Protein Metabolism in the Adult Domestic Cat (*Felis catus*)

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ABSTRACT

The main objective of this study was to provide information for the determination of the protein and amino acid requirements of the adult domestic cat by the factorial method. Data are presented on four factors for a factorial model: (1) the endogenous amino acids excreted in the faeces, (2) the amino acids which are required for the replacement of hair, (3) the body amino acids which are catabolised and ultimately voided in the urine and (4) the excretion of the urinary amino acid felinine.

Endogenous ileal nitrogen and amino acid excretions were determined under the conditions of protein-free and peptide (enzymatically hydrolysed casein, molecular weight < 10,000 Da) alimentation while the relative contribution of the hindgut to total endogenous gut excretion was investigated in a separate study by feeding cats a protein-free diet with or without added antibiotics. Endogenous ileal nitrogen and amino acid nitrogen excretions (mean ± SEM) of 2.4 ± 0.27 and 1.9 ± 0.13 mg/g food dry matter intake, respectively, were found for the cats fed the protein-free diet, whereas higher excretions of 3.6 ± 0.73 ($P = 0.12$) and 3.6 ± 0.76 ($P = 0.03$) mg/g food dry matter intake were obtained under the condition of peptide alimentation. Significantly ($P < 0.05$) higher endogenous ileal amino acid excretions, for the enzymatically hydrolysed casein-fed cats compared with those fed the protein-free diet, were found for methionine, aspartic acid, serine, glutamic acid, proline, valine and isoleucine, with the differences in excretions of glycine, alanine, leucine and histidine being significant at the 6 % level. Most of the endogenous faecal amino acid excretions were unaffected by the inclusion of the antibiotics in the protein-free diet, although bacterial numbers were significantly ($P < 0.01$) reduced (69 %). Antibiotics addition led to significantly higher faecal endogenous excretions of nitrogen, taurine, threonine, serine and histidine but significantly lower excretions for methionine and lysine.

Nitrogen and amino acids required for the growth of hair were estimated by the measurement of hair growth and loss rates for adult male and female domestic short-haired cats, and the nitrogen and amino acid composition of cat hair. Hair growth rate was determined using the mid-side patch technique in combination with the ratio of hair on the mid-side area to total hair on the body to allow conversion of mid-side hair growth rates to hair growth rates over the entire body. Hair loss rate was determined in a separate experiment by quantitative collection of ingested and non-

ingested hair loss. The mid-side hair growth and the hair loss rates showed a sinusoidal pattern throughout the year. The peak hair growth rate for the female cats was reached earlier than that for the male cats. Hair loss rate was 75 d out of phase with hair growth rate. The estimate for the total amount of hair growth throughout the year (32.7 $g \cdot kg^{-1}$body weight) was similar to the estimate for the total amount of hair loss throughout the year (28.1 $g \cdot kg^{-1}$body weight). The amino acid composition of cat hair was determined by conventional 24 h acid hydrolysis and by a compartmental model which took into account the simultaneously occurring processes of hydrolysis and degradation of amino acids over time. Four colours of cat hair, were also analysed (24 h hydrolysis) to determine if there was an effect of hair colour on amino acid composition. There was no significant ($P > 0.05$) effect of hair colour on the amino acid composition of cat hair. Amino acid nitrogen accounted for 94 and 99 % of the total nitrogen when determined by conventional 24 h hydrolysis and the compartmental model, respectively. The accurate amino acid composition of cat hair is presented.

Endogenous urinary excretions of total, urea, ammonia and creatinine nitrogen in the adult cat were determined by feeding cats a protein-free diet or by regression to zero protein intake of urinary nitrogen metabolite excretions for adult cats fed four levels of dietary protein. The mean (± SEM) endogenous total, urea and ammonia nitrogen excretions for the cats fed the protein-free diet were 360 (± 11.3), 243 (± 8.8) and 27.6 (± 1.06) $mg \cdot kg^{-0.75}$body $weight \cdot d^{-1}$, respectively. Lower mean (± SEM) estimates of 316 (± 53.9) and 232 (± 43.4) $mg \cdot kg^{-0.75}$body $weight \cdot d^{-1}$ were obtained for the endogenous excretions of total and urea nitrogen, respectively using the regression technique while a higher endogenous urinary ammonia nitrogen excretion of 33.7 (± 5.68) $mg \cdot kg^{-0.75}$body $weight \cdot d^{-1}$ was found. The differences between the two techniques were not statistically significant. Daily excretions of creatinine nitrogen were not significantly ($P = 0.64$) different (mean ± SEM, 16.2 ± 0.46 and 17.5 ± 1.19 $mg \cdot kg^{-0.67}$body weight, respectively) for the two methods. The chemical body composition of male and female adult cats was determined to obtain an estimate of the pattern of endogenous amino acid catabolism. Dehaired empty cat bodies were subjected to analysis for dry matter, lipid, ash, crude protein, amino acids, and several minerals (Ca, P, K, Mg, Fe, Mn and Zn). The chemical body composition was found to be similar between male and female cats except for the amount of crude protein which was significantly ($P < 0.05$) higher on a wet tissue (21.7 ± 0.35 % vs. 20.0 ± 0.60 %),

lipid-free matter (24.1 ± 0.22 % vs. 23.0 ± 0.22 %) and lipid-free dry matter (80.7 ± 0.23 % vs. 78.3 ± 0.32 %) basis in the male cats compared to the female cats. There was no significant ($P > 0.05$) effect of gender on the whole body amino acid composition (lipid-free dry matter or mol % basis) except for cysteine which was significantly ($P < 0.05$) lower in the male cats in comparison to the female cats.

A review on the urinary amino acid, felinine, which can be found in cat urine is presented. The biological significance of felinine to the animal is still a matter for speculation, but its function as a precursor to a pheromone seems likely. To obtain a standard for quantification experiments, several published synthesis procedures reported leading to (±)-felinine were evaluated for their yield. Most of the procedures were found to produce an amino acid isomeric with felinine. The yield for the only evaluated synthesis procedure shown to produce felinine was found to be low. A new higher yielding method for the synthesis of (±)-felinine is presented. In a separate study, normal urinary excretions of felinine by entire male, castrated male, entire female and spayed female cats were determined and were found to be (mean ± SEM) 122 ± 23.6, 41 ± 8.4, 36 ± 7.3 and 20 ± 3.8 $\mu mol \cdot kg^{-1}$ body weight$\cdot d^{-1}$, respectively.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3 PART 1

CHAPTER 3 PART 2

CHAPTER 3 PART 3

CHAPTER 4 PART 1

Table

CHAPTER 4 PART 2

Table

CHAPTER 5 PART 1

Table

CHAPTER 5 PART 3

Table

LIST OF FIGURES

CHAPTER 1

CHAPTER 3 PART 1

CHAPTER 3 PART 2

CHAPTER 4 PART 1

CHAPTER 5 PART 1

Figure

CHAPTER 5 PART 2

Figure

CHAPTER 5 PART 3

Figure

CHAPTER 6

Figure

GENERAL INTRODUCTION

The domestic cat (*Felis catus*) is a member of the Felidae family of the order Carnivora. Although the majority of the species in order Carnivora (meat eaters) are omnivores, the Felidae family consists of animals which are strictly-carnivorous. A meat diet throughout the evolution of the domestic cat has resulted in several metabolic peculiarities in this animal which are not found in animals belonging to other families in this order.

