.07	0.01	0.023	129.01	0.0004
Pr > f	.01	.05	NS	.05	.01	.05	.05	.001	NS
Diet (D)	156.82	685.18	122.69	0.1	122.69	0.01	0.63	9405.1	0.36
Pr > f	.001	.01	.001	.001	.001	.001	.001	.001	.001
CL x D	4.73	20.13	0.24	0.0	0.24	0.0	0.0	1.51	0.0005
Pr > f	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error	10.92	60.64	1.76	0.001	0.38	0.0003	0.0051	5.31	0.021

Table 2: Treatment means ± s.e. (N) for parameters determined in the 16 day coprophagy prevention experiment with meadow voles. Parameter means with the same letter are not significantly different ($p > .05$, Duncan's multiple range test). ADMD = apparent dry matter digestibility, DE = digestible energy.

Parameter	COPROPHAGOUS		NONCOPROPHAGOUS	
	HIGH QUALITY	LOW QUALITY	HIGH QUALITY	LOW QUALITY
Body wt. chg.				
(g)	4.21 ± 0.8 (9)^{A}	-0.9 ± 1.15 (7)^{B,C}	0.71 ± 0.98 (9)$^{B}_{A}$	-2.19 ± 1.48 (9)^{C}
(%)	9.06 ± 1.83 (9)^{A}	-1.6 ± 2.42 (7)^{B}	1.72 ± 2.31 (9)	-5.84 ± 3.63 (9)^{B}
Consumption				
(g/day)	7.63 ± 0.45 (9)^{B}	11.49 ± 0.47 (5)^{A}	7.23 ± 0.4 (9)^{B}	11.45 ± 3.63 (9)^{A}
(g/day/g vole)	0.158 ± 0.01 (9)^{B}	0.262 ± 0.01 (5)^{A}	0.164 ± 0.005(9)^{B}	0.287 ± 0.02 (9)^{A}
Fecal Prod.				
(g/day)	1.85 ± 0.11 (9)^{B}	6.21 ± 0.28 (5)^{A}	1.87 ± 0.17 (9)^{B}	6.39 ± 3.63 (9)^{A}
(g/day/g vole)	0.038 ± 0.001(9)^{B}	0.141 ± 0.007(5)^{A}	0.043 ± 0.005(9)^{B}	0.159 ± 0.001(9)^{A}
Digestibility				
ADMD (%)	79.5 ± 0.01 (9)^{A}	46.0 ± 0.006(5)^{B}	73.1 ± 0.04 (9)^{A}	44.3 ± 0.01 (8)^{B}
DE (%)	79.2 ± 0.73 (9)^{A}	43.3 ± 0.87 (5)^{B}	79.6 ± 1.07 (9)^{A}	42.7 ± 0.65 (8)^{B}
DE Intake				
(kcal/g$^{.75}$)	1.48 ± 0.03 (9)^{A}	1.22 ± 0.06 (5)^{B}	1.49 ± 0.05 (8)^{A}	1.29 ± 0.07 (8)^{B}

Table 3: Mean square values from 2x2 ANOVA for parameters determined in the 16 day coprophagy prevention experiment with meadow voles. Significant ($p < .05$) and non-significant (NS) p values are reported below the appropriate mean square. ADMD = apparent dry matter digestibility, DE = digestible energy.

Source	BODY WT. CHG.		CONSUMPTION		FECAL PROD.		DIGESTIBILITY		DE INTAKE
	(g)	(%)	g/day	g/day/g	g/day	g/day/g	ADMD	DE	$kcal/g^{.75}$
Collar (CL)	28.98	306.0	0.01	0.0	0.01	0.0	7.95	13.31	0.01
pr > f	.05	.05	NS	NS	NS	NS	NS	NS	NS
Diet (D)	67.61	835.02	3.54	0.04	22.42	0.01	659.51	8368.3	1.41
pr > f	.01	.001	.05	.001	.001	.01	.001	.001	.0001
CL x D	2.59	4.57	0.01	0.0	0.01	0.0	0.99	15.77	0.01
pr > f	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error	5.49	58.42	0.88	0.0016	0.23	0.0004	8.63	9.79	0.024

Table 4: Treatment means ± s.e. (N) for parameters determined in the 16 day coprophagy prevention experiment with pine voles. Parameter means with the same letter are not significantly different ($p > .05$, Duncan's multiple range test). ADMD = apparent dry matter digestibility, DE = digestible energy.

