ANALYSIS OF THE UTILIZATION OF FOOD RESOURCES BY THE AFRICAN WOOD MOUSE *HYLOMYSCUS DENNIAE ENDOROBAE* (RODENTIA: MURIDAE) FROM IHURURU FOREST, KENYA

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other University or for any other award.

Signature…….

Date……

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SUPERVISORS APPROVAL

We, the University supervisors confirm that the work reported in this thesis was carried out by the candidate under our supervision and that we approve it for submission for examination.

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DEDICATION

This thesis is dedicated to my children for standing with me and persevering the difficult situation of me being a mother, a teacher and a scholar.

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ACRONYMS AND ABBREVIATIONS

a.s.l Above Sea Level

BL Body Length

BMR Basal metabolic rate

BW Body weight

CAE Caecum

CL Caecum Length

C V Coefficient of variation

DUO Duodenum

GIT Gastrointestinal tract

EAWS East Africa Wildlife Services

KWS Kenya Wildlife Services

LI Large intestine

Max Maximum

Min Minimum

OTU Operational Taxonomic Unit

RF Relative Frequency

SEM Standard Error of the Mean

SI Small Intestine Length

SL Stomach length

SV Stomach Volume

TGL Total Gut Length

UNEP United Nations Environmental Program

WFO World Food Organization

OPERATIONAL DEFINITION OF TERMS

Digestibility	A measure of the efficiency of digestion and absorption of various nutrients present in a food resource.
Phenotypic plasticity	A reversible change in animals to cope with a wide range of diets.
Phenotypic flexibility	A non reversible change in animals to cope with a wide range of diets.
Biodiversity	The total variety of life forms within an ecosystem or on earth.
Gut capacity	Stomach volume.
Nest trap	Sherman trap used for trapping study animals.

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ABSTRACT

Hylomyscus denniae endorobae is a rodent important in ecosystems as predator, prey, seed disperser, determinant of forest tree growth and structure as well as a contributor to biodiversity which subsequently plays a role in natural livelihood and national development. Fragmentation of tropical rain forest continues to pose a serious threat to species diversity, which leads to decreased natural income, primary production and general breakdown of an ecosystem. This in turn influences food resources and energy available to animals such as *H. d. endorobae.* With habitat destruction, *H. d. endorobae* will get to human habitat, destroy stored seed crops and transmit diseases. Evaluating gut morphology changes in response to different diets gives an insight into how animals can survive with changes in the natural habitat. The study was done to evaluate how *H. d. endorobae* adapts to different diets in the face of food scarcity in order to make informed decisions on conservation of the species. The purpose of this study was to analyze utilization of food resources by *H. d. endorobae*. Thirty male rodents (*H. d. endorobae*) weighing between 35-50g were trapped from Ihururu forest in Nyeri, Kenya, one of the natural habitats of the rodent species experiencing a lot of anthropogenic activities. Microscopic examinations of faeces collected from the trapped animals were done to establish the diet of the animal species in its natural habitat. The individuals were dissected and morphological measurements taken to establish gut size. Slides of different gut sections were microscopically examined to establish the number and length of villi. Nine other adult male rodents were randomly grouped into threes, caged individually and fed on 80g of different diets for six months to determine the influence of diet on gut morphology, digestibility and absorption efficiency. Results showed that *H. d. endorobae* consumes 70.7% seed, 22.6% plant leaf and 6.8% animal matter. There was no correlation between the mean body weight (40.1 ± 3.9g) and the mean total gut length (62.6 ±1.3cm) of field collected animals. The mean gut lengths were 62.6 ± 1.3cm, 58.5 ± 4.5cm, 55.7 ± 1cm, and 57.1 ± 2.3cm for field collected animals, wheat, kale and omnivore diets, respectively. Also, gut length did not show any significant differences ($p = 0.889$) between *H. d. endorobae* fed on different diets. Regression analysis showed no significant difference between diet and stomach volume ($P = 0.205$). There was significant correlation ($p < 0.05$) between diet and number of villi in the duodenum and caecum of field collected animals and those fed on wheat and large intestine and caecum of those fed on kale. Also, there was significant correlation ($p < 0.05$) between diet and length of villi in all regions of the gut except in the caecum ($P = 0.232$) of animals fed on omnivore diet. Diet influenced digestive efficiency ($p = 0.007$) with kale diet having the lowest efficiency (77.69%) compared to wheat diet (95.12%) or omnivore (94.29%). These results suggest that *H. d. endorobae* meets its energy demands with minimal gastrointestinal changes and probably increased food intake. It is recommended that the natural habitats of *H. d. endorobae* should be maintained and conserved to prevent its migration to human habitats and probable species erosion.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Rodents are small mammals with short ears, legs and long tails (Thomas, 1926; Carleton, 2006). They belong to order *Rodentia*, suborders *Hystricomorpha*, *Sciuromorpha*, *Castorimorpha*, *Cystorimorpha* and *Anamaluromorpha* and several families among them, family *Muridae* (Wilson and Reeder, 2005). Murids are small slender bodied rodents with a scaled tail, pointed snout and prominent whiskers. They are brown in colour, but some have black grey or white markings. They have excellent senses of smell and hearing, and are either herbivores or omnivores. Murids are classified into 5 sub-families, 140 genera and 640 species (Musser and Carleton, 2005).

Hylomyscus denniae endorobae is a montane woodland mouse (Musser and Carleton, 2005) broadly distributed in the highlands of southern and eastern Africa from Angolan Plateau through the mountains of northern Zambia and Tanzania to the Albertine Rift and Central East Africa systems in eastern Zaire, Burundi, Rwanda, Uganda and Kenya. In Kenya, there are 11 operational taxonomic units (OTUs) of *Hylomyscus* on the upper slopes of Mount Kenya. *Hylomyscus denniae endorobae* inhabits sub-tropical or tropical moist mountains (Musser and Carleton, 2005), a habitat characterized by tall indigenous trees with open canopy and dense low vegetation. Although *H. d. endorobae* is typically a granivore, it will consume other food types including insects and plant leaves, when seed availability is low due to habitat change (Rodgers and Gorman, 1995).

Hylomyscus has a small body and high mass specific metabolic rate (Rodgers and Gorman, 1995). It is susceptible to changes in nutrient and energy availability. Increased food intake can partially or fully compensate for low- quality high fiber food (low digestibility or low nutrient content) such that the energy and nutrient requirements of small mammals may still be met (Naya *et al.*, 2004). However, this compensation is not always sufficient to maintain the energy balance and since quality of available food changes with season, small mammals might use adaptations which increase the amount of energy and nutrient from their low quality diets, if their population numbers are to remain relatively stable (Hammond and Wunder, 1993). Small mammals like meadow voles have three options of maintaining the digestibility of low quality diet during times of high energy need. They may change the composition of their diet, increase food intake, or modify their gastrointestinal morphology or physiology to meet their increased energy needs. Small mammals may use a combination of these strategies to compensate for declining diet quality and nutrient availability. For example, under moderate energy demands (40-50% increase in BMR), meadow voles (*Microtus pennyslivanicus)* increased food intake and mucosal mass of the small intestine and caecum but, the length of GIT tissue was not affected (Derting and Bogue, 1993).

It is important to investigate how the *Hylomyscus denniae endorobae* adapts to different food resources when natural diets dwindle. This will help to show the need to conserve natural habitats in order to prevent migration of the rodent into human habitats where they can transmit diseases and destroy stored grains or prevent their death hence maintain their population.

The digestive system is one of the most expansive tissues of the body (Cant *et al*., 1996) and thus adjusting gut size to its functional demands could represent an important energy saving mechanism. Many rodent species occur in highly variable environments and consume poor quality diets (Karasov and McWilliams, 2005). This leads to increase in hind gut length, number of villi, shortening and blunting of cecal villi. Digestible energy is the part of energy absorbed in digestive tracts and it is a measure of expressing feed stuff nutrients available in order to determine energy requirements of animals (Schneider and Flatt, 1995; Willougby, 1999). There are two basic methods of measuring digestibility; direct and indirect methods. The direct method measures total food intake with collection of total faeces while indirect method uses a marker or indicator, with sufficient samples of feces (Wallougby, 1999). Food availability and quality limit the acquisition of energy, whereas gut morphology and physiology determine the absorption efficiency and the rate of nutrient and energy uptake (Weiner, 1992).

Phenotypic flexibility may occur in the gut due to change of diet quality. Anthropogenic disturbances affect most forest ecosystems through loss of original habitat, reduction in habitat batch size and increasing isolation of habitat patches (Andren, 1994), which in turn affects nutrient resource availability to rodents. Utilization of food resources and phenotypic flexibility is well studied in rodents such as *Otomys* (Vorontsov, 2003) and *Microtus pennsylvanicus* (Hammond and Wunder, 1993) but not *Hylomyscus* (Naya *et al.*, 2008). The current study focused on the effect of diet on gut morphology, digestive and absorption efficiency in *H. d. endorobae.*

1.2 Statement of the problem

Fragmentation of natural habitats by humans is posing a threat to wildlife such as rodents, subjecting them to temperature fluctuations, starvation, and exposure to predators (Tainter *et al.*, 2004). Rapid environmental changes such as habitat degradation and loss cause mass extinctions and sudden drops in biodiversity, resulting in the collapse of ecosystems (Andren, 1994; Suurkula, 2010; Sabiney *et al*., 2010; Shah, 2011; MEA, 2005; IUCN, 2011) leading to spread of zoonotic diseases, food, water and wood fuel shortages and can impose substantial costs at local and national level. Loss of plant species means loss of food resources for animal species, including rodents, which have to adapt to alternative food resources (Manley, 2008) for sufficient energy to promote healthy growth and the overall survival of wild animal population. If habitats are destroyed and the rodent does not adapt, then it will move into human habitat and transmit diseases such as Hantavirus, plague as well as destroy stored seed crops. The rodents may die, leading to erosion of species. Kenya's Ihururu Forest, a habitat rich in animal species (KWS, 2005) is under threat of deforestation, posing a danger of increased isolation and degeneration leading to scarcity of natural food resources for wild rodents such as *Hylomyscus denniae endorobae.* It is important to investigate how the animal adapts to different foods to help prevent migration or death.

1.3 Justification

Rodents are important in ecosystems as prey to reptiles, birds and civets; predator of invertebrates; as pollinators of plants, seed dispersers, regulators of insect populations and disease transmitting agents as well as determinants of forest tree growth and structure (Tainter *et al*., 2004). They also contribute to biodiversity, which plays a role in natural

livelihood and national development, by protecting water catchments, providing raw materials for textile and paper manufacturing industries, timber for furniture and construction, wood fuel and harbuoring wild animals. There is therefore need to conserve their habitat. If the habitat is conserved, the rodent will not migrate to human habitats so spread of zoonotic diseases and destruction of seed crops will be prevented.

Efficient utilization of food resources and ability to adapt to new food items as the original food sources dwindle minimizes the dependence of rodents on the unpredictable seed crops and leaves of forest trees which are at a risk of extinction due to anthropogenic activities. To adapt to new or scarce food resources, rodents either adjust energy requirements or change gut morphology. Measuring gut morphology changes gives an indication about how the animal adapts to different food resources which can be an indicator of its survival. Studies have shown that crypt depth, mucosal thickness, number and length of villi of the gut change in response to diet (Kristy *et al*., 2005). Although the ecology and population dynamics of *H. d. endorobae* have been studied (Carleton and Stanley, 2005; Naya, 2008), information on utilization of food resources and morphophysiological adaptations is deficient. This study sought to establish adaptations of the gut of *Hylomyscus denniae endorobae* in response to different food items. The study provides information useful in developing models for species distribution and as part of informed opinion to guide the Ministry of Wildlife and Natural Resources and the National Museums of Kenya in the development of good management and rodent conservation methods.

1.4 Research questions

i) What food resources are exploited by *Hylomyscus denniae endorobae* in its natural habitat?

ii) What is the relationship between gut length and body weight of *Hylomyscus. denniae endorobae*?

iii) What is the relationship between stomach volume and the type of diet of *Hylomyscus denniae endorobae*?

iv) What is the influence of the type of diet on number and length of gut villi of *Hylomyscus denniae endorobae*?

v) What is the influence of the type of diet on digestive and absorption efficiency of *Hylomyscus denniae endorobae*?