Basic biological, physiological and biochemical investigations into the metabolism of the domestic cat and determination of the nutritional needs of this species have mainly been carried out over the last two decades. Whereas several of the cat's metabolic peculiarities have been documented, there is still a paucity of quantitative information on the nutritional requirements of this companion animal. This is especially so for the requirements for protein and amino acids during the adult life stage.

The main objective of this study was to provide information to allow the determination of the protein and amino acid requirements of the adult domestic cat by the factorial method. Information is presented on four factors required for the formulation of a factorial model: (1) the endogenous amino acids excreted in the faeces, (2) the body amino acids which are catabolised and ultimately voided in the urine, (3) the amino acids which are required for the replacement of hair and (4) the requirements for amino acids for the excretion of the urinary amino acid felinine.

CHAPTER 1

REVIEW OF LITERATURE

1.1 INTRODUCTION

The current phylogenetic relationship for the members of the order Carnivora is divided into two suborders, the aquatic Pinnipedia and the terrestrial Fissipedia. The former suborder consists of three families and includes animals like the seal, walrus and sea lion. The latter suborder, Fissipedia, consists of two superfamilies, the Canoidea (or Arctoidea) and the Feloidea (or Aeluroidea) which contain five and three families, respectively (Fig 1.). Divergence of these two suborders is believed to have occurred in the late Eocene to early Oligocene period, around 35 million years ago (Romer 1966, Carroll 1988).

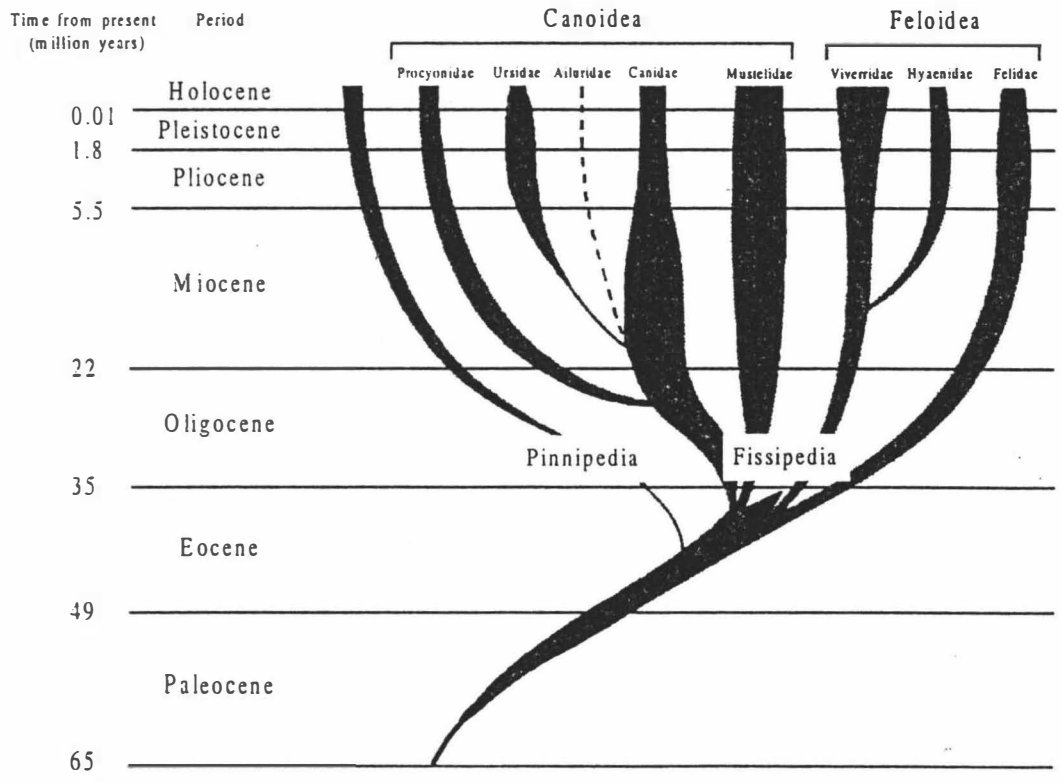

Figure 1. Evolution of the order Carnivora (Adapted from Romer 1966 and Morris and Rogers 1989).

The evolutionary changes in the order Carnivora, as measured by the rate of evolution of the chromosomes, is amongst the lowest of all mammals with an average age of the Carnivora being three times that of the primates (Bush *et al.* 1977). The Feloidea (which include the Viverrids, Hyaenids and Felids) underwent rapid evolution to fully specialised forms, which have not changed for millions of years (MacDonald *et al.* 1984, Morris and Rogers 1989). All the members of the subfamily Feloidea are virtually strict flesh eaters and, therefore, true carnivores. The Canoidea (which include Procyonids, Ursids, Ailurides, Canids and Mustelids) show a greater dietary diversity than the Feloidea. Mustelids (e.g. weasels and mink) are carnivores while the Canids (e.g. dogs), Ursids (e.g. bears) and Procyonids (e.g. racoon) are omnivores. The Ailurids (pandas) have even adapted an herbivorous dietary habit. It is evident that the order Carnivora consists of animals with widely different dietary habits and that the scientific name Carnivora (meat eaters) is not entirely appropriate.

The Felids have evolved as strict carnivores and this dietary habit has resulted in a specific metabolism of this family which cannot be found in Canids or Ailurids. Comparison of the nutritional requirements of the dog and the cat, members of the Canidae and Felidae, respectively supports this view. The cat, unlike the dog, is unable to regulate catabolic enzymes (for amino acids) in the liver and, therefore, requires a diet high in protein. The cat cannot synthesise niacin from tryptophan, convert carotene to vitamin A, convert enough linoleic acid to arachidonic acid or synthesise sufficient taurine. These metabolic adaptations are believed to have occurred largely because of the strictly-carnivorous nature of the cat and all the above nutrients which the cat cannot synthesise can be found in a diet consisting of animal flesh. The dog on the other hand, has retained the ability to synthesise the above mentioned nutrients, which is consistent with the view that this animal is an omnivore/carnivore.

Domestication of the cat may have been as early as 3000 BC (Zeuner 1963) and is believed to have occurred accidentally after recognition of the value of the cat in protecting the food storage facilities of man, in particular stocks of cereals, from small animals (Röhrs 1987). Nowadays the carnivorous cat is often kept in an environment where it cannot obtain its natural food and it is the responsibility of the owner to provide the animal with the nutrients it requires. It becomes imperative, therefore, that the food supplied to the animal is nutritionally balanced and meets all the requirements of the animal. The basic requirements for individual nutrients, therefore, is of primary importance in determining the adequacy of a diet for cats.

This review first considers protein metabolism in mammals with special emphasis on the general metabolic pathways of amino acids. The protein metabolism of the strictly-carnivorous cat is discussed as it differs from omnivores (rat and man) and the non strict-carnivore, the dog. The different methods for the determination of protein and amino acid requirements of mammals are discussed and finally the information which is needed to determine the protein and amino acid requirements of adult cats by the factorial method is presented.