Parameter	COPROPHAGOUS		NONCOPROPHAGOUS	
	HIGH QUALITY	LOW QUALITY	HIGH QUALITY	LOW QUALITY
Body wt. chg.				
(g)	1.4 ± 0.58 (7)$^{A}_{A}$	-1.08 ± 0.64 (7)$^{A,B}_{B,C}$	0.06 ± 0.61 (7)$^{A}_{A,B}$	-3.66 ± 1.25 (8)$^{B}_{C}$
(%)	5.55 ± 2.33 (7)^{A}	-4.67 ± 2.98 (7)^{B,C}	0 ± 2.36 (7)^{A,B}	-11.88 ± 3.48 (8)^{C}
Consumption				
(g/day)	4.61 ± 0.25 (7)$^{A}_{B}$	5.29 ± 0.36 (7)$^{A}_{A}$	4.63 ± 0.26 (7)$^{A}_{B}$	5.39 ± 0.49 (7)$^{A}_{A,B}$
(g/day/g vole)	0.176 ± 0.01 (7)^{B}	0.228 ± 0.01 (7)^{A}	0.172 ± 0.01 (7)^{B}	0.21 ± 0.02 (7)^{A,B}
Fecal prod.				
(g/day)	0.99 ± 0.09 (7)$^{B}_{B}$	2.77 ± 0.2 (7)$^{A}_{A}$	0.98 ± 0.07 (7)$^{B}_{B}$	2.85 ± 0.27 (7)$^{A}_{A}$
(g/day/g vole)	0.038 ± 0.52 (7)^{B}	0.121 ± 0.01 (7)^{A}	0.036 ± 0.002 (7)^{B}	0.112 ± 0.01 (7)^{A}
Digestibility				
ADMD (%)	78.6 ± 1.10 (7)$^{A}_{A}$	47.7 ± 1.29 (7)$^{B}_{B}$	78.9 ± 0.56 (7)$^{A}_{A}$	47.2 ± 0.95 (7)$^{B}_{B}$
DE (%)	81.5 ± 0.81 (7)^{A}	47.1 ± 1.17 (7)^{B}	81.5 ± 0.58 (6)^{A}	44.2 ± 2.03 (6)^{B}
DE Intake				
(kcal/$g^{.75}$)	1.4 ± 0.07 (6)^{A}	0.97 ± 0.06 (6)^{B}	1.39 ± 0.07 (6)^{A}	0.87 ± 0.04 (6)^{B}

Table 5: Treatment means ± s.e. (N) for parameters determined in the 16 day cecectomy experiment with meadow voles. Parameter means with the same letter are not significantly different ($p > .05$, Duncan's multiple range test). ADMD = apparent dry matter digestibility, DE = digestible energy.