1.5 Null Hypotheses

i) Gut morphological characteristics in *Hylomysus denniae endorobae* are not altered by diet.

ii) Digestive efficiency in *Hylomyscus denniae endorobae* is independent of diet.

1.6 Objectives

1.6.1 General objective.

To analyze food resource utilization and gut morphology adaptations by *Hylomyscus denniae endorobae*.

1.6.2 Specific objectives

i) To determine the food resources exploited by *Hylomyscus denniae endorobae* in its natural habitat.

ii) To determine the relationship between gut length and body weight of *Hylomyscus denniae endorobae.*

iii) To determine the relationship between diet and stomach volume of *Hylomyscus denniae endorobae*.

iv) To analyze the influence of diet on shape, number and length of villi of *Hylomyscus denniae endorobae.*

v) To determine food digestibility in *Hylomyscus denniae endorobae* and nutrient absorption efficiency of different diets.

1.7 Significance of the study

The study demonstrated that *Hylomyscus denniae endorobae* is an omnivore, mainly granivore and that diet has no influence on total gut length and duodenal morphology. Diet influences stomach volume, number and length of villi. These changes increase energy demands of the species which leads to increase in food intake. The information on adaptability of the digestive tract and utilization of various food resources is useful to the Ministry of Wildlife and wildlife conservationists, for incorporation in appropriate scientific advice in developing wildlife management policies, biodiversity and small mammal conservation methods. These will protect Ihururu Forest, prevent fragmentation and hence improve its biodiversity. The information is useful for future researchers and

conservationists in understanding adaptations of animal species in response to habitat destruction.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of *Hylomyscus denniae endorobae*

Hylomyscus denniae endorobae, also known as African wood mouse, is a small mammal of the genus *Hylomyscus*. The genus has 12 species provisionally arranged in six species groups (*H. aeta, H. alleni, H. anselli, H. baeri, H. parvus and H. denniae*). *Hylomyscus denniae* is a complex group of three such species *(H. d. denniae, H. d. endorobae and H. d. anselli* (Musser and Carleton, 2005; Carleton, 2006).

2.2 Habitat of the African wood mouse

Rodents inhabit all habitats of the world except the Antarctica. All habitats of eastern Africa contain one or more rodent species but marshes, forests, or savannahs are the most favourable. Most rodents are nocturnal while some are crepuscular. Some nocturnal species may be seen foraging during the day in cases of high population densities (Oguge, 1996). All rodents rely heavily on scent in regulation of their social behavior. *Hylomyscus denniae endorobae* is a forest dwelling montane woodland mouse that inhabits highland forests of eastern and southern Africa. In Kenya, it inhabits the western, eastern and central highlands including the Ihururu Forest, Aberdares and Mount Kenya (Carleton and Stanley, 2005; Carleton, 2006). Although *H. d. endorobae* is of least conservation status (Schlitter *et al.,* 2008), destruction and fragmentation of its natural habitats may eventually lead to a decline in its population. The habitats of *H. d. endorobae* are disappearing due to

immense pressure from loggers and farmers (EAWS, 2010). Forest removal results to crippling drought and consequent famines (EAWS, 2010). The forests harbour a huge variety of flora and fauna, including small rodents and their destruction causes migration of the small animals to human habitats, death and hence erodes biodiversity (Kenya Forests Working Group, 2011).

2.3 Role of rodents in the ecosystem

Small mammals have a large impact on vegetation communities (Sommer *et al.,* 2008). Rodents such as *Acomys* disperse large nut-like seeds in rich years so nut bearing tree species such as Proteaceae, Cederbergensis and Concavum of South Africa have mast seeding (Midgley *et al.*, 2002). Scatterhoading of seeds by rodents allows regeneration of forest trees, to maintain the natural forest structure and quality (Jansen *et al*., 2006). Small mammals play crucial roles in natural ecosystems as predators, prey, seed dispersers, pests and grazers (Smith, 2005). Studies have shown that small mammal rodents pollinate about 85 species of plants such as Proteaceae, Malatomataceae and the African lily (*Massonia depress*) (Wester *et al.,* 2009). Zoophilous flowers are very sturdy with strong styles and stamens and a stigma located about 10mm from the nectar. They are situated at ground level and produce highly viscous nectar (Wester *et al.,* 2009), which facilitates lapping by rodents.

A healthy diverse community of small mammals serves as an indicator of the ecological condition of a forest habitat compared to other wildlife species since small mammals are very sensitive to habitat alterations (Sommer *et al.,* 2008). Rodents keep the population of

pest species of insects low and can serve as food for large game species and humans (Green *et al*., 2005; Hole *et al*., 2005)

2.4 Biodiversity

Biodiversity is the degree of variation of life forms within a given ecosystem, biome or entire planet (Tuomisto *et al*., 2010). The biodiversity of an ecosystem depends on climate, geography and the presence of other species (UNEP, 2009). Species abundance distribution is the basis of all ideas about species diversity and the raw material for all diversity measures. Species diversity contains two conceptual distinct components. The first component is species richness, which is defined as total number of species in the community (Larsson, 2011; *Wikipedia*, 2014). The second has been described by the terms equitable and evenness which refers to the degree to which relative abundances of individuals among the different species are similar (Larsson, 2011; *Wikipedia*, 2014). Tropical forests are the main biodiversity hotspots (Francis, 2008).

The rate of deforestation in the tropics is unprecedented (William, 1999). In the next few decades, tropical forest species will increasingly become confined to habitats comprising forest remains and natural ranges. Forest cover in Kenya has since increased from 1.7% - 5.9% but has not reached the United Nations recommended 10% cover (Kenya Forest Working Group, 2012). The growing interest in the effect of habitat fragmentation on evolutionary and ecological process is generated in part by concerns about the fate of threatened and endangered species whose habitats are becoming increasingly fragmented (Gaines *et al.*, 1992).

Loss of biodiversity leads to the collapse of ecosystems, causing large-scale agricultural problems that threaten food supplies to hundreds of millions of people (Suurkula, 2010; Shah, 2011). It also increases the spread of wildlife pathogens to humans, shortage of wood fuel, reduced water quality and supply and can impose substantial costs at local and national level.

Extensive forest destruction in Kenya has contributed to climate change evidenced by unpredictable rains and prolonged droughts, resulting in loss of biodiversity and low crop production (http:www.ncdc.noaa.gov/, 2013). Food shortage has left many dead and millions hungry (WFO, 2011). To ensure the preservation of many species, conservation biologists need a deeper understanding of the effect of diet on gut morphology and availability of energy to rodents. It is therefore crucial to determine gut morphological changes in *Hylomyscus denniae endorobae* to develop strategies to generate desirable outcome such as sustainable use.

2.5 Food habits of rodents

Some rodents have acquired specialized food habits, physical characteristics or physiological processes suitable for occupying a particular eco-niche (Oguge, 1996; Kingdon, 1997; Wilson and Reeder, 2005). Majority of the rodents are terrestrial, some are arboreal, while others are fossorial. Many of the rodents are herbivorous, feeding on grass (gramivores), roots, leaves (folivores), seeds (granivores) and fruits (frugivores). Some are omnivores, feeding on a variety of foods including animal items while a few are insectivores. Omnivores are intermediate, feeding on grains, leaves and insects (Adler, 1994; Oguge, 1996; Kingdon, 1997). *Hylomyscus denniae endorobae* is a herbivore

granivore (Carleton and Stanley, 2005), but not completely granivorous. Studies have shown that it feeds on wild seed when food is plenty and supplements its diet with plant leaves and animal matter when the climate is unfavorable (Carleton and Stanley, 2005). Its natural habitat should be maintained to ensure food availability for reproductive efficiency.

2.6 Food digestibility

Digestibility is one of the factors that determine the nutritive value of a feed. Digestibility data offers the insight into proper feeding of animals. Food intake is relatively more important than digestibility in determining the overall nutritive value because highly digestive feeds are of little value unless consumed by animals in question. However, digestibility usually provides a fairly reliable index of nutritive value because more digestive feeds are normally consumed to a greater extent than less digestible feeds. Only hydrolyzed portions of the feed get into the circulation of the animal and also measures of digestibility are somewhat easier to obtain than measures of intake. Digestibility is affected by feed intake, particle size, chemical composition, feed processing, climate, age and exercise (Schneider and Flatt, 1995).

Animals digest better when feed is limited than when they receive full feed (Okin and Mathison, 1991). More feed increases rate of feed movement through the tract, thus allowing less time for digestion and absorption. Different content of chemicals of similar feed affect digestibility, whereby some diminish the opportunity for the digestive enzymes to come into contact with their respective substrates. Addition of relatively small quantities of specific nutrients, for example protein or carbohydrates, enhances complete feed digestibility (Luginbuhl *et al*., 1994). Digestibility is higher under high environmental

temperatures than cold temperatures, due to high mean retention time of feed stuffs in the tract (Cranford *et al.,* 2000). Cold climate imposes higher energy demands leading to increased food intake (Cranford *et al*., 2000). According to Munger and Toad, 2007; Ruff, 2007, digestibility of a given nutrient can be calculated as follows:

$$\text{Nutrient digestibility} = \frac{\text{Nutrient intake - Nutrient in feces}}{\text{Nutrient intake}} \times 100$$

Digestible diet stimulates growth of the small intestine which further enables faster digestion and absorption of the food. Diet rich in indigestible material leads to increase in stomach and caecum size, but does not affect the dimension of the small intestine (Naya *et al*., 2008). However, non-fermentable material in diet increases mass of the small intestine than other digestive chambers (Liu and Wang, 2007). Wheat bran has non-soluble polysaccharides and only cause increase in colon and caecum dimensions (Hansen *et al.,* 1992).

Diet with high lactose content dilates the caecum (Liu and Wang, 2007). Fermentation generates short chain fatty acids which increase the daily production of epithelial cells three to four fold in the intestine of rats (Liu and Wang, 2007). High protein diet causes a decrease in gut size and a decrease in small intestine mass (Wang *et al.*, 2006). Low diet quality decreases digestive efficiency leading to low amount of energy available. This interferes with the reproductive efficiency of the species minimizing it survival. It is therefore crucial to determine digestibility of foods utilized by *Hylomyscus denniae endorobae* to devise strategies of conserving it.

2.7 Gut morphology adaptation

Differences in food habits among mammals are often reflected in their alimentary canal (Ellis *et al*., 1994). Populations of the same species show differences in gut morphology and are mainly related with differences in diet composition (Sassi *et al*., 2007). Variations in intestine length within a species are expressed as an adaptation to changes in food quantity and quality in relation to energy demands of the animals (Myrcha, 1995). No change in gut morphology due to diet quality was recorded in *Avircola terrestris* (Lee and Hauston, 1993) and *Phylotis darwini* (Sabat and Bozinovic, 2000). Changes in the morphology of the gastrointestinal tract (GIT) may be subtle and could well involve changes in the physical length, mass and function (Cranford *et al*., 2000). Phenotypic flexibility is a way by which animals cope with a much wider range of conditions during various life cycle events than fixed morphology would allow (Piersma and Lindstrom, 1997). Flexibility is more important than the maintenance of high, constant capacities and a large digestive tract size, since the later would involve high maintenance costs and comparatively lower energy savings (Bozinovic *et al*, 2010).

Flexibility in rodents occurs in species with different food habits (from strict herbivores to omnivores that mainly prey on vertebrates) and life history traits. There is accumulating evidence that the functional size of organs and aspects of metabolic physiology of an individual may show flexibility over time scales of weeks and even days, depending on the physiological status, environmental conditions and behavioural goals (Cranford *et al*., 2000). Mice take 12 weeks (Paglialunga *et al*., 2007), *Apodemus* and *Microtus*, 21 days (Sagher *et al.,* 1990) and dogs, one year (Sheffy *et al.,* 1989). Studies have indicated that changes from feeding on seeds and small invertebrates to cellulose diets of vegetative parts

of plants result in several evolutionary modifications in digestive tracts of muroid rodents (Vorontsov, 2003), and that intra-specific variations occur in the morphometry of the GIT in *Praomys* sub-populations (Gitonga, 2007). Both increased energy demand and lower diet quality determine changes in gut length and mass mainly at the level of the small intestine and caecum (Hammond and Wunder, 1993). Changes involve disappearance of some villi, and reduction in size and number of crypts (Karasov and Mac William, 2005).