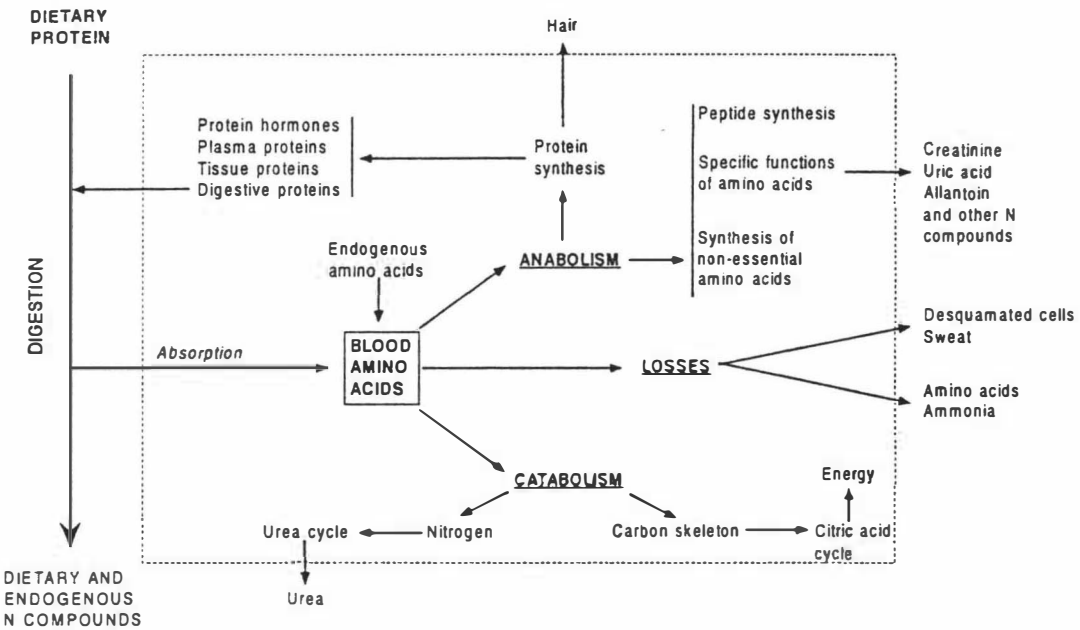

Figure 2. Schematic representation of protein metabolism in mammals (Adapted from Munro 1964).

1.2 MAMMALIAN PROTEIN METABOLISM - AN OVERVIEW

The processes in an organism by which nutritive material is built up into living matter or by which living matter is broken down into simpler substances to perform specific functions can be described as metabolism. H. N. Munro describes protein metabolism as: "the history in the body of some 20 amino acids which occur in the proteins of the diet" (Munro 1964). Protein metabolism in mammals, therefore, includes the digestion and absorption of dietary protein, anabolic and catabolic processes of amino acids and nitrogenous compounds derived from amino acids in the mammalian body and the excretion of nitrogenous compounds from the body. Figure 2 shows a simplified representation of protein metabolism in mammals. Comprehensive reviews dealing with various aspects of protein metabolism with special emphasis to mammals have

been published by Munro and Allison (1964a,b), Munro (1969a, 1970a), Schepartz (1973), Cole *et al.* (1976), Florkin *et al.* (1980), Waterlow and Stephen (1981), Neuberger and Van Deenen (1982), Newsholme and Leech (1983), Linder (1985), Bock *et al.* (1989) and Hunt and Groff (1990).

Due to the diversity of the subject of protein metabolism, a complete integrated picture of protein metabolism is not presented in this thesis. There are, however, some general pathways along which amino acids and other nitrogenous compounds are metabolised by mammals which form the basis of the metabolism of protein. As these pathways are mainly associated with various tissues, the focus of this overview will be on the metabolism of amino acids and other nitrogenous compounds by the gastrointestinal tract, liver, muscle, kidney, brain and adipose tissue. The emphasis, furthermore, will be directed towards protein metabolism in monogastric mammals.

1.2.1 Synthesis and breakdown of body proteins

Protein in mammalian tissues is constantly replaced by newly synthesised protein. This process in called protein turnover and is the result of two processes, protein degradation and protein synthesis. In young growing mammals there is a net accumulation of protein in the body which occurs as a result of an imbalance between the rates of protein synthesis and degradation with the former exceeding the latter. In adult mammals, when growth has effectively ceased, these two processes counterbalance each other resulting in no net change in the body protein. Protein turnover, furthermore, occurs in every cell of the body with the exception of erythrocytes and varies among tissues with a more rapid turnover of visceral proteins as compared to muscle protein (Buttery and Vernon 1980, Newsholme and Leech 1983, Hunt and Groff 1990). Because the process of protein turnover is not completely efficient there is, even under the most favourable of conditions, a continual loss of amino acids from the body (Young 1991).

The processes involved in the synthesis of proteins take place in the cytoplasm of the cell and involve interactions between ribosomes, different types of ribonucleic acids (messenger, transfer and ribosomal), amino acids and enzymes as well as energy in the form of adenosinetriphosphate (ATP) and guanosinetriphosphate. The order of events leading to the formation of protein in the body has been described extensively (Korner 1964, Munro 1976, Pain 1978, Newsholme and Leech 1983, Simon 1989).

The breakdown of proteins in the body is believed to be accomplished by

proteolytic digestion of proteins in lysosomes which are present in the cytoplasm of the cell. Lysosomes contain a number of proteinases and peptidases able to completely hydrolyse protein (Waterlow *et al.* 1978, Hunt and Groff 1990). The cytoplasm of cells, furthermore, contain several soluble proteinases also able to hydrolyse protein (Buttery and Vernon 1980, Newsholme and Leech 1983, Mayer and Doherty 1986, Simon 1989, Hunt and Groff 1990).

Whole body protein turnover per unit of metabolic body weight varies little between mammalian species (Waterlow 1984, Reeds 1988). Growing animals, however, have a higher whole body protein turnover rate than adult animals of the same species (Reeds 1988). This higher turnover of body protein during growth of mammals seems to be a wasteful process as deposition of protein is accompanied by an increase in the rate of protein breakdown. Protein turnover rates also vary between different tissues of the body. Waterlow (1984) collected data from the literature on protein turnover in tissues of the rat and showed that the protein turnover in liver, muscle, skin and small intestine account for 82 % of the total turnover of protein in the body of the rat with protein turnover in muscle and liver accounting for 50 % of the total turnover. Other factors which influence turnover rates of body protein are food intake, exercise and injury (Waterlow 1984)

Several explanations have been put forward for the occurrence of protein turnover in mammals (Goldberg and St. John 1976, Dean 1980, Newsholme and Leech 1983). Firstly, degradation of protein is necessary to prevent the accumulation of incorrectly synthesised or denaturated proteins. Secondly, the concentrations of proteins can be changed rapidly by modification in the rate of synthesis or degradation. The latter is particularly important in the metabolism of nutrients where enzymes are involved. Thirdly, during periods of insufficient food intake, protein degradation may be required to provide energy and sufficient amino acid precursors for the synthesis of those proteins that are absolutely essential.