Parameter	CONTROL (S)		CECECTOMIZED (CX)	
	HIGH QUALITY	LOW QUALITY	HIGH QUALITY	LOW QUALITY
Body wt. chg.				
(g)	2.34 ± 1.3 (8)$^{A}_{A}$	-2.79 ± 0.72 (8)$^{B}_{B}$	1.5 ± 0.74 (9)$^{A}_{A}$	-2.4 ± 1.27 (8)$^{B}_{B}$
(%)	3.51 ± 2.57 (8)	-4.99 ± 1.1 (8)	3.7 ± 1.77 (9)	-5.05 ± 2.69 (8)
Consumption				
(g/day)	6.69 ± 0.5 (8)$^{C}_{C}$	8.82 ± 0.79 (8)$^{B}_{B}$	6.69 ± 0.39 (9)$^{C}_{C}$	10.79 ± 0.57 (8)$^{A}_{A}$
(g/day/g vole)	0.126 ± 0.01 (8)	0.166 ± 0.01 (8)	0.145 ± 0.16 (8)	0.22 ± 0.01 (8)
Fecal prod.				
(g/day)	1.54 ± 0.13 (8)$^{C}_{A}$	4.63 ± 0.46 (8)$^{B}_{B}$	1.93 ± 0.13 (9)$^{C}_{C}$	6.72 ± 0.37 (8)$^{A}_{A}$
(g/day/g vole)	0.029 ± 0.001 (8)	0.086 ± 0.07 (8)	0.042 ± 0.002 (9)	0.135 ± 0.01 (8)
Digestibility				
ADMD (%)	77.0 ± 0.35 (8)$^{A}_{A}$	48.0 ± 0.83 (8)$^{C}_{C}$	71.1 ± 0.84 (8)$^{B}_{B}$	37.7 ± 1.29 (8)$^{D}_{D}$
DE (%)	78.7 ± 0.6 (8)	46.3 ± 1.11 (8)	74.9 ± 0.87 (8)	37.1 ± 1.26 (8)
DE Intake				
($kcal/g^{.75}$)	1.16 ± 0.05 (8)^{A}	0.87 ± 0.04 (8)^{B}	1.17 ± 0.04 (8)^{A}	0.89 ± 0.04 (7)^{B}

Table 6: Mean square values from 2x2 ANOVA for parameters determined in the 16 day cecectomy experiment with meadow voles. Significant ($p < .05$) and non-significant (NS) p values are reported below the appropriate mean square. ADMD = apparent dry matter digestibility, DE = digestible energy.

Source	BODY WT. CHG.		CONSUMPTION		FECAL PROD.		DIGESTIBILITY		DE INTAKE
	(g)	(%)	g/day	g/day/g	g/day	g/day/g	ADMD	DE	kcal/$g^{.75}$
Cecectomy (CX)	0.26	0.57	6.237	0.0009	9.947	0.007	419.03	628.03	0.0009
pr > f	NS	NS	NS	.001	.001	.001	.001	.001	NS
Diet (D)	162.55	609.77	81.40	0.025	129.14	0.046	8061.4	9051.1	0.56
pr > f	.001	.001	.001	.001	.001	.001	.001	.001	.001
CX x D	3.058	0.13	7.99	0.002	5.94	0.002	38.92	53.77	0.0004
pr > f	NS	NS	NS	NS	.01	.001	.05	.05	NS
Error	8.83	38.14	2.62	0.0005	0.75	0.0002	6.54	7.72	0.014

Table 7: Treatment means ± s.e. (N) for parameters determined in the 16 day cecectomy experiment with pine voles. Parameter means with the same letter are not significantly different ($p > .05$, Duncan's multiple range test). ADMD - apparent dry matter digestibility, DE - digestible energy.

Parameter	CONTROL (S)		CECECTOMIZED (CX)	
	HIGH QUALITY	LOW QUALITY	HIGH QUALITY	LOW QUALITY
Body wt. chg.				
(g)	0.80 ± 0.25 (9)^{A}	-3.84 ± 1.51 (5)^{A,B}	-1.24 ± 0.42 (5)^{A}	-1.6 ± 0.5 (2)^{B}
(%)	3.13 ± 1.01 (9)^{A}	-15.9 ± 5.89 (5)^{B}	-5.96 ± 2.53 (5)^{B}	-7.1 ± 2.6 (2)^{B}
Consumption				
(g/day)	4.45 ± 0.28 (9)^{C}	7.04 ± 0.34 (5)^{B}	4.7 ± 0.38 (5)^{C}	9.86 ± 0.61(2)^{A}
(g/day/g)	0.19 ± 0.01 (9)^{C}	0.33 ± 0.04 (5)^{B}	0.2 ± 0.02 (5)^{C}	0.45 ± 0.06(2)^{A}
Fecal prod.				
(g/day)	0.98 ± 0.07 (9)^{D}	4.01 ± 0.11 (5)^{B}	1.3 ± 0.15 (5)^{C}	5.64 ± 0.04(2)^{A}
(g/day/g)	0.042 ± 0.002(9)^{C}	0.183 ± 0.01 (5)^{B}	0.056 ± 0.01 (5)^{C}	0.255 ± 0.02(2)^{A}
Digestibility				
ADMD (%)	78.1 ± 0.59 (9)^{A}	42.5 ± 3.37 (5)^{B}	72.4 ± 1.79 (5)^{A}	42.5 ± 3.96(2)^{B}
DE (%)	80.6 ± 0.63 (9)^{A}	40.7 ± 3.32 (5)^{B}	75.3 ± 2.03 (5)^{A}	41.3 ± 4.02(2)^{B}
DE Intake				
(kcal/g$^{.75}$)	1.45 ± 0.05 (9)^{A}	1.01 ± 0.04 (5)^{B}	1.4 ± 0.06 (5)^{A}	1.69 ± 0.37 (2)^{A}