Studies have also shown that seasonal changes in gut size are commonly related with seasonal changes in diet and quality or environmental temperatures (Delvelle and Busch, 2003; Derting and Horning, 2003). Large hindguts are associated with increased capacity of organs that contribute the most to nutrient and energy extraction from a particular diet (Ellis *et al*., 1994). Recent studies show that small intestine length flexibility differs between experimental factors in laboratory but not wild rodents and that wild rodents show no differences in flexibility of length of small intestine between species feeding on different food diets (Naya *et al.*, 2008). Plasticity adjusts gut structure and function to seasonal differences in nutrition and maintains optimized gut function under differing feeding regimes (Starck, 2003). Information on the morpho-physiology of an organism provides a framework to examine the influence of biotic and abiotic factors on organism distribution and predict the impact of environmental changes (Cooke, 2013). Without the morpho-physiological knowledge niche descriptions become limited. Information on gut morphology of *Hylomyscus denniae endorobae* will be integrated into ecosystem management and development of tools to solve conservation problems.

2.8 Effect of diet on gut morphology

High quality diet increases the length of the intestine (Olkowski *et al.,* 2005). The length and weight of jejunum of malnourished rats increase with high quality diet since concentrated diet stimulates growth of the small intestine (Nieto *et al*., 2002). This might be a compensatory mechanism to increase the absorptive capacity and assimilate any nutritional benefit from a hypo-protein diet (Nieto *et al*., 2002). A decrease in gut length with herbivore diet can be due to other factors; Low quality diet giving low energy for tissue growth or due to individual differences within the species population. Earlier research findings indicated that rats of the same species show differences in gut size and are mainly related to differences in diet composition (Sassi *et al*., 2007).

Increase in length of intestinal villi is due to intestinal epithelial cells which are derived from cells situated in the crypt base (Gordon *et al.,* 1993). The cells proliferate by mitosis in the crypts, differentiate as they migrate upwards and reach the villus tip where they are shed into the intestinal lumen within 48 hours after formation. Crypt depth increases as an animal grows then decreases with age. Deeper crypts are associated with increase in the proliferation and rate of upward migration of epithelial cells (Gordon *et al*., 1993). No changes occur in mucosal thickness of mature and aged mice. Wheat protein reduces the height of villi in rats probably due to a first encounter phenomenon and might have immunological as well as morphological effects. Crude protein is the major nutrient among the diet macronutrients for the development of the morphological features of the villi and epithelial cells. Kale has very low protein with high fiber proportion. High fiber increases the number of goblet cells leading to the thickening, shortening and atrophy of villi. High fat diet, saturated fat diet or cholesterol enriched diets increase villi length in wild mice

(Nieto *et al*., 2002). In mammals, there are about 10-40 villi per mm^2 of intestinal tissue. Villi are prevalent at the beginning of the small intestine and diminish in number towards the end of the digestive tract (Dahlke *et al*., 2003).

2.9 Effect of gut morphology on digestion and absorption efficiency

Digestive efficiency is a measure of food quality and nutrient availability (Bozinovic *et al*., 2004; Karasov and Mc Williams, 2005). The rate and efficiency of energy acquisition have been proposed as major constraints on energy budgets (Karasov and Mac Williams, 2005). Research has shown that the small herbivorous rodent, *Microtus branditi* has a long large intestine and caecum than omnivore rodents and that gut size changes with seasons, food quality, temperature, reproductive status and other internal or external factors (Song and Wang, 2006). Plasticity adjusts gut structure and function to seasonal differences in nutrition and to maintain optimized gut function under differing feeding regimes (Starck, 2003). *Ethomys chrysophilus*, a herbivore-granivore, has relatively longer intestines in wetter seasons (Korn, 1991). Gut morphology and physiology determine both digestive absorption efficiencies and energy intake rates (Weiner, 1992). The effect of gut morphology on absorption of different diets, however, is seldom tested. Although research on the morphology and morphometric modifications of gastrointestinal tract has been reported on rodents such as the South American omnivorous rodent *Akodon azarae* (Delvalle *et al.*, 2006) and the leaf-eared mouse, *Phyliotis darwini* (Naya *et al*., 2005), little has been documented on the physiology of *Hylomyscus denniae endorobae.*

2.10 Mineral nutrient ions

Mineral ions are essential for various functions such as teeth formation, bone mineralization, nerve impulse transmission and osmotic pressure balance in vertebrates. Absorbability of dietary minerals depends on the food, while absorption depends on the absorptive capacity of the intestines, which is affected by mineral reserves such as calcium reserves, hormonal regulation or previous dietary mineral supply (Asvarujanon *et al*., 2005). Urinary and fecal loss of endogenous minerals lowers mineral biodiversity. Certain foods such as those rich in phosphorus increase the likelihood of absorbed calcium being incorporated into bone whereas anions such as sulphate and chloride, organic ligands and excess protein or sodium increase the loss of calcium in urine and thus hinder its incorporation into bone. However, excess phosphorus may cause undesirable ectopic calcification (Asvarujanon *et al*., 2005).

Absorption of mineral salts occurs actively in the upper small intestine, passively in the lower small intestine and very little in the large intestine in mammals (Asvarujanon *et al*., 2005). Vitamin D facilitates mineral ion absorption whereas some dietary proteins such as phytates in wheat bran and most seeds, oxalates in spinach and tannin in tea can form insoluble complexes with calcium, thereby reducing absorbability. More calcium, zinc, magnesium and iron ions are absorbed in the absence of phytates (Bowen, 2008**).** The study sought to investigate the influence of different diets on mineral ion absorption efficiency by *Hylomyscs denniae endorobae.*

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection site of experimental animals

The experimental animals were the African wood mouse *Hylomyscus denniae endorobae* which inhabits highland forests of eastern and southern Africa such as the central highlands of Kenya. They were collected from Ihururu (Nyeri) Forest at grid -0.40989S and 36.9051E (Appendix I). Ihururu is a small village in the central highlands of Nyeri County, Kenya. The region has a mean annual temperature of 18^0C, wind 5.8km⁄h, rainfall of between 1700mm and 2600mm mostly falling between March and August (Nyeri-Meteorological department, 2011). The landscape is hilly with some slopes rising up to the peak at 3000m above sea level such as the Aberdares. The region has two vegetation types; forest vegetation dominated by trees on the hill and secondary grass mosaic on the lowland. Due to anthropogenic activities in Ihururu forest, little natural vegetation survives locally apart from that on the hill which typically comprises tall Euphobiceae, "muna", "muirungi" and thick undergrowth. Down the slope, vegetation is less natural, comprising exotic trees such as cypress, pine, wattle, acacia and eucalyptus as well as kikuyu grass. Fauna of the region include civets, hawks, owls, colobus monkeys, sykes monkeys, sanj sun squirrels, blotched genet, porcupines, insects, other rodents such as *Lemniscomys, Lophuromys, Dendromus* and *Praomys.*

3.2 Trapping of experimental animals

The rodents were trapped using mammal box trap also called Sherman live trap (Sherman, 2009) measuring 25 x 14x 8cm, during the month of June, 2011. Labeled traps were baited with about 5g of peanut butter on oat owing to its availability and affordable cost. Traps were set in the evening at 6.00 pm and opened at 6.00 am of each collection day. The animals were identified as far as possible (Carleton, 2006), weighed to the nearest 0.1g, using a pesola spring balance (PESOLA, Switzerland) and a ziploc bag, sexed by observing the genitals and morphometric measurement taken using a graduated ruler. In total, thirty nine animals were trapped, thirty of which were used for field experiments and the remaining nine were carried to the laboratory where they were subjected to various treatments as described below.

3.3 Experimental design

3.3.1 Trapping of experimental animals

A grid measuring 50 × 50 metres was laid in Ihururu Forest through stratified sampling. Two 50m long parallel transects were laid at right angles with a two-trap trapping station every 10m using the Pocelline method (Wilson *et al*., 1998) to ensure trapped animals were from the same microhabitat. Only adult male animals were used; young males and females were not used in this study because gastrointestinal tract of sub-adults and lactating females may differ due to their increased energy needs for growth or lactation (Gebczynska and Gebczynski, 2001). Age was determined using body weight, with adults weighing about 30g or more and the location of testes where young immature mice had

inguinal testes while mature mice had scrotal testes.

3.3.2 Laboratory experiments

The experiments comprised three treatments with three replicates each. Nine male animals weighing between 35g and 50g were randomized into three groups of three individuals each and caged individually. The experimental animals were fed on rodent pellets for eleven days to adjust prior to presentation of test foods which composed of wheat alone, kale alone and a mixture of wheat, kale and insect as a control. The animals were kept in an animal house at mean temperatures of 22^0C. They were presented with 80g of test food that was replaced after every four days. The animals were fed for six months after which they were sacrificed for histological procedures.

3.3.3 Collection of fecal matter

Data was collected for determining the feed utilized by *Hylomyscus denniae endorobae* in its natural habitat. Thirty nest traps housing a rodent each were sampled. About 5g of fecal matter was collected from each nest trap *H. d. endorobae*. A total of 150g of fecal matter was collected, sun dried and pooled into three pools of 50g each. The dry fecal matter was then ground into fine powder using improvised ‘tabaka’ soft stones. Out of the 50g, 10g of fecal matter from each pool was used for fecal analysis. A total of 30g of fecal matter from thirty animals was analyzed.

3.3.4 Analysis of fecal matter

Fecal matter from field collected animals was analyzed for epidermis identification using the protocol proposed by Williams (1996), with modifications. Three scats each weighing 10g were selected from each of the 50g pool of feces. About 2.5 ml of 30% alcohol was added to 10g of fecal ground powder and left to settle for four hours. The supernatant was removed and 2.5 milliliters of boiling water added to the mixture after which the solution was left to settle for five hours. This method separates the epidermis from mesophyll of the leaves as fat is removed. Epidermis was bleached using 50% sodium hypochlorite. The tissue was fixed using Safranin solution (1:1 in ethyl alcohol 96%) then observed under the light microscope at x100 magnification. Each slide was examined under four different views and epidermal matter counted. In total, twelve views were observed for each fecal sample. The mean relative frequency (RF) of epidermis in each fecal pool sample was recorded and the mean relative frequency ratio calculated. Epidermis identification parameters used were: Stem epidermis: absence of hairs, stomatal guard cells, stomata and thorns; Leaf epidermis: presence of macro and micro hairs, prickle hairs, thorns, papillae, silica bodies, stomata and stomatal guard cells, and Root epidermis: presence of root hairs. Animal epidermis was identified using epithelial tissue cells that form a continuous sheet (*en. wikipedia org. / wiki*/ plant 2011).

3.3.5 Determination of gut morphology

Thirty individuals of *Hylomyscus denniae endorobae* were euthanized using chloroform (Barry, 1998), dissected according to Sowash (2009) and the entire digestive tract removed. The stomach was catheterized at the pyloric sphincter and just proximal to the

duodenum, voided and fixed in 10% neutral buffered formalin. The intact length of the gastrointestinal tract sections was determined to the nearest 0.1cm by gently straightening(without stretching) and laying flat each section of tissue along a metric ruler (Perkins, 2004; Shaw, 2012) to obtain the total gut length (TGL).

Gut sections were separated into stomach, duodenum, small intestine, caecum and large intestine (Perkins, 2004; Shaw, 2005). The sections were processed for histology by dehydrating them with graded ethanol then making slides. For each intestinal tissue sample, cross sections measuring 2mm were prepared and staining done with hematoxylin and eosin. The sections were then examined under the light microscope at ×100 magnification for shape, number and length of intestinal villi and results recorded. Further, for each intestinal cross section, eight intact, well oriented crypt villus units were selected for examination. The microscopic examinations were conducted in triplicates (24 measurements for each sample section). Morphometric measurements were performed to obtain villi length, crypt depth and mucosal thickness using a transparent graduated ruler under the light microscope (Hammond *et al*., 1996). The villus height was measured from the tip to the villus-crypt junction. The crypt depth was defined as the depth of the invagination between adjacent villi. Villi bases were counted to determine the number of villi per millimeter of each section. The procedure was repeated twice for each section and the mean for each parameter determined.