1.2.2 The role of the liver

The liver is involved in almost all aspects of protein metabolism. Its basic metabolic functions are the removal or modification of toxins, the regulation of concentrations of nutrients before these materials enter the peripheral circulation and the production of transport and plasma proteins. The amino acid pool available to the liver for metabolism originates from three sources (Popper and Schaffner 1957): amino acids

absorbed from the intestine following hydrolysis of ingested and secreted protein, amino acids made available by intra- and extra-lysosomal protein breakdown, and synthesis of amino acids. Amino acids originating from these three sources can either be used to synthesise proteins, peptides or other nitrogen containing compounds, yield glucose, fatty acids or energy, or can be used for the synthesis of non-essential amino acids.

Anabolic functions of the liver include, amongst others, the synthesis of liver and plasma proteins and peptides. Plasma proteins and peptides have a high turnover rate in comparison to other body proteins and the liver is the site for the synthesis of all albumin and fibrinogen and much of the globulins in plasma (Popper and Schaffner 1957, Peters 1983, LeBouton 1986). The liver, furthermore, conjugates bile acids with glycine and/or taurine which are used in the digestion of fat (Popper and Schaffner 1957, Newsholme and Leech 1983, Hunt and Groff 1990). The formation of creatine in the liver, is partly accomplished by two enzymatic reactions involving methionine and arginine (Narayanan and Appleton 1980). The liver is also involved in the metabolism of purine compounds of which the end product in humans, higher apes and Dalmatian dogs is uric acid and in other mammals, including dogs other than Dalmatians, is allantoin (Allison and Bird 1964, Van den Berghe 1981, Hunt and Groff 1990).

Most amino acids appear to be catabolised predominantly in the liver except for the branched chain amino acids (valine, leucine and isoleucine) which are rapidly catabolised in other tissues, especially muscle (Felig 1975, Goldberg and Chang 1978, Newsholme and Leech 1983, Crim and Munro 1984, Häussinger and Gerok 1986, Hunt and Groff 1990). The catabolism of amino acids in the liver can be divided into two stages (Krebs 1964). In the first stage, the α-amino group of an amino acid is enzymatically removed, leaving behind the corresponding α-keto acid analog of the incoming amino acid. The ammonia is converted to urea in the urea or ornithine cycle whereafter the urea is transported via the blood to the kidney for excretion. Although some of the enzymes involved in the synthesis of urea can also be found in several other organs, insignificant amounts of urea are formed extrahepatically making the liver the primary site for the formation of urea (Visek 1979). The urea or ornithine cycle is universal among mammals (Munro 1969b, Visek 1979). The α-keto acid formed by the deamination of the incoming amino acid in the liver is degraded to an intermediate product (pyruvate, acetoacetyl-CoA, oxaloacetate, furmarate, succinyl-CoA or α-ketoglutarate) which can be used in the tricarboxylic acid (TCA) cycle (Krebs 1964). In

the second stage, these intermediate products are used in the TCA-cycle which results in the formation of CO_2 and energy in the form of ATP. The intermediate products formed by degradation of the deaminated amino acids in the liver, can also be used for the formation of glucose, fatty acids or ketone bodies. The amino acids which can be degraded to pyruvate, oxaloacetate, furmarate, succinyl-CoA or α-ketoglutarate, 15 in all, can be converted to glucose and are, therefore, called glucogenic amino acids. The amino acids yielding acetoacetyl-CoA (phenylalanine, tyrosine, leucine, lysine and tryptophan) can yield ketone bodies and are named ketogenic amino acids. The division of ketogenic and glucogenic amino acids is not absolute since the catabolism of two amino acids (phenylalanine and tyrosine) gives rise to both glucose and ketone bodies at the same time while tryptophan can either be glucogenic or ketogenic (Krebs 1964, Lehninger 1982, Broquist 1984).

TABLE 1
Essentiality of amino acids in mammals.

Essential	Conditionally essential	Non-essential
Arginine[a]	Cysteine	Alanine
Histidine	Tyrosine	Aspartic acid
Isoleucine		Asparagine
Leucine		Glutamic acid
Lysine		Glutamine
Methionine		Proline
Phenylalanine		Glycine
Threonine		Serine
Tryptophan		
Valine		

[a]Essential for several growing and adult mammals (e.g. cat and dog).

The liver can also catabolise amino acids for the synthesis of other amino acids. Of the 20 amino acids present in mammalian protein, the liver can synthesise 10-11 amino acids, depending on the mammalian species, which are named non-essential or dispensable amino acids. The carbon skeleton for the synthesis of non-essential amino acids can originate from carbohydrates, fats or amino acids while the nitrogen can originate from either ammonium ions or the amino group of α-amino acids (Krebs 1964). The other amino acids cannot be synthesised by mammals and are, therefore, termed essential or indispensable amino acids and as a result have to be derived from the diet. Two amino acids, cysteine and tyrosine are synthesised from methionine and phenylalanine in the liver, respectively. In the case of inadequate dietary intake of the

latter two essential amino acids, the former two non-essential amino acids may become essential and for this reason, cysteine and tyrosine are often called conditionally essential amino acids. Table 1 shows the essential, conditionally essential and non-essential amino acids for different mammalian species. The latter classification is based on the ability of mammals to synthesise the amino acid and, therefore, does not include criteria like adequacy of synthesis for normal physiological processes or adequacy of synthesis for different life stages. Other classifications taking these latter considerations into account have been published by Jackson (1983) and Laidlaw and Kopple (1987).

1.2.3 The role of muscle tissue

Muscle constitutes the largest single tissue in the body of mammals and contains the largest reserve of amino acids, both free and covalently bound in protein. The movement of these amino acids between plasma and muscle is important in the normal homeostatic regulation of plasma nutrients, and is essential for survival during crisis such as starvation. By virtue of being the largest single tissue in the mammalian body, it can be anticipated that muscle tissue plays a significant role in the overall metabolism of the body (Young 1970, Munro 1978, Goldspink 1980).

As mentioned, muscle is the predominant site for the metabolism of the branched-chain amino acids. Here the breakdown of these amino acids generates amino groups whose accumulation in the organism would be toxic. Unlike the liver, muscle tissue lacks the enzymes to dispose of ammonia as urea. To dispose of the ammonia generated on breakdown of amino acids in muscle tissue, either branched-chain amino acids or amino acids released by muscle proteolysis, the amino acids alanine and glutamine have a vital role. Ammonia and glucose can be metabolised to form alanine in muscle tissue which is released into the general circulation. The alanine is taken up by the liver where, upon transamination, the ammonia is converted in the urea cycle to urea while the carbon skeleton of alanine is used for glucose synthesis. This glucose is released into the blood stream and can be taken up again by muscle tissue for the formation of alanine. This cycle is better known as the glucose-alanine cycle and its role is to transport ammonia generated following the catabolism of amino acids in muscle tissue to the liver (Felig 1975, Goldberg and Chang 1978, Newsholme and Leech 1983, Harper *et al.* 1984). Another route to dispose of ammonia generated upon catabolism of amino acids in muscle tissue, is the formation of glutamine which is formed by enzymatically binding ammonia and glutamate. In the kidney, the ammonia