Table 8: Mean square values from 2x2 ANOVA for parameters determined in the 16 day cecectomy experiment with meadow voles. Significant ($p < .05$) and non-significant (NS) p values are reported below the appropriate mean square. ADMD = apparent dry matter digestibility, DE = digestible energy.

Source	BODY WT. CHG.		CONSUMPTION		FECAL PROD.		DIGESTIBILITY		DE INTAKE
	(g)	(%)	g/day	g/day/g	g/day	g/day/g	ADMD	DE	$kcal/g^{.75}$
Cecectomy (CX)	0.14	4.67	4.58	0.01	1.97	0.004	35.21	24.49	0.13
pr > f	NS	NS	.01	.05	.0001	.01	NS	NS	NS
Diet (D)	41.36	644.45	50.98	0.13	52.99	0.11	5231.6	6622.1	0.17
pr > f	.01	.01	.001	.001	.001	.001	.001	.001	.05
CX x D	14.21	233.67	6.25	0.012	1.63	0.003	30.6	32.25	0.51
pr > f	NS	NS	.01	.05	.0001	.01	NS	NS	NS
Error	4.15	79.58	0.64	0.01	0.06	0.0003	220.17	20.68	0.04

Table 9: Coprophagy frequency means ± s.e. (N) for intact and cecectomized (CX) voles on high and low quality diet. Significant differences between diet types are reported ($p < .05$, Wilcoxon Rank Sum Test).

Species	HQ	LQ	p
Meadow voles			
intact	12.13 ± 0.89 (7)	18.93 ± 1.21 (7)	.002
cx	12.17 ± 0.73 (6)	16.95 ± 2.19 (6)	.042
Pine voles			
intact	15.2 ± 1.33 (6)	16.87 ± 1.63 (6)	NS
cx	14.01 ± 1.07 (6)	13.49 ± 1.14 (3)	NS

The vita has been removed from the scanned document

Nutritional Implications of Coprophagy and Cecal Function in Two Microtine Rodents (*Microtus pennsylvanicus* and *Microtus pinetorum*)

by

Eric O. Johnson

(ABSTRACT)

Coprophagic behavior and cecal function were examined for the meadow vole (*Microtus pennsylvanicus*) and the pine vole (*Microtus pinetorum*) as diet quality was manipulated. The nutritional response of the voles to a high and low quality diet was measured after the coprophagic or cecal component was eliminated from the digestive process. The nature of the nutritional response was determined by measuring food consumption, fecal production, diet digestibility, energy intake, body weight dynamics, and reingestion frequency.

The prevention of coprophagy lead to lower diet digestibility and body weight loss as compared to controls, but did not reduce energy intake by pine or meadow voles. The deletrious effects of coprophagy prevention were similar in magnitude for both quality diets. It was concluded that coprophagy provided select dietary nutrients but was not critical for maximum energy or protein intake under these dietary conditions. Cecum removal decreased diet digestibility but did not significantly influence body

weight dynamics since food consumption and passage rate increased to compensate for the digestibilty perturbations induced by cecectomy. However, the effects of cecum removal on diet digestibility were more severe on the low quality diet. This suggested that cecal function became more important as diet quality decreased. The removal of the cecum probably disrupted exsisting mechanisms for internal digesta separation and selective retention that serve to improve fibrous forage digestibility. Consequently, cecectomized voles on the low quality diet reingested a smaller proportion of feces than did intact voles on the same diet.