3.3.6 Determination of stomach volume (SV)

After measuring and noting the length of the gut in the thirty field collected individuals, each was sectioned, stomach contents removed, saline flushed then filled with water using

a syringe and the water volume measured to the nearest 0.1millilitres (Burnnet, 2004). The volume of water was taken to be equivalent to the stomach volume. The procedure was repeated for each individual and the mean measurements recorded.

3.3.7 Determination of digestive efficiency

The experiment consisted of three treatments with three replicates for each. Nine animals were placed into three groups (A, B and C) of three animals each, and fed on three different diets. Group A was fed on wheat and water, Group B fed on leafy kale and water while Group C was fed on a mixture of wheat, leafy kale, locust and water. The digestibility of nutrients was determined by analyzing fecal matter using Murray and Ingols (1994) analysis method. After the pretreatment feeding trials, each animal was weighed and placed in its cage at ambient temperature (22^0C) with a tray and polythene bag placed below for collection of fecal matter and urine. The animals were deprived of food for 24 hours then presented with eighty grams of test food and water *ad libitum,* each according to diet grouping.

After four days the food remains were collected, weighed, then oven dried until constant mass and weighed to the nearest 0.1mg to determine the amounts ingested. The experimental animals were reweighed to the nearest 0.1g. Feces were collected from each group, and weighed to the nearest 0.1g. Before reweighing, all fecal samples were oven dried until constant mass was obtained. The procedure was repeated six times during which a total of eighteen samples per group were collected and the means obtained. Initial digestive efficiency was measured as apparent dry matter digestibility which was calculated as;

$$\frac{\text{Dry food consumed (MFO) - Dry faeces produced (MFE)}}{\text{Dry food consumed (MFO)}} \times 100$$ (Munger and Toad, 2007; Ruff, 2007; Cranford *et al*., 2000).

3.3.8 Determination of absorption efficiency

During the four days of experimental feeding, urine samples were collected using transparent polythene paper placed on the trays under the cages and stored in labeled vials. Each urine sample was then analyzed using a flame photometer to determine the amount of sodium, potassium and calcium ions. Three different urine samples were collected from each animal of the three groups fed on different diets and analyzed separately. The procedure was repeated twice and the mean amount of the different ions for each group obtained and recorded. Because digestive efficiency calculations assume that an animal maintains a constant body mass over a discrete interval, any animals with mass losses in excess of 15% from their initial body weight during the pretreatment trials were not used in the actual feeding experiment.

3.3.9 Morphological adaptability of the digestive tract

Experimental animals were fed for six months according to diet treatments, after which they were euthanized using chloroform (Barry, 1996), dissected as outlined in section 3.3.5 above and histological examination of the gut done. Morphometric measurements were done as outlined in section 3.3.5 for each individual in the group and means obtained for the group. The experiment was replicated twice for each section.

3.4 Data analysis

Data collected was processed using the Statistical Package for Social Sciences (SPSS). Mean values of gut morphological parts were calculated to show trends in the variables such as gut length and gut capacity. Analysis of variance (ANOVA) was used to compare the mean gut lengths of individuals relative to head- body length (BL) and body weight (BW). It was also used to compare the effect of digestive and absorption efficiency between diets and effect of diet on gut length, and number of villi. Using correlation coefficient test, data was analyzed to determine the relationship between body size and stomach volume. It was also used to determine the relationship between diet and gut morphology. Digestibility data obtained was analyzed to obtain average weight of food consumed and feces released in each group. Correlation and regression analyses were done to determine the influence of diet on gut length, stomach volume and effect of diet on digestibility and absorption.

CHAPTER FOUR

RESULTS

4.1 Diet analysis of field collected *Hylomyscus denniae endorobae*

Diet analysis of scat from field collected feces showed that in its natural habitat, *H. d. endorobae* consumes seeds (70.67), leaves (22.56%) and animal matter (6.77%) (Table1).

Table 1. Proportion (%) of epidermal parts (units) in twelve quadrats of fecal scats of *Hylomyscus denniae endorobae*.

Scat	Number of views	Seed coat epidermis	Leaf epidermis	Animal epidermis
X	12	33	12	4
Y	12	27	8	3
Z	12	34	10	2
Total	36	94	30	9
Mean (x)		2.61	0.83	0.25
R F		70.67%	22.56%	6.77%

30g of fecal material was pooled into three; X, Y and Z. Each pool was analyzed three times giving a total of 12 views. (n = 30).

The mean number of seed coat epidermis was higher (2.61 ± 0.192) compared to that of leaf epidermis (0.833 ± 0.129) and animal matter epidermis (0.25 ± 0.732). The results showed a statistically significant difference (F = 5.323, df = 29, p = 0.0297) between different epidermal matter in the diet of *Hylomyscus denniae endorobae* in its natural habitat.

4.2 Relationship between body weight and total gut length

The mean body weight of field collected animals was 40.1 ± 3.8 while the mean total gut length was 62.6 ± 1.3cm (n=30) (Figure 1). There was no statistically significant correlation between body weight (BW) and total gut length (TGL) (r = -0.214, df = 29, p = 0.255). Before commencement of feeding the mean weights for experimental animals were; 43.0 ± 2.1g, 37.7 ± 2.6g and 40.3 ± 2.5g for groups A, B and C respectively. After feeding the experimental animals on different diets for six months the mean body weights were; 56.6 ± 4.7g (n =3) for the animals fed on wheat (group A), 44.1 ± 3.3 g (n =3) for those fed on kale (group B) and 53.4 ± 3.6g (n =3) for those fed on a mixture of wheat, kale and insect diet (group C). Weight increase index for animals fed on wheat was 13.7g, on kale 6.4g and for those fed on a mixture of wheat, kale and insect 13.1g.

The average total gut length was 58.5 ± 4.5cm (n=3) for animals fed on wheat, 55.7 ± 1.0cm (n=3) for those fed on kale and 57.2 ± 2.1cm for those fed on omnivorous diet (Table 2). The results showed no statistically significant correlation between body weight and total gut length for animals fed on wheat diet (r = 0.382, p = 0.220, df = 2), for those fed on kale, (r = 0.729, p = 0.480, df = 2) and for those fed on omnivorous diet (r = 0.639,

p = 0.559, df = 2). One way ANOVA showed no statistically significant effect of diet on body weight (F = 3.838 df = 2, p = 0.083) and total gut length (F = 5.231 df =2, p = 0.084).

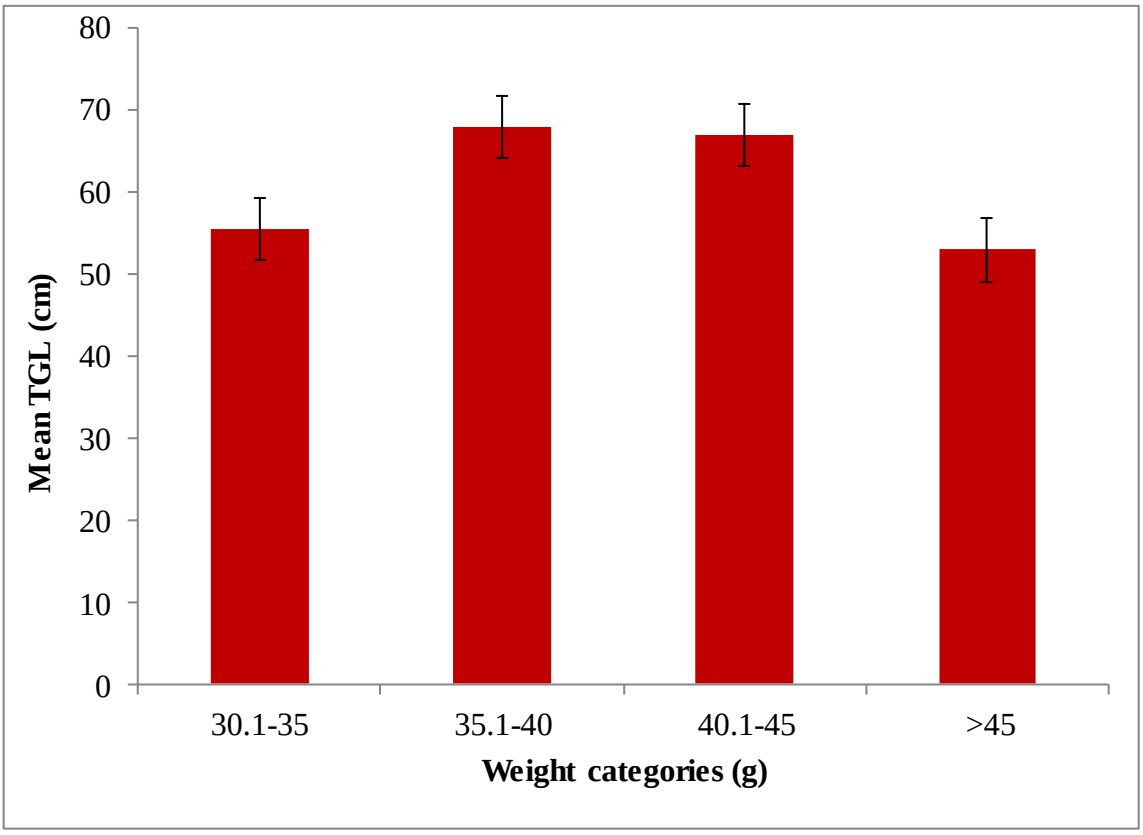

Figure 1: Mean body weight (g) and mean total gut length (cm) of field collected *H. denniae endorobae*

Table 2. Mean body weight (g) and total gut length (cm) of laboratory maintained *Hylomyscus denniae endorobae*.

PARAMETER	Laboratory maintained animals after six months (n=3)		
	Wheat	Kale	Omnivorous diet
Initial BW (g)	42.95 ± 2.14	37.68 ± 2.62	40.31 ± 2.47
Final BW (g)	56.64 ± 2.71	44.11± 3.28	53.36 ± 2.64
Index of increase (%)	31.9	16.9	32.4
Initial TGL (cm) (field collected)	62.62±1.33	62.62±1.33	62.62±1.33
Final TGL (cm)	58.87± 4.49	56.67± 1.02	57.20 ± 2.13
Index of change (%)	-5.99	-9.5	-8.66

4.3 Relationship between body length (BL) and total gut length (TGL) of field collected and laboratory maintained *Hylomyscus denniae endorobae*

The mean total body length (BL) of field collected animals was 104.6 ± 8.4cm (n=30) with a range of 101.6 - 107.6cm while their total mean gut length was 62.6 ± 1.3g (n = 30) with a range of 56.1 - 69.1cm. There was a wide range of variation in the total gut length among individuals used in the study (Tables 3 and 4). Pearson correlation showed no statistically significant correlation between body length and total gut length of field collected animals

(r = 0.260, p =0.166, df = 29). Also, the findings showed no statistically significant correlation between BL and TGL of laboratory maintained animals fed on wheat diet (r = 0.807, df = 2, p = 0.403, kale (r = 0.772, p = 0.439) and omnivorous diet (r = 0.997, p = 0.480).

Table 3. Mean body length and mean total gut length (cm) of field collected *Hylomyscus denniae endorobae* (n = 30).

PARAMETER	Mean BL (g)	Mean TGL (g)
Field collected animals	104.7± 8.4	62.6 ± 1.3
S.E.M	0.84	1.31
Coefficient of variation	61.5	53.1

Table 4. Mean body length (BL) and mean total gut length (TGL) in cm of laboratory maintained animals.

Laboratory maintained animals (n = 3)						
	Wheat		Kale		Wheat + kale + insect	
	BL	TGL	BL	TGL	BL	TGL
1	115	67.1	111	57.6	110	50.0
2	115	58.3	102	54.4	112	54.4
3	111	52.3	107	54.0	116	67.2
Range	111 - 115	52.3 - 67.1	102 - 111	54 - 57.6	110 - 116	50 - 67.2

Key: BL - body length, TGL - Total gut length.