from glutamine can be liberated and excreted in the urine. The production of glutamine by muscle tissue in this manner, continually drains the available pool of glutamate in this tissue and amino acids taken up from the blood or generated by the breakdown of muscle protein can be used to replenish this pool (Ruderman 1975, Goldberg and Chang 1978, Newsholme and Leech 1983, Crim and Munro 1984). The generated α-keto acids formed during deamination of amino acids in muscle tissue can be used to synthesise glutamate or are released into the blood where they can be taken up by other tissues. The α-keto acid of leucine is an exception in that this keto acid is preferentially used for the production of energy in muscle tissue (Goldberg and Chang 1978, Hunt and Groff 1990). This latter amino acid, furthermore, has a special regulatory function on protein turnover in muscle tissue (Goldberg and Chang 1978, Christensen 1982, Hunt and Groff 1990). The ability of muscle tissue to control the rate of conversion of amino acids to either alanine or glutamine has been postulated to be part of a complex regulatory mechanism that provides alanine for hepatic gluconeogenesis, glutamine for acid/base balance and glutamine for the small intestine, or other rapidly-dividing cells when required (Newsholme and Leech 1983).

During the turnover of muscle protein, the amino acid 3-methylhistidine which is derived from the actin and myosin in muscle tissue is released. This amino acid cannot be re-used by mammals and is transported to the kidney where it is quantitatively excreted in the urine. The amino acid 3-methylhistidine, therefore, provides a measure of the breakdown rate of muscle protein (Young and Munro 1978, Newsholme and Leech 1983, Crim and Munro 1984).

1.2.4 The role of the kidney

The kidney's role in protein metabolism cannot be overemphasised because of this organ's role in freeing the body of nitrogenous waste products which have accumulated during the metabolism of amino acids by other tissues. Nitrogenous waste products excreted by the kidney include urea formed in the liver, creatinine resulting from the spontaneous decomposition of creatine, 3-methylhistidine released during the breakdown of muscle protein and uric acid or allantoin formed in the liver by the breakdown of purines. Also, in the urine of normal mammals, small amounts of amino acids are present which have escaped reabsorption by the kidney.

In addition of ridding the body of nitrogenous waste products, the kidney is actively involved in the metabolism of amino acids. Ammonia from glutamine,

produced in the different tissues of the body, is enzymatically liberated in the kidney and quantitatively excreted in the urine. The resulting glutamate can be released into the blood stream or further metabolised by the kidney. Excretion of ammonia in the kidney is important in controlling the acid/base balance of the body fluids (Newsholme and Leech 1983, Halperin *et al.* 1990, Hunt and Groff 1990).

The kidney is the only organ besides the liver that possesses all the enzymes necessary for the formation of glucose. All reabsorbed amino acids can be metabolised to form glucose in the cells of the kidney although the dispensable amino acids alanine, aspartate, glycine, glutamate and proline are predominantly used (Wirthensohn and Guder 1986, Hunt and Groff 1990).

Other important metabolic processes occurring in the kidney are the formation of arginine from citrulline which originates from the intestine (Morris 1992), the formation of alanine and serine (Felig 1975, Cahill *et al.* 1981) and the hydrolysis of glutathione into its constituent amino acids (Christensen 1982, Ormstad and Orrenius 1983).

1.2.5 The role of the gastrointestinal tract

The main role of the gastrointestinal tract in mammalian protein metabolism is the digestion and absorption of ingested nutrients. Digestion of proteins in the gastrointestinal tract is accomplished by the hydrolysis of the protein by various enzymes which are secreted by the stomach, pancreas and small intestine of mammals. Non-specific proteinases secreted in the stomach, and the endo-, carboxy- and aminopeptidases excreted into the small intestine, hydrolyse the ingested protein to oligopeptides, tripeptides, dipeptides and amino acids at the site of absorption. Absorption of tripeptides, dipeptides and amino acids occurs along the entire small intestine and is accomplished by several transport mechanisms in the enterocytes (Friedrich 1989, Webb 1990). Most tri- and dipeptides absorbed by the enterocyte are hydrolysed in the cell (Scharrer and Wolffram 1987, Hunt and Groff 1990) and together with the absorbed amino acids can be metabolised in the enterocytes or released into the hepatic portal vein. Unabsorbed proteins, peptides and amino acids of dietary and endogenous origin at the end of the small intestine enter the large intestine where they can be metabolised by the microflora of the large intestine. No significant absorption into the portal blood of peptides or amino acids occurs in the large intestine (Meyer, 1990, Schmitz *et al.* 1991, Darragh *et al.* 1994).

The gastrointestinal tract is actively involved in the metabolism of dietary and endogenous amino acids. The amino acids glutamine, glutamate, asparagine and aspartate can be deaminated by the intestine. Glutamine is required by rapidly-dividing cells (Krebs 1980) where it donates the amide nitrogen for the synthesis of purine nucleotides. Aspartate is also used in the formation of purines and in the formation of pyrimidine nucleotides (Newsholme and Leech 1983). Excess ammonia generated by the deamination of these amino acids is either absorbed as such into the portal vein or converted to alanine for transportation through the hepatic portal vein to the liver where the ammonia is processed in the urea-cycle (Newsholme and Leech 1983, Crim and Munro 1984). The intestine also synthesises citrulline and proline from glutamine (Newsholme and Leech 1983, Morris and Rogers 1982, Morris 1992).

1.2.6 The role of other body tissues

Tryptophan, tyrosine and glutamate are important precursors for the neurotransmitters serotonin, dopamine, noradrenaline, adrenaline and γ-aminobutyric acid in neurons. The amino acids glutamate, aspartate and glycine have been shown to act as neurotransmitters in the brain (Newsholme and Leech 1983, Crim and Munro 1984, Hunt and Groff 1990). Inactivation of the latter three neurotransmitters frequently involves the deamination of these amino acids with the ammonia released upon deamination being converted to glutamine and released in the blood for metabolism by other tissues (Newsholme and Leech 1983).

The amino acid metabolism of adipose tissue may be similar to that of muscle tissue (Newsholme and Leech 1893, Crim and Munro 1984). It has been shown that adipose tissue is capable of metabolising the branched-chain amino acid leucine with the subsequent release of glutamine and alanine (Tischler and Goldberg 1980).

1.3 FELINE PROTEIN METABOLISM - AN OVERVIEW

The domestication of the cat (*Felis catus*) may have been as early as 3000 BC, although the evidence has been described as ambiguous (Zeuner 1963). In any case, the cat was clearly domesticated around 1600 BC by the Egyptians who regarded the cat as a sacred animal. The Egyptians collected and kept various recognised species of cats among which were the jungle cat (*Felis chaus*) and the African wild cat (*Felis libyca*). It has been suggested that the present-day breeds of domestic cats are descendants of the

European wild cat (*Felis silvestris*) and the African wild cat (*Felis libyca*) (Robinson 1971, Clutton-Brock 1981, MacDonald *et al.* 1984).