4.4 Relationship between diet and stomach volume

Field collected *Hylomyscus denniae endorobae* had a mean stomach volume of 3.9 ± 0.2 cm^3, with a range of 1.0cm^3 to 6.1cm^3. The mean stomach volume for laboratory maintained animals fed on wheat was 3.87 ± 0.1cm^3 with a range of 2.5cm^3 to 4.7cm^3, while those fed on kale had a mean of 3.9 ± 0.1cm^3 with a range of 3.9 to 4.2cm^3. Laboratory maintained animals fed on omnivorous diet had the smallest mean stomach volume of 2.0 ± 0.02cm^3 (Table 5).

Table 5. The mean stomach volume (cm^3) of field collected and laboratory maintained animals fed on different diets

	Mean stomach volume (cm^3)			
	Field collected animals n = 30	Laboratory maintained animals n = 3		
		Wheat diet	Kale diet	Omnivorous diet
	3.85± 0.19	3.89 ± 0.05	3.9 ± 0.06	2.0 ± 0.02
Mean BW (g)	40.1 ± 3.8	56.6 ± 2.7	44.1 ± 3.3	53.4 ± 2.6
Ratio of SV/ WB	0.1	0.07	0.09	0.04

Regression showed no significant difference between diet and stomach volume (t =-1.852, df= 3, p = 0.205 at the 0.05.

4.5 Relationship between body weight and stomach volume of *H. d. endorobae*

The mean total body weight of field collected animals was 40.1 ± 0.4 with a stomach volume of 3.9 ± 0.2 (Table 6). The results showed a highly significant correlation between body weight and stomach volume of field collected animals (r = 1.000, df = 29, p = 0.000 at the 0.01 level) (2- tailed). Regression analysis showed a significant slope between BW and SV (b = -2.0861, p = 0.00132). For laboratory maintained animals, there was no significant correlation between body weight and gut capacity for those fed on wheat and

kale diets (r = -0.698, p = 0.508 and r = -0.456, p = 0.363). However, there was significant negative correlation for laboratory animals fed on a mixture of wheat, kale and insect diet (r = - 0.997, p = 0.050, df = 2).

Table 6. The mean body weight (g ± S.E) and stomach volume (cm^3) of field collected and laboratory maintained animals.

Parameter	Field collected animals	Laboratory maintained animals after six months		
		Wheat	Kale	Omnivorous diet
Mean BW (g)	40.057± 3.83	56.64± 2.71	44.11±3.28	53.36 ± 2.64
Mean SV (cm3)	3.853±0.188	3.870±0.059	3.90±0.06	2.00±0.021
Pearson's correlation (r)	1.000	-0.698	0.456	-0.997
P value	0.000	0.508	0.363	0.050
	n = 30	n = 3	n = 3	n = 3

4.6 Relationship between stomach volume (SV) and mean TGL of *H. d. endorobae*

Correlation coefficient showed no statistical difference between stomach volume and TGL of field collected animals r = -0.873, df = 29, p = 0.263. Results showed no statistically significant difference between total gut length and stomach volume of laboratory maintained animals fed on wheat (r = -0.925, df = 2, p = 0.249), kale (r = 0.606, p = 0.585) and a mixture of wheat, kale and insect (r = 0.698, p = 0.508).

4.7 Effect of diet on total gut length

The mean gut lengths for *Hylomyscus denniae endorobae* fed on wheat, kale and omnivore diet were 58.5 ± 4.5, 55.7 ± 1.0 and 57.1 ± 2.5 respectively (Figure 2). The results showed no statistically significant effect of diet on the total gut length ($F= 0.120$, $df = 8$, $p = 0.889$) between groups. However, numerical results showed a lower total gut length in animals fed on kale compared to those fed on wheat and omnivore diets while those fed on wheat and omnivore diets had numerically similar gut lengths. Also, those fed on kale and omnivore diet had statistically similar gut lengths (Figure 2).

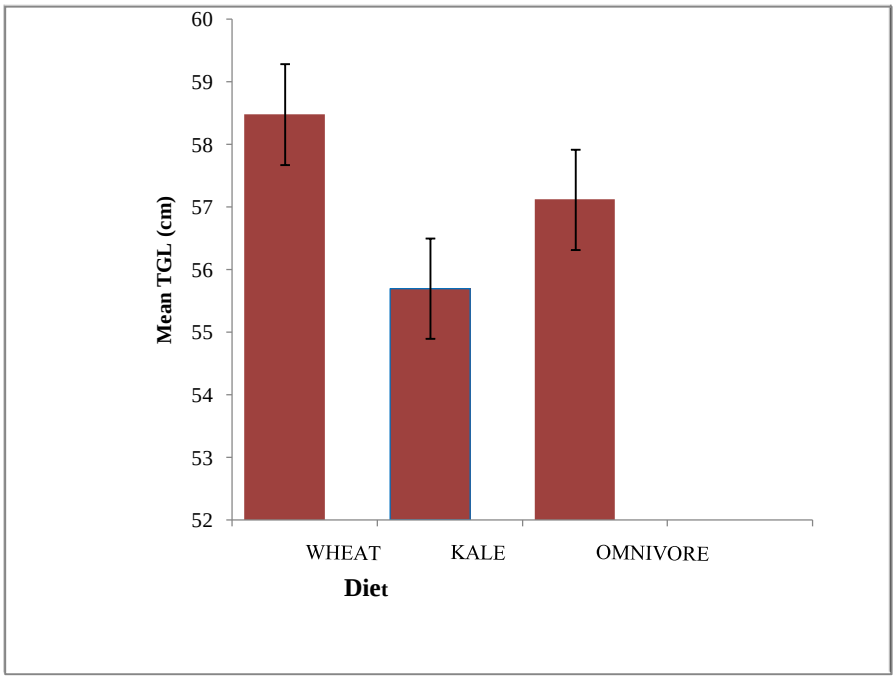

Figure 2: Mean total gut length (cm) of *Hylomyscus denniae endorobae* fed on wheat, kale and omnivore diets (n = 3 for each group)

4.8 Digestive and absorption efficiency

Laboratory maintained animals fed on wheat diet consumed a relatively high mean amount of food compared to amounts consumed by those fed on omnivorous diet and those fed on kale. Experimental animals fed on kale produced a relatively high amount of dry feces compared to those fed on either wheat or omnivorous diets (Table 7). *Hylomyscus denniae endorobae* fed on wheat diet had a digestive efficiency of 95.12%, those fed on kale diet had 77.69% while those fed on the omnivorous diet had 94.29% (Table 7 and Figure 3).

Table 7. Digestive efficiency (%) of *Hylomyscus denniae endorobae* fed on wheat, kale and a mixture of wheat, kale and insect.

Diet	Dry weight (g) of food consumed	Dry weight (g) of feces produced	Digestive efficiency (%)
Wheat	38.53 ± 3.02	1.88 ± 0.2	95.12
Kale	8.65 ± 1.02	1.93 ± 0.08	77.69
Wheat + Kale + locust	27.50 ± 0.47	1.57 ± 0.02	94.29

Results showed a statistically significant effect of diet on digestive efficiency between groups ($F= 236.083$ $p = 0.00011$). Also, there was a statistically significant difference in digestive efficiency between kale and a mixture of wheat, kale and insect ($F= 94.01$, $df = 4$, $p = 0.0096$), and wheat and kale ($F = 94.01$ $df = 4$, $p = 0.0105$). However, the percentage

Values showed no statistically significant difference in digestive efficiency between wheat and a mixture of wheat, kale and insect ($F = 44052.22$, $df = 4$, $p = 2.27$).

Pearson correlation coefficient showed a highly significant correlation between diet and digestive efficiency between groups ($r = 0.361$, $df = 53$, $p = 0.007$).

4.9 Nutrient absorption efficiency

Using polythene papers placed on trays under cages, about one milliliter of urine was collected according to group diets and kept in labeled vials. Standardization of the flame photometer for sodium, potassium and calcium was done then urine samples analyzed for the mineral ions. Analysis of urine for ions showed that animals fed on kale secreted more sodium ions (32.03µm), compared to those fed on wheat (23.4µm) and omnivorous diets (18.86µm). Urine samples from animals fed on omnivorous diet had more calcium ions excreted (28.86µm) compared to samples from those fed on wheat (14.2µm) and from those fed on kale (16.5µm). They also had more potassium ions excreted (6491µm) followed by kale (5276µm) then wheat (4348µm) relative to amounts of mineral ions present in test feed Tables 8 and 9).

Table 8. Mean mineral ions (µm± S.E) in 1ml of urine from laboratory animals fed on different diets with different amounts of mineral ions.

	Amount of mineral ions in urine (µm)		
Diet	Sodium	Calcium	Potassium
Wheat	23.4	14.2	4348
Kale	32.03	16.50	5276
Omnivore	18.86	28.86	6491

.

Table 9. Assumed amount of mineral ions in 80g of feed given to laboratory animals

Diet	Sodium	Calcium	Potassium
Wheat	3848	32000	249600
Kale	3168	6688	134400
Locust	none	none	traces

The proportions of mineral ions were calculated based on the nutrient database figures by Sirah (2011) and Bilgichi *et al*., (2007).

The results showed a highly significant effect of diet on absorption efficiency of sodium ions ($F = 37.85$, $p = 0.00354$), calcium ions ($F = 17.655$, $p = 0.0137$) and potassium ions (F 74.921, $p = 0.0009$).

4.10 Shape and mean number of villi in different gut regions of field collected and laboratory maintained *Hylomyscus denniae endorobae*

The mean number of villi in a 5mm section of field collected *Hylomyscus denniae endorobae* was 7.0 ± 0.14 in the duodenum, 11 ± 0.16 in the small intestine, 6 ± 0.11 in the large intestine and 4.0 ± 0.1 in the caecum (Table 9). The mean number of villi in a 5mm section of laboratory rodents fed on wheat was 12.0 ± 0.03 in the duodenum, 10.0 ± 0.05 in the small intestine, 8.0 ± 0.03 in the large intestine and 7.0 ± 0.05 in the caecum. Those fed on kale diet had a mean of 9.0 ± 0.04 in the duodenum, 11.0 ± 0.03 in the small intestine, 10.0 ± 0.05 in the large intestine and 7.0 ± 0.05 in the caecum while those fed on a mixture of wheat, kale and insect diet had a mean number of 8.0 ± 0.03 in the duodenum, 9.0 ± 0.04 in the small intestine, 6.0± 0.05 in the large intestine and 7.0 ± 0.11 in the caecum (Table 9). Villi in all gut segments of field collected animals had a normal slender shape with pointed tips and a thick mucosal layer (Plates 1 and 2) while villi of animals fed on wheat became rugged and more blunted, particularly those in the large intestine and caecum (Plates 3 and 4).

Table 10. The mean number of villi (± SE) in different regions of the gut of field collected and laboratory maintained *Hylomyscus denniae endorobae*.

Region of gut	Field collected animals (n=30)	Laboratory animals (n=3)		
		Wheat	Kale	Omnivorous diet
Duodenum	7.0 ±0.14	12.0±0.03	9.0±0.04	8.0 ±0.03
Ileum	11.0 ±0.16	10.0±0.05	11.0±0.03	9.0 ±0.04
Large intestine	6.0 ± 0.11	8.0 ±0.03	10.0±0.05	7.0± 0.05
Caecum	4.0 ±0.10	7.0±0.05	7.0 ±0.05	6.0 ±0.11

The results showed no significant effect ($P > 0.05$) of diet on the number of villi in different regions of the gut. There was no significant correlation between the number of villi in different gut regions of field collected animals and the number of villi in the small intestine ($p = 0.225$) and large intestine ($p = 0.166$) for animals fed on wheat, duodenum ($p = 0.154$) and small intestine ($p = 0.105$) for those fed on kale and duodenum ($p = 0.0944$), small intestine ($p = 0.1879$), large intestine ($p = 0.2869$) and caecum ($p = 0.0991$) for those fed on omnivore diet. However, there was significant correlation between the number of villi in the duodenum ($p = 0.0321$) and caecum ($p = 0.0406$) of field collected animals and laboratory maintained animals fed on wheat, the large intestine ($p = 0.0130$) and caecum ($p = 0.0171$) of those fed on kale diets.