The domestic cat (*Felis catus*) is a member of the Felidae family which consists of 37 species divided over four genera (*Felis*, *Panthera*, *Neofelis*, *Acinonyx*). The members of this family are flesh-eaters i.e. strict-carnivores, unlike other members of the order Carnivora like the Canidae (dogs), Ursidae (bears) and Procyonidae (racoons) (MacDonald *et al.* 1984, Morris 1985). It is this strictly-carnivorous diet that is believed to be the cause of several specialisations in the cat's metabolism (Morris and Rogers 1982, MacDonald *et al.* 1984, Halle and Gebhardt 1990, Lowe and Markwell 1995). Examples of these unique features, some of which will be discussed more fully later, are the inability of the cat to: regulate catabolic enzymes of amino acid metabolism, synthesise niacin from tryptophan, convert carotene to vitamin A, convert sufficient linoleic acid to arachidonic acid, synthesise several urea cycle intermediates in sufficient quantity, convert sufficient cysteine to taurine and to use the alternative glycine for bile acid conjugation instead of taurine. All the nutrients mentioned above, which the cat cannot synthesise, can be found in the diet of a carnivore. Another aspect of the peculiar metabolism of the domestic cat is that this animal and several other members of the Felidae family excrete several unique sulphur-containing amino acids in their urine of which the function(s), at present, are largely unknown.

1.3.1. Feline development

Adult body weights of members of the Felidae family range from 1.5 - 280 kg (Parker 1990). The body weight range among adult domestic cats (*Felis catus*), however, is much smaller and is in the order of 2 - 6 kg. This is unlike domestic dogs (*Canis familiaris*) which have an adult body weight range of 1 kg (Chihuahua) up to 50 kg (Great Dane and Newfoundlander).

The kitten, like the puppy, is blind and deaf at birth and is covered with hair. The hair coat at birth is important in maintaining the kitten's body temperature as the new-born kitten has a small amount of subcutaneous fat (Widdowson 1965). Kittens are relatively immature at birth in comparison to puppies and babies but more mature than pigs as can be seen from Table 2. The kitten is born at a metabolic age which is higher than the puppy, piglet and rat pup but about half the age of the human baby. The piglet is very immature at birth in comparison to the other four species. Human babies on the other hand are born at a relatively old age in metabolic terms and are

relatively mature at parturition.

The protein content of the mother's milk varies among the five species with rat's milk containing the highest amount of protein and human milk the lowest. A higher percentage of protein in the milk, however, does not seem to coincide with higher metabolic growth rates of the young (Table 2). The piglet has the highest growth rate per unit of metabolic weight while pig's milk has a relatively low protein content in comparison to the other species. The kitten in this respect has a similar growth rate as that of the dog and rat. Human infants, although mature at birth grow at a relatively slow rate.

TABLE 2
Developmental data for different animal species.[a]

	Species				
	Man	Pig	Dog	Cat	Rat
Birth					
Weight (kg)	3.400	1.340	0.310	0.114	0.006
Metabolic age[b] (days)	87.2	26.2	32.5	42.1	22.2
Maturity[c] (%)	4.86	0.67	3.10	3.17	1.31
Milk protein content (%)	1.1	5.9	7.1	9.5	12.0
Daily gain during suckling period					
(g/day)	30.0	180.0	36.8	12.4	1.0
(% of body weight)	0.5	3.1	3.1	3.1	5.9
($g/kg^{0.83}$/ day)	7.1	41.8	31.5	26.2	29.4
Average mature weight (kg)	70.0	200.0	10.0	3.6	0.4
Average life span (years)	70	16	13-17	13-17	2-3
Average metabolic life span[b] (years)	22.2	3.5	8.0	10.6	3.2

[a]Data calculated or adapted from Altman and Dittmer (1962), Widdowson (1965), Oftedal (1986), Taylor (1980) and Loveridge (1987).
[b]Metabolic age=$(t-3.5)/A^{0.27}$ where t=time after conception, A=mature body weight (Taylor 1980).
[c]Maturity = (immature weight/mature weight)*100 (Taylor 1980).

There is a large variation in the average metabolic life span of the five species with the cat having a long metabolic life span in comparison to the pig, dog and rat but short in comparison to man.

It can be concluded that the cat is born at an average metabolic age and stage of maturity compared to other mammals. Although the protein content of cat milk is high in comparison to other mammals, the growth rate of kittens during the suckling period is similar to other mammals. The metabolic life span of cats is high in comparison to the dog, pig, rat but low in comparison to man.

1.3.2 Peculiarities of feline protein metabolism

Over the past 50 years, the amino acids deemed to be essential for growth of various animal species, ranging from protozoa to man, have been determined (Meister 1965). There is great uniformity across animal species as to the amino acids which can and cannot be synthesised by the animal. The cat, in this respect, is no different from other animals in that the same 10 α-amino acids (Table 1) are essential in its diet (Van den Oord and Lafeber 1963, Rogers and Morris 1979). In addition to these 10 α-amino acids, the β-sulphonic amino acid, taurine can also be regarded as essential for the cat because it has a critical role in preventing central retinal degeneration (Rogers and Morris 1983, MacDonald *et al.* 1984, Baker and Czarnecki-Maulden 1991). The following sections will discuss some of the peculiarities of feline protein metabolism.

TABLE 3
Minimum dietary crude protein requirements for growing and adult animals of several mammalian species.[a]

Species	Growing	Adult	Reference
	(g/kg dry matter)		
Man	80	50	NRC (1980)
Dog	143	80	NRC (1985)
Rat	167	58	NRC (1978)
Cat	240	140	NRC (1986)
Fox	240	160	NRC (1981)
Mink	310	200	NRC (1981)

[a]Ideal protein, meeting all the known essential amino acid requirements except for the growing fox and mink, and the adult cat, fox and mink. All diets contain 20.9 MJ ME/kg dry matter.

1.3.2.1 Inability to regulate amino acid catabolic enzymes

The protein requirement of cats has been noted by many researchers to be considerably higher than that for other non strictly-carnivorous and omnivorous animals such as the dog, rat and man (Scott 1960, Scott 1981, Rogers and Morris 1983, Burger *et al.* 1984, MacDonald *et al.* 1984, Halle and Gebhardt 1990). The minimum crude protein requirements for several growing and adult mammals are shown in Table 3. It can be seen that the protein requirements, expressed as a percentage of the diet, for the strict-carnivores (cat, mink and fox) are between 1.5 to 4.0 times higher for growth and for maintenance than those for the omnivores (man and rat) and the omnivore/carnivore, the dog.

The reason for the higher protein requirement of the cat has been attributed to the

inability of this animal to regulate amino acid metabolising enzymes in the liver. When most animals, including dogs, rats and humans, ingest a diet high in protein, the activities of the amino acid catabolising enzymes in the liver increase to cope with the higher flux of amino acids. The activity of the urea cycle enzymes also increases to metabolise the increased ammonia generated upon the catabolism of the amino acids (Allison 1955, Munro 1970b, Schimke 1970, Das and Waterlow 1974). In a classic study, Rogers *et al.* (1977) compared the activity of three urea cycle enzymes, several nitrogen catabolic enzymes, and gluconeogenic and lipogenic enzymes in adult cats fed either a high protein diet, a low protein diet or starved for five days. There was little change in the activities of the nitrogen catabolic enzymes and the enzymes of the urea cycle between cats receiving the high protein diet, low protein diet or cats starved for five days. The authors compared the hepatic enzyme activities of the cats with literature values for the rat and concluded that the activity of the hepatic enzymes of the cat are set at such a level to cope with a high protein diet. Rowsell *et al.* (1979) subsequently confirmed some of the findings of Rogers *et al.* (1977) while Fau *et al.* (1987) showed that the adaptational response to an excess dietary intake of methionine exists in cats but that it is more limited than that found in rats.