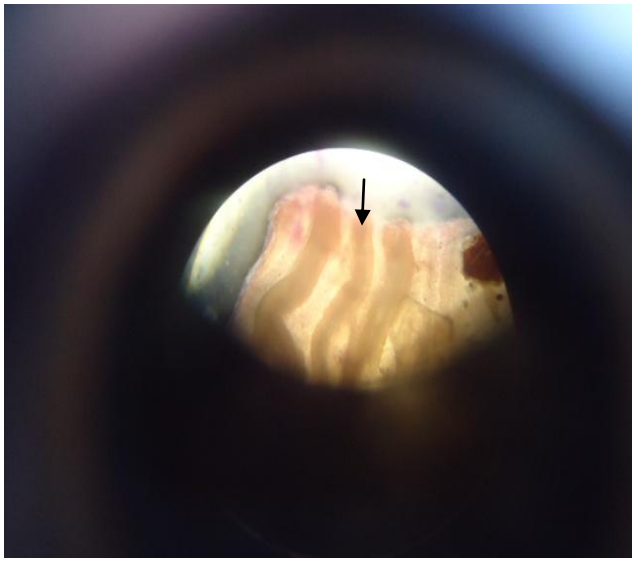

Plate 1. Large intestinal villi of field collected animals as seen under the light microscope (mag x100). Arrow points to the tip of villus

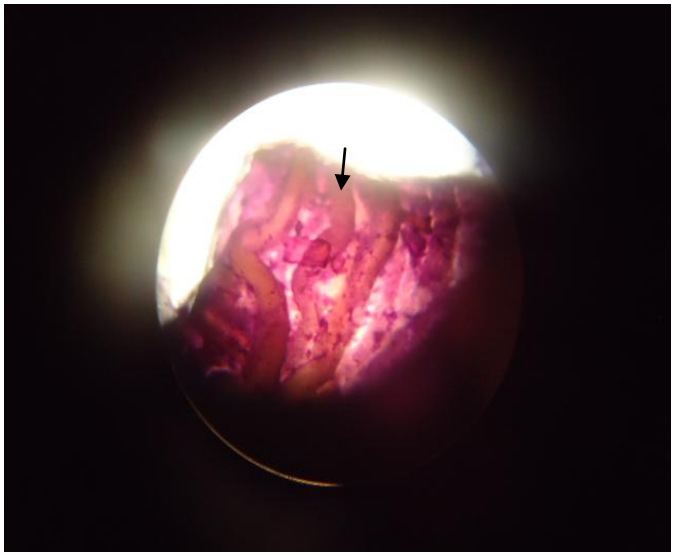

Plate 2. Cecal villi of field collected animals as seen under the light microscope (mag x100). Arrow points to the tip of villus

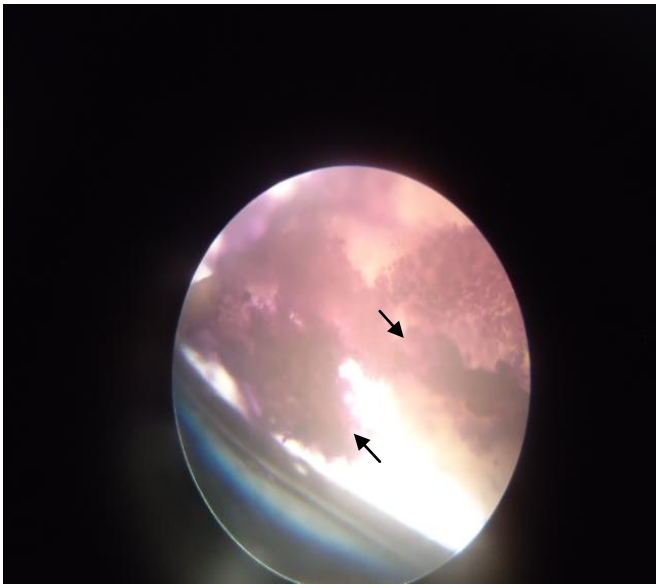

Plate 3. Large intestinal villi of animals fed on wheat as seen under the light microscope (mag x100). Arrows point to the tips of villi

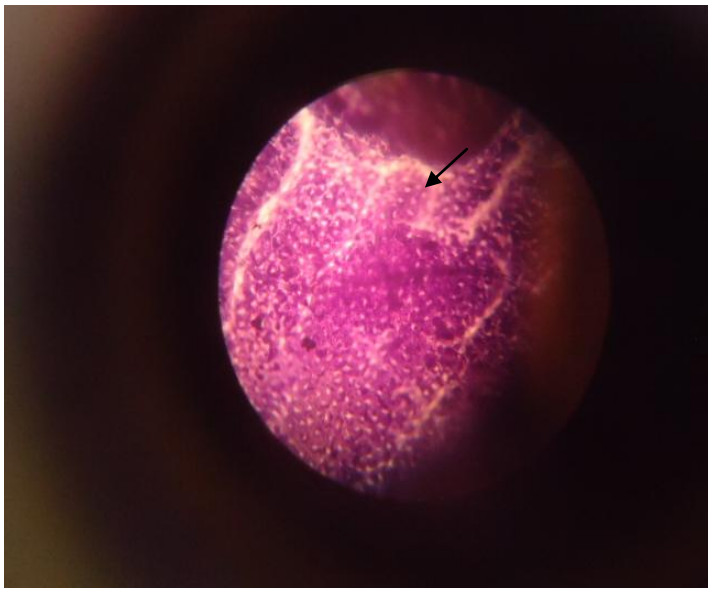

Plate 4: Cecal villi of animals fed on wheat as seen under the light microscope (mag x100). Arrow points to the tip of villus

4.10.1 Relationship between diet and length of villi in different regions of the gut of laboratory maintained *Hylomyscus denniae endorobae*

Field collected *Hylomyscus denniae endorobae* had mean villi lengths of 2.7 ± 0.04mm, 3.17 ± 0.23mm, 5.1 ± 0.02mm and 2.2 ± 0.1mm (mg x100) for duodenum, small intestine, large intestine and caecum, respectively (Table 10). Experimental rodents fed on wheat diet had mean villi length of 2.4 ± 0.01mm in the duodenum, 2.2 ± 0.1mm in the ileum, 1.3 ± 0.32mm in the large intestine and 2.0 ± 0.11mm in the caecum. Those fed on kale diet had mean villi length of 2.0 ± 0.02mm in the duodenum, 2.5 ± 0.14mm in the small intestine and 3.1 ± 0.21mm in the large intestine while those fed on a mixture of wheat, kale and insect diet had a mean villi length of 2.9 ± 0.23mm in the duodenum, 3.0 ± 0.13mm in the small intestine, 2.2 ± 0.15mm in the large intestine and 1.8 ± 0.11mm in the caecum (Figure 4).

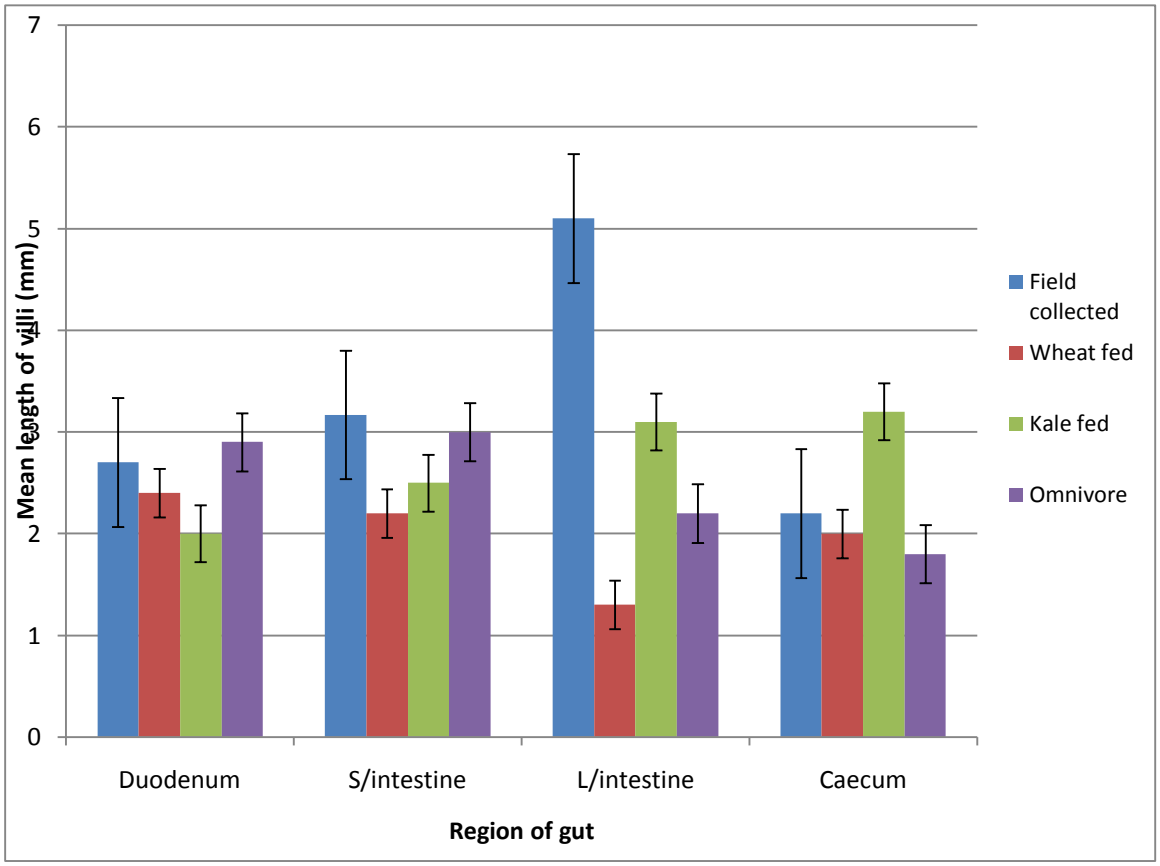

Figure 3: The mean length of villi (mm ± S.E) in different gut regions of field collected and laboratory maintained animals fed on different diets

The values of villi length in various regions of the gut of field collected mice and those of laboratory maintained animals fed on different diets were tested for associations. The results showed statistically significant correlation between diet and villi length in the small intestine ($r = -0.832$, $p = 0.00$), large intestine ($r = -0.994$, $p = 0.000$) but not in the caecum ($r = 0.165$, $p = 0.232$) of animals fed on wheat. Also, correlation coefficient showed a significant correlation between diet and villi length in the duodenum ($r = -0.492$, $p = 0.000$), small intestine ($r = -0.853$, $p = 0.000$), large intestine ($r = -0.973$, $p = 0.000$) and caecum ($r = 0.849$, $p = 0.000$) of animals fed on kale. The results also showed a significant

correlation between diet and villi length in the duodenum (r = -0.597, p = 0.000), small intestine (r = 0.289, p = 0.000), large intestine (r = 0.991, p = 0.034) and caecum (r = -0.319, p = 0.019) of animals fed on a mixture of wheat, kale and locust. Results showed significant effect of diet on the length of villi in the large intestine of animals fed on wheat (F = 3.5308, p = 0.0465), kale (F = 16.327, p = 0.00202) and a mixture of wheat, kale and insects (F = 4.082, p = 0.04918). There was significant effect of diet on length of villi in the caecum of animals fed on kale diet (F = 0.575, p = 0.0447). This was the only region where increase in villi length occurred.

4.11 Relationship between the number and length of villi in different regions of the gut of field collected and laboratory maintained *Hylomyscus denniae endorobae*

Correlation coefficient showed no statistically significant correlation between the number and length of villi in the duodenum (r = 0.002, df 29, p = 0.99), small intestine (r = 0.220, p = 0.244), large intestine (r = 0.253, p = 0.177) and caecum (r = 0.034, p = 0.860) respectively, for field collected animals. Also, there was no statistically significant correlation between the number and length of villi in the duodenum of animals fed on wheat (r = -0.37, df = 23, p = 0.864). However, there was a statistically significant correlation between the number and length of villi in the small intestine (r = 0.502, p = 0.012), large intestine (r = 0.612, p = 0.001) and caecum (r = -0.753, p = 0.000) of experimental animals fed on wheat. The results showed no statistically significant correlation between the number and length of villi in the duodenum, small intestine, large intestine and caecum of laboratory animals fed on kale, or between the number and length

of villi in various regions of the gut of laboratory maintained animals fed on a mixture of wheat, kale and insect.