These studies show that the cat has a limited ability to adapt hepatic enzymes to dietary changes. The non-adaptive nitrogen metabolising enzymes in the liver of the cat, furthermore, seem to be permanently set to handle a high protein diet which results in a high obligatory nitrogen loss even when cats are fed low protein diets (Morris and Rogers 1986).

1.3.2.2 Arginine metabolism

Several years ago, Morris and Rogers (1978a,b) demonstrated that cats developed severe hyperammonaemia and died within several hours after the ingestion of an arginine-free diet. The inclusion of ornithine in the diet protected the cat from hyperammonaemia but did not allow growth. The addition of citrulline to the arginine-free diet prevented hyperammonaemia and allowed normal growth of kittens (Morris *et al.* 1979).

The cause for the hyperammonaemia when an arginine-free diet is fed to cats can be found in the metabolism of the urea cycle intermediates. In other mammals, arginine is synthesised in the kidney from citrulline which is formed in the small intestine. For the synthesis of citrulline, the small intestine takes up large amounts of glutamine

which is converted to ornithine and subsequently to citrulline, involving several enzymatic reactions (Morris and Rogers 1982, MacDonald *et al.* 1984, Baker and Czarnecki-Maulden 1991, Morris 1992). Rogers and Phang (1985) and Morris (1985) showed that the enzymes, pyrroline-5-carboxylate and ornithine aminotransferase, which are involved in the synthesis of ornithine and citrulline in the intestine, have a very low activity in the cat in comparison to the rat. The cat, therefore, is unable to produce sufficient ornithine and citrulline to synthesise arginine and to maintain the urea cycle when an arginine-free diet is fed. As the catabolism of amino acids proceeds on an arginine-free diet, the ammonia produced cannot be converted to urea resulting in hyperammonaemia (Morris and Rogers 1978a,b). Addition of either ornithine or citrulline to an arginine-free diet will prevent hyperammonaemia because intermediates are provided to maintain the urea cycle which will process the toxic ammonia to urea. The reason that citrulline supplementation of an arginine-free diet supports normal growth and ornithine supplementation does not, is a result of the low activity of one of the two enzymes, ornithine aminotransferase, which is necessary for the conversion of ornithine to citrulline. Citrulline is readily converted to arginine in the kidney of the cat but because of the low activity of ornithine aminotransferase in the intestine of the cat not enough citrulline reaches the kidney when an arginine-free diet supplemented with ornithine is fed. When citrulline is added to an arginine-free diet, however, the kidney can synthesise arginine to allow comparable growth to cats fed a complete protein diet (Morris and Rogers 1982, MacDonald *et al.* 1984, Baker and Czarnecki-Maulden 1991).

A reason for the unique arginine metabolism of the cat as compared to other mammals has been given by MacDonald *et al.* (1984). They argued that because the cat has had a relatively constant diet consisting of animal protein throughout its evolution, there has been no need to synthesise intermediates of the urea cycle. Rather, there has been a need, in the cat, to use amino acids for gluconeogenesis as animal tissues are relatively low in carbohydrates. Thus, the metabolic need is not to make ornithine or citrulline when nitrogen intake is high because dietary arginine would provide the amino acids needed in the urea cycle as well as arginine needed for protein synthesis. The need, rather, is to deplete ornithine and citrulline during the post-absorptive state so that urea synthesis can be reduced to prevent excessive catabolism of amino acids. In omnivores, the need to regulate urea synthesis is greater because there are times when the animal ingests a low protein diet and has to adapt to conserve nitrogen.

During starvation or protein-free feeding of cats, endogenous arginine will supply intermediates for the urea cycle and, therefore, no hyperammonaemia will occur under these conditions. Work done on the mink (Leoschke and Elvehjem 1959) and the ferret (Deshmukh and Shope 1983) indicates that this is a metabolic adaptation consistent with strictly-carnivorous mammals.

1.3.2.3 Taurine metabolism

Taurine, 2-amino ethanesulphonic acid, is an amino acid regarded as an essential nutrient for the cat (Knopf *et al.* 1978, Morris *et al.* 1990). Taurine deficiency in cats has been shown to cause clinical blindness with central retinal degeneration (Hayes *et al.* 1975a,b), cardiomyopathy (Pion *et al.* 1987), platelet hyperaggregability (Pronczuk *et al.* 1988), and impaired neutrophil function (Schuller-Levis and Sturman 1988). Essentiality of taurine in the diet of the cat arises from differences in the rate of synthesis and the rate of loss of taurine from the body (Knopf *et al.* 1978, Burger and Barnett 1982, Morris *et al.* 1990).

Taurine is normally made from cysteine in the liver of mammals and involves the enzymes cysteine dioxygenase and cysteine sulphinic acid decarboxylase (Hayes and Sturman 1981, Hayes 1988, Morris *et al.* 1990). In man, monkeys and cats, the activity of the enzyme cysteine sulphinic acid decarboxylase, is low in comparison to dogs and rats (Hayes and Sturman 1981, Worden and Stipanuk 1985). The low activity of this enzyme has been suggested to limit synthesis of taurine from cysteine in cats (Knopf *et al.* 1979, Worden and Stipanuk 1985, Hayes 1988, Baker and Czarnecki-Maulden 1991). Morris *et al.* (1990) suggested that the low activity of enzymes may not be the only explanation for the low synthesis of taurine in the cat but also high fluxes involving cysteine along alternate metabolic pathways. Recently, cysteic acid has been found to be an excellent precursor of taurine (Edgar *et al.* 1991, Edgar *et al.* 1994) indicating that the enzyme cysteine sulphinic acid decarboxylase does not seem to limit taurine synthesis from cysteic acid since this enzyme is responsible for the conversion of cysteic acid to taurine. This would indicate that the other enzyme responsible for the conversion of cysteine sulphinic acid to cysteic acid, cysteine dioxygenase, limits taurine synthesis in the cat.

Besides the low rate of synthesis of taurine, the cat loses large amounts of taurine from its body. Cats have a unique bile acid metabolism whereby conjugation of bile acids occurs predominantly with taurine (Rabin *et al.* 1976) unlike most mammals,

such as man, in which conjugation occurs with both glycine and taurine, or rabbits which conjugate all of their bile acids with glycine (Hayes 1988). When taurine becomes limiting, the cat is unable to spare taurine by conjugating bile acids with glycine, as other mammals. The obligatory use of taurine to produce bile acids results in a continuous loss of taurine from the body of the cat which is proportional to the fraction of bile acids not re-absorbed by the intestine. Together with the loss of taurine in the urine, these losses exceed the rate of synthesis of taurine, resulting in a negative taurine balance (Hayes and Sturman 1981, Hickman *et al.* 1992). From an evolutionary point of view, the cat has had no need to synthesise or conserve taurine, as animal tissues are rich in taurine. Apparently the cat has made adaptations in its metabolism of taurine according to the availability of taurine.