CHAPTER FIVE

DISCUSSION

5.1 Diet of *Hylomyscus denniae endorobae* in its natural habitat

Diet of field collected animals comprised mainly of seed coat epidermis, with low proportion of leaf epidermis. Animal matter was present in lowest proportions. This implies an omnivorous diet, mainly granivore. *Hylomyscus denniae endorobae* feeds mainly on wild seeds, some plant leaves and a little animal matter mainly, invertebrates (Carleton and Stanley, 2005). This is in line with earlier research findings that wild mice are granivores feeding primarily on wild dicotyledonous seeds, and plant leaves when wild seeds are scarce (Rodgers and Gorman, 1995). Collection of animals of study was done during the rainy season, in the month of June when seed was plenty.

5.2 Relationship between body weight and total gut length

The results showed no significant correlation between body weight and total gut length of field collected animals and experimental animals fed on wheat representing granivore diet, kale for herbivore diet and a mixture of wheat, kale and locust for omnivore diet. Seed has a high proportion of carbohydrate, which is deposited in form of glycogen and fats leading to increase in body weight and a decrease in gut length due to pressure of overlying mass. High protein and fat diet provide high energy density which causes increase in body weight but causes a decrease in intestinal mass. This is in agreement with findings by Ferraris *et al.* (1992) and Wang *et al*. (2006) that gut length is independent of body weight in rats fed on non-carbohydrate and high carbohydrate diets for several weeks. Further

findings have shown that diet rich in indigestible material does not affect the dimension of the small intestine (Naya *et al*., 2007). The increased body weight of experimental animals was due to readily available energy and protein provided by the diets. Also, the experimental animals were confined in cages and hence low energy expenditure. The low body weight in kale fed *Hylomyscus deniae endorobae* could have been due to kale diet having a substantial amount of indigestible carbohydrates which lowered the energy density, leading to a lower body weight compared to wheat and omnivore diets.

The difference in weight between wheat fed animals and those fed on a mixture of wheat, kale and insect could have been due to high protein content in the omnivore diet ranging between 20.4-26g/80g (Bilgichi *et al*., 2007; Sirah, 2011) which may have caused a decrease in the small intestine mass leading to a lower weight compared to wheat diet. The experimental animals may have used their large intestines for food storage and nutrient extraction and still met their daily energy needs hence no increase in their stomach and caecum lengths resulting in no change in total gut length.

5.3 Relationship between diet and stomach volume

There was no significant difference between diet and stomach volume between groups of animals fed on different diets. The mean stomach volume of *Hylomyscus denniae endorobae* fed on omnivore diet was lower compared to field trapped animals. This is not in line with earlier findings that diet rich in indigestible material causes an increase in stomach and caecum size (Naya *et al*., 2007). The apparent decrease in stomach volume of experimental animals fed on omnivore diet was probably due to mice preferring locust and wheat which are rich in protein, lipid and soluble starch, digested in the foregut and

stomach. Concentrated diet enables faster digestion and absorption of food (Allen, 2007). In the natural habitat, insects are hard to find and seeds may be seasonally available. The expected increase in stomach volume of animals fed on kale diet also did not occur. Small rodents have been reported to cope with low food quality by increasing food intake and increase in villi length rather than by increase in gut length (Grant *et al.*, 1998; Wilson *et al*., 1998). The high protein content in wheat diet and increase in cecal villi length may have led to similar stomach volumes for animals fed in wheat and kale diets.

5.4.1 Effect of herbivorous diet on gut length

Animals fed on herbivorous diet (kale) showed no significant change in gut length at colon and caecum level. This was not in line with the expected results of herbivorous rodents that have been reported to have a relatively longer colon and caecum, shorter proportion of small intestine and greater digestive tract capacity (Vorontsov, 2003). Strict herbivores such as *Microtus brandti* have longer large intestine and caecum compared to granivores and omnivores (Song and Wang, 2006). Omnivores and granivores have varied large intestine and caecum depending on the proportions of seed, vegetation and animal foods in their diets. The hindgut is more important for herbivores than omnivore and granivore rodents where it is used for food fermentation and acts as an indicator for food habits (Kerrin, 2010). *Hylomyscus denniae endorobae* is a granivore. These findings imply that *H. d. endorobae* may not survive if exposed to farm land habitats such as tea farms, where only plant leaf is available as feed. Herbivores rely upon a more continual flow of its low quality, high fiber diet throughout the anterior portion of the gut prior to retention, fermentation, and assimilation of nutrients in the caecum and colon (Kerrin, 2010). Cecal

capacity is of greater importance than that of other gut sections in herbivorous rodents (Del velle and Busch, 2003). From the results on the total gut length, it appears that *H. d. endorobae* meets high energy demands by increasing feed intake and cecal villi length.

5.4.2 Effect of wheat diet on gut length

There were no clear trends in the relationship between wheat diet and mean gut length. Resistant starch and high fiber content in wheat delays intestinal transit time, enabling digestion and absorption to occur without changes in gut length. This is in line with earlier findings that omnivore rodents are more flexible than herbivore and granivore rodents (Browkoska, 1995). Increase in digestive organ size due to increase in diet fiber content has been found to occur in *Microtus branditi, Akodon azar*ae, *Microtus agrestis, Microtus pennsylvanicus and Meruxies ungulculatus* (Sagher *et al*., 1990; Derting and Bogue, 1993; Hammond and Wunder, 1993; Delvelle and Busch, 2003; Song and Wang, 2006). The study findings suggest that wheat diet has similar effects on gut length as the natural diet of *Hylomiscus denniae endorobae.* The results on gut flexibility are also in line with earlier findings that there are no differences in small intestine length between herbivores, omnivores and insectivores in wild rodents (Naya *et al.,* 2008). Wheat bran has insoluble polysaccharides which affect colonic fermentation and increase butyrate concentration. Resistant starch delays intestinal transit time and has little or only modest effects on fecal bulking. Passage rate determines intake in wild rodents although it was not measured. The present results imply increased feed intake and faster passage rates which limit utilization of nutrients (Wang *et al.,* 2000) which in turn lowers the performance of the species.

5.4.3 Effect of omnivorous diet on gut length

This study showed that *Hylomyscus deniae endorobae* fed on a mixture of wheat, kale and locust had the shortest large intestine length compared to those fed on wheat and kale. This could probably be due to the mice preferring wheat and locust to kale as was observed during the collection of food remains which had a larger proportion of kale compared to wheat and very little or no locust at all. Locusts have a higher amount of protein (10g per 20g of body weight) but very low carbohydrates compared to wheat (14g per 80g of protein and 50g per 80g of carbohydrates) (www. annecollins.com/sodium diet/sodium grain-wheat.htm). Protein, lipid and soluble starch diet is normally digested in the foregut and midgut. In the natural habitat, insects are hard to find and wild seeds may be seasonally available resulting to *H. d. endorobae* consuming plant leaves. This is based on the fact that plant seeding and locust reproduction depend on climatic factors such as rainfall and sunlight which are seasonal (Nyeri meteorological report, 2011). Wheat bran and kale have non-soluble polysaccharides which are non-fermentable and cause an increase in colon and caecum dimensions. Their fermentation produces fatty acids which cause an increase in daily epithelial cell production 3-4 fold in rats (Wong *et al*., 2006). This was not so probably due to high protein content (20.4 per 80g) in the omnivorous diet.

5.5.1 Digestibility

Analysis of digestibility of food items provides vital information on amount of food digested and therefore relative values of the food items (Schneider and Flatt, 1995). The findings that digestibility was highest in *Hylomyscus denniae endorobae* fed on wheat, but

lowest in mice fed on kale implies that grains are more important in the diet of the rodents since they contain mainly carbohydrates which provide energy for growth and development of body muscles, reproductive systems and the functioning of the organ systems. Wheat diet has more resistant starch which delays intestine transit time hence maximum digestion and absorption. The results imply that digestibility of the diet was affected by the nature of the food stuff composition. Dry fecal weight for animals receiving the control diet (mixture of wheat, kale and locust) was lower than the dry fecal weights for rodents fed on wheat and kale diets. High fecal bulking was expected in omnivore diet but was not the case probably because of the high protein and fat in the omnivore diet which masked some of the wheat bran effects. In the presence of animal matter, fecal output was low, translating to high digestive efficiency. This is in line with earlier findings that omnivore diet is better digested hence high digestive efficiency (Jobling, 1994). In the case of kale, digestibility was influenced by low carbohydrates resistant to digestion in the small intestine.

Digestibility is affected by such factors as feed intake, particle size chemical composition, climate, feed processing, age and exercise. Differences in composition and amounts of nutrient present in feed stuffs leads to different levels of digestibility hence affecting the levels of digestible energy available to an animal (Glengross *et al*., 2005). This supports the study results which showed that resistant starch in kale lowered digestibility. Different content of certain chemicals of similar feed affect digestibility; some chemicals diminish the opportunity for the digestive enzymes to come into contact with their respective substrates. Addition of relatively small quantities of specific nutrients such as protein or soluble carbohydrate may enhance full digestibility (Luginbuhl *et al*., 1994).

5.5.2 Nutrient absorption

The study showed significant effect of diet on absorption of sodium ions, calcium and potassium ions for all diets. Also, there was significant difference in absorption of potassium ions in all diets but not for sodium and calcium ions. Regression coefficient showed significant correlation between diet and absorption of calcium ions. Digestibility and absorption of mineral ions was affected by the different diets, with wheat having the lowest excreted calcium ions compared to potassium and sodium ions. This conforms with earlier findings that phytates in wheat bran inhibit absorption of calcium ions in rats (Asuarujanon *et al*., 2005) and that a diet with more crude oil forms fatty acid salts, thereby depressing calcium absorption in particular and ash digestibility in general (Murray *et al*., 2000). Several factors such as cell wall constituents and ether extracts can act as mineral sinks and decrease calcium digestibility in rats. Oxalate-calcium ratio in wheat diet could have been more availing little calcium for digestion and absorption, allowing more to be excreted in feces, and hence the small amount of calcium in urine (Morris and Savage, 2003).

5.6 Effect of diet on the number and shape of villi

Increase in the number of villi increases the surface area for absorption, to compensate for the decrease in gut and villi length due to diet (Dahlke *et al*., 2003). Growth of new villi being an energy demanding process, mice increase their rate of food intake hence will migrate to new habitats or die if food is not available. The study also showed that villi of wild mice fed on wheat protein became more blunted and broader than those fed on kale diet. This increases the surface area for absorption of nutrients. This result is in line with

early findings that wheat protein makes rat villi to become more blunted and broader and that change is irreversible especially if the first encounter was in mature life (Dahlke *et al*., 2003). Wheat protein antigens and bacterial growth cause villi atrophy showing the protrusion to be more blunted, broader, with an increased surface area. If there is bacterial or other9infections in the caecum, there may be changes of an immunological nature, a delayed immune reaction involving secretion of lg A or the production of antibodies, with the intestinal villi taking on a different morphological structure as a defense against foreign protein, thus taking on the appearance of a normal villus of the same immunological status. Intestinal villi of field collected animals were seen under the microscope as finger-like protrusions extending from the terminal web area of the cells with slightly blunted ends.

Although dietary composition may affect villous morphology and its arrangement (Chiou *et al.*, 1994), it also depends on species and genetic strain (Moore *et al*., 1996). Villi in the colon and caecum were shorter, broader and more rugged compared to villi in the proximal region of the gut. Wild seed is rich in proteins and carbohydrates mainly digested in the foregut hence the small intestine had more villi for absorption compared to duodenum and large intestine.

The marked difference in shape and number of the distal intestinal villi could be attributed to the fact that protein does not stay as long in the proximal intestinal region as it does in the distal region, and by the time that part is reached the protein could have been broken down by the various intestinal enzymes. The effects could be due to not only the undigested protein but also to the various breakdown products, peptides and other potent

chemicals resulting from digestion, some of which may be toxic and have detrimental effect on the villi (Dahlke *et al*., 2003).

5.7 Effect of diet on length of villi

There was significant correlation between diet and the length of villi in all regions of the gut of experimental animals except duodenal and cecal villi of those fed on wheat diet. Villi lengths decreased for all diets in the gut regions, with wheat diet causing the greatest decrease of 3.8mm in villi of the large intestine except for kale diet where cecal villi increased by 1.0mm. This contrasts earlier findings that non-soluble polysaccharides in wheat generate short chain fatty acids during fermentation process which increases the daily epithelial cell production three to four fold in the intestine (colon-jejunum) of rats and that saturated fat diet or cholesterol enriched diets increase villi length in wild mice (Wong *et al*., 2006). Also, the results do not agree with research by (Kelly *et al*., 2012) that animals feeding on high fiber diet have longer small intestinal villi than those consuming low fiber diets to increase surface area for maximum absorption of nutrients.

Wheat protein reduces height of villi in rats probably due to a first encounter phenomenon and might have immunological as well as morphological effects (Gu and Li, 2004). Probably wheat had low crude amino acids, which is the major macronutrient for the development of the morphological features of the villi and epithelial cells. Low protein, high fiber diet caused a reduction in mean villi length. This could have also been caused by the wheat protein and low crude oil in the omnivore diet and low crude oil in wheat and kale diets which reduce epithelial cell proliferation. An increase in villi length, epithelial

thickness and crypt depth improves gut morphology by increasing the surface area for absorption (Hedemann *et al*., 2006; *Wikipedia*, 2009). A decrease in villi length reduces the surface area for absorption of nutrients in mice. This can lower utilization efficiency, leading to low reproductive efficiency thereby minimizing survival of the animals.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study reports the utilization of food resources by *Hylomyscus denniae endorobae* trapped from Ihururu Forest in Nyeri, Kenya and fed at the Zoological Science laboratories, Kenyatta University. The following conclusions were drawn from the study:

i) *Hylomyscus denniae endorobae* is a granivore. Its diet in the natural habitat had a higher proportion of seed coat epidermis compared to leaf and animal matter epidermis. This implies that *H. d. endorobae* may not survive if exposed to habitats where only animal matter is available.

ii) Total gut length (TGL) is independent of body weight in *H. d. endorobae*. Field collected animals had varied body weights but approximately the same total gut lengths. After feeding, the body weights changed but total gut lengths remained unchanged, a clear indication that body weight has no influence on total gut length.

iii) Diet has no influence on total gut length (TGL) in *H. d. endorobae*. There was no change in total gut length for laboratory animals. The total gut length remained almost the same as that of field collected animals. This implies that the species may not cope with changes in food resource availability in fragmented natural habitats.

iv) Diet influences the number and length of villi. Wheat, kale and insect diets used in the study caused an increase in the number of villi in all gut regions and a decrease

in villi Lengths in all gut regions except for cecal villi length of animals fed on kale diet which increased. This increases energy demand of *H. d. endorobae* hence they may not survive in a habitat with limited food resources.

v) Diet influences digestive efficiency. Omnivore diet showed high digestive efficiency in *H. d. endorobae*. The digestion of proteins, carbohydrates and lipids occurs in the stomach, duodenum and small intestine, where the surface area is large for faster absorption. This implies that *H. d. endorobae* density may decrease in exposed habitats with little food resources.

vi) Absorption efficiency of mineral ions is influenced by diet. Phytates and oxalates in Kale and wheat bran inhibit absorption of calcium ions by *H. d. endorobae,* which may cause osteoporosis, poor nerve impulse conduction, poor heart and skeletal muscle cell contraction and inadequate production of transmitter release hormone. This suggests that *H. d. endorobae* may not survive in natural habitats changed into farm land.

6.2 Recommendations

Wildlife conservationists such as the National Museums of Kenya and the Kenya Wildlife Services should formulate a policy to ban human settlement and small scale farming, logging and charcoal burning in Ihururu Forest. Protection of natural habitats of *Hylomscus denniae endorobae* will enable survival of the species. This will prevent species erosion, increase species diversity and improve ecosystem productivity.

6.3 Suggestions for future work

(i) Effect of temperature, and age on digestibility of food resources and gut length in *Hylomyscus denniae endorobae* should be investigated in order to avail more information on gut flexibility and evolution of the species.

(ii) Effect of bacterial growth on villi morphology should be investigated to avail data for an accurate account of the effect of diet on villi size and shape.

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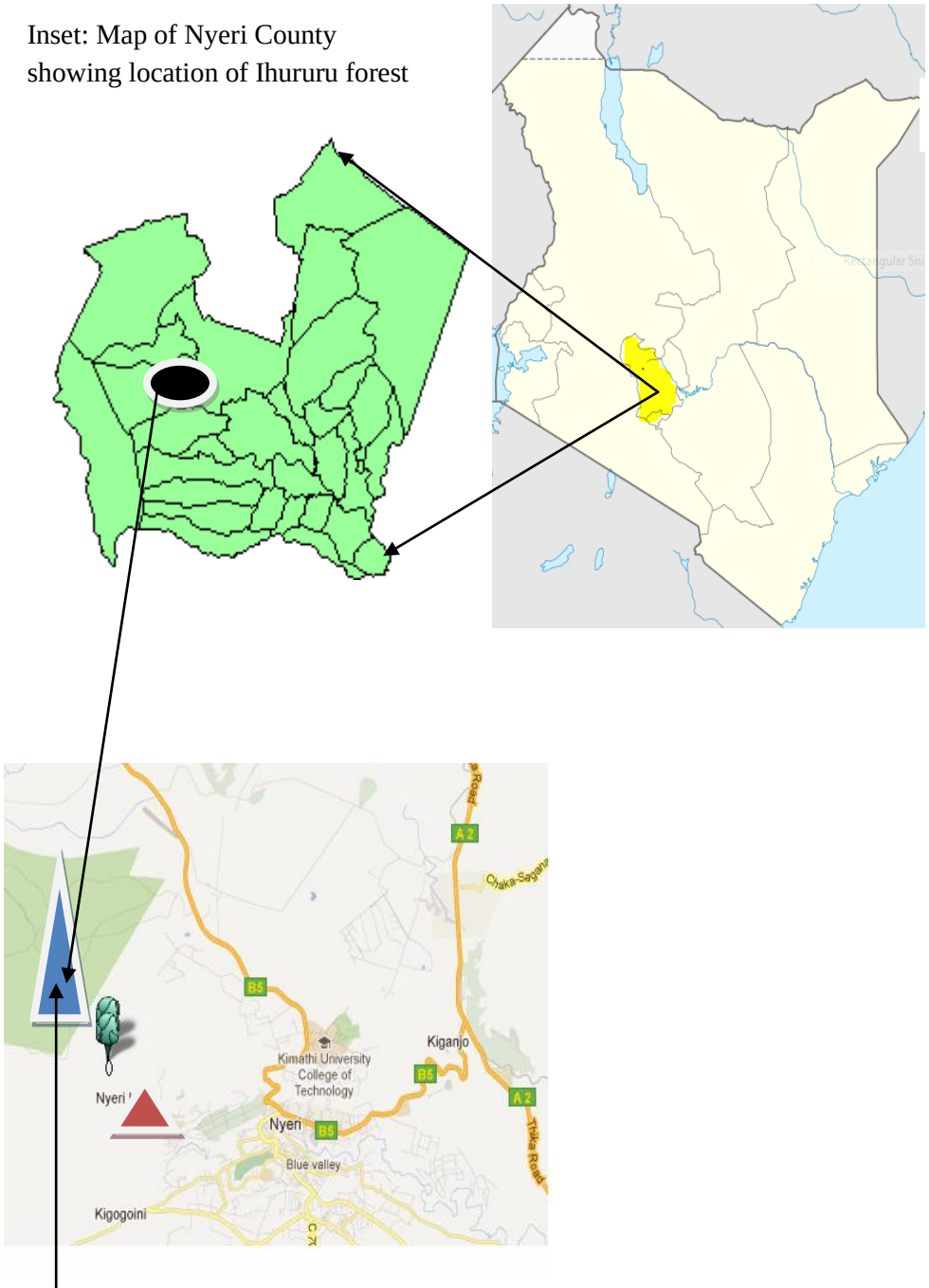
APPENDICES

Appendix i: **Map of Kenya showing location of Nyeri County**



Ihururu forest (Adopted from Nyeri, Physical planning, 2011)

Appendix ii: Photograph showing *Hylomyscus denniae endorobae*

Appendix iii: Live Sherman traps used for trapping study animals

Appendix iv: Temperature of Ihururu forest over the trapping period (11th-25thnd June 2011)

DATE	TIME	PLOT	GROUND NOW (˚C)	GROUND MAX (˚C)	GROUND MIN (˚C)	TREE NOW (˚C)	TREE MAX (˚C)
6/11/2011	8:04	1	17.2	-	-	16.0	-
6/12/2011	7:17	1	15.5	19.1	5.3	13.4	25.2
6/13/2011	8:00	1	13.0	19.3	9.5	10.1	24.4
6/14/2011	8:00	1	15.1	15.6	11.9	24.9	20.6
6/14/2011	10:18	2	16.5	17.3	16.1	15.9	25.1
6/14/2011	13:45	4	16	17.2	15.1	17.7	18.3
6/15/2011	8:17	1	-	38.9	17.1	13.3	26.7
6/15/2011	9:45	2	16.4	17.4	16.4	12.9	19.7
6/15/2011	-	4	15.3	16.3	14.7	15.3	18.3
6/16/2011	18:05	1	17.4	17.4	15.6	16.7	22.8
6/16/2011	-	2	15.7	16.7	15.7	13.4	18.0
6/16/2011	11:53	4	14.5	16.3	14.7	13	15.1
6/17/2011	6:45	1	15.1	17.4	15.1	11.3	22.8
6/17/2011	9:00	2	15.4	16.4	15.3	17.8	19.3
6/17/2011	9:43	4	14.4	15.1	14.2	16.6	15.8
6/18/2011	6:57	1	15.7	20.4	15.7	13.2	23.7
6/18/2011	9:32	2	15.9	17.2	15.8	14.9	23.1
6/18/2011	10:16	4	14.8	15.9	14.7	13.9	16.0
6/19/2011	7:38	1	15.7	18.0	15.7	13.1	21.7
6/19/2011	9:38	2	15.9	16.8	15.8	15.4	12.9
6/19/2011	10:31	4	14.9	15.7	14.8	14.1	14.6
6/20/2011	7:37	1	16.0	18.4	16	13.4	22.9
6/20/2011	10:30	2	16.2	16.9	16.2	13.7	20.7
6/20/2011	11:03	4	1.9	15.9	14.9	13.4	18.8
6/21/2011	8:00	1	15.8	17.3	15.8	13.7	18.3
6/21/2011	12:00	2	15.9	16.4	15.8	14.4	16.3
6/21/2011	9:48	4	14.8	15.3	14.8	13.2	14.3
6/22/2011	7:38	1	15.7	22.4	15.3	12.7	21.1
6/22/2011	9:43	2	15.7	23.5	15.7	13.4	18.9
6/22/2011	10:29	4	14.3	12.2	14.3	13.2	16.0
6/23/2011	7:42	1	15.1	17.7	15.1	13.3	22.7
6/23/2011	9:13	2	15.6	16.4	15.6	16.2	20.1
6/23/2011	9:43	4	14.6	15.3	14.3	14.7	16.9
6/24/2011	7:36	1	15.3	19.4	15.3	12.9	22.5
6/24/2011	9:24	2	15.7	16.8	15.7	15.6	20.4
6/24/2011	-	4	14.8	15.7	14.7	15.6	19.1
6/25/2011	8:01	1	15.3	19.4	15.3	13.2	22.5
6/25/2011	9:48	2	16.1	17.2	15.8	17.6	20.7

Appendix v: Table showing proportion of Nutrients in 100g of each experimental diet

	Experimental quantities						
	Fiber	Protein	Carbohydrate	Lipid	Na+	Ca2+	K+
Wheat	A lot	8-14g	48-56g	14-18g	4.8mg	40mg	312.0mg
Kale	1.6g	2.4g	5.7- 8g	0.6g	3.96mg	8.36mg	168.0mg
Locust	none	8g/20g	2.4g/20g	9.6g/20g	none	none	Traces

The proportions were calculated based on the nutrient database figures by Sirah (2011) and Bilgichi *et al*., (2007).