Recently, it has been shown that the type of diet influences the taurine status of the cat. Douglas *et al.* (1991) and Earle and Smith (1991a) have shown that to maintain the same blood taurine levels, cats fed canned moist foods require a higher concentration of taurine in their diet than cats fed dry cat foods. Hickman *et al.* (1992) showed that heat-processing of a canned cat food will result in a greater loss of taurine from the intestine than the same food but unprocessed. Baker and Czarnecki-Maulden (1991), citing unpublished work by Wright and Sturman, reported that an inhibitor of taurine uptake was formed during heat-processing and that this inhibitor may reduce taurine absorption in the intestine, affect reabsorption of bile acids and/or affect reabsorption in the renal tubule. Hickman *et al.* (1992) and Kim *et al.* (1995) showed that dietary protein source (soya bean vs. casein) affects taurine balance in cats. Kirk *et al.* (1994) measured the release of cholecystokinin (CCK), a hormone stimulating gallbladder contraction, in the plasma of cats fed different types of diets and showed that the type of diet influences the secretion of CCK with the known taurine-depleting diets causing a higher secretion of CCK. These latter results were confirmed by Backus *et al.* (1995) with these authors concluding that dietary proteins and possibly other dietary factors increase the secretion of CCK by interfering with the feedback inhibition of CCK release, thereby increasing the loss of taurine through an increased loss of bile acids. From these studies it seems that some diets will deplete cats of taurine by increasing bile acid excretion in conjunction with a possible reduced reabsorption of bile acids. The increased deconjugation of bile acids found in the large intestine of cats fed taurine-depleting diets (Anantharaman-Barr *et al.* 1994, Backus *et al.* 1994), is in accordance with this view.

1.3.2.4 Tryptophan metabolism

Most mammals derive their niacin (nicotinic acid) requirements from metabolism of the essential amino acid tryptophan. The cat is unusual in this respect in that tryptophan cannot replace nicotinic acid in the diet and cats die within 20 days when fed diets high in tryptophan but lacking in nicotinic acid (Da Silva *et al.* 1952).

There are four pathways by which tryptophan is metabolised in mammals (Mercer and Silva 1989). The oxidation of tryptophan by the kynurenine-glutarate pathway in the liver is quantitatively the most important accounting for approximately 99 % of whole body tryptophan metabolism (Bender 1982, Mercer and Silva 1989). Among the end products of the kynurenine-glutarate pathway are nicotinic acid and glutarate. The cat possesses all the enzymes involved in the kynurenine-glutarate pathway (Morris and Rogers 1982, Mercer and Silva 1989) and on this basis it would be anticipated that the cat could utilise tryptophan for niacin synthesis. However, the activity of the first unique enzyme in the formation of glutarate, picolinate carboxylase, is 30 to 50 times greater in the liver of the cat than the rat (Suhadolnik *et al.* 1957, Ikeda *et al.* 1965). The inability of the cat to utilise tryptophan for synthesis of nicotinic acid, therefore, is not due to a deficiency in one of the enzymes involved in the synthesis of nicotinic acid but rather is due to a more active alternative metabolic pathway.

The cat would not normally be in a position where niacin would be limited, as animal tissues are relatively high in niacin. Animal tissues in comparison to plant tissues are also high in tryptophan. The high activity of the alternative pathway of tryptophan metabolism (to glutarate) in the cat results in a very low excretion of the metabolites of this amino acid in this animal's urine (Da Silva *et al.* 1952, Leklem *et al.* 1969, Mercer and Silva 1989) and prevents tryptophan and its intermediates from reaching toxic levels in the body of the cat (Morris and Rogers 1982). The alternative metabolism of tryptophan in the cat, as compared to other mammals, may be the reason for the low incidence of bladder cancer in cats as metabolites of tryptophan have carcinogenic properties (Mercer and Silva 1989, Morris and Rogers 1989).

1.3.2.5 Urinary excretion of unusual sulphur-containing amino acids.

Several unusual sulphur-containing amino acids have been isolated from cat urine. Datta and Harris (1951) found an unknown amino acid in cat (*Felis catus*) and ocelot (*Felis pardalis*) urine which was later isolated, characterised and named felinine by

Westall (1953). Another amino acid, isovalthine, was discovered and isolated from cat urine by Mizuhara and Oomori (1961). The latter authors also found a sulphur-containing amino acid in the urine of cats which they named isobuteine (Oomori and Mizuhara 1962). The structures of these three amino acids are similar with all three possessing a cysteine moiety and being branch-chained (Fig. 3).

FELININE ISOVALTHINE ISOBUTEINE

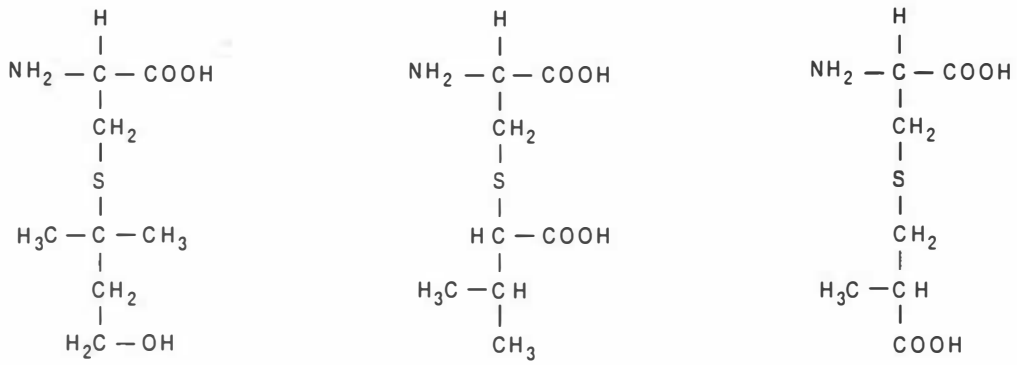

Figure 3. Molecular structure of felinine (Westall 1953), isovalthine (Mizuhara and Oomori 1961) and isobuteine (Oomori and Mizuhara 1963).

The concentration of felinine in the urine of the cat has been reported to be extremely high. Westall (1953) found a concentration of approximately 1.0 g of felinine/L in the urine of a male cat while Shapiro (1963) reported a felinine concentration of 1.7 and 1.0 g/L in the urine of a male and female cat, respectively. An even higher concentration of 1.9 g/L urine was recorded by Tallan *et al.* (1954) for a male cat while Avizonis and Wriston (1959) reported a urinary felinine excretion of 8.0 g/L in a cat fed a cystine enriched diet for three days. It must be noted, however, that these values should be interpreted with some caution as the methods for detection of felinine (paper chromatography and ion-exchange chromatography) used in all the above studies may not have allowed accurate quantification of felinine. Based on these concentrations MacDonald *et al.* (1984) concluded that the dietary sulphur amino acids required for synthesis of felinine in adult cats is in the order of 50 to 75 mg (0.05 % of the diet). The sulphur amino acid requirements of adult cats are reported to be 0.3 % of the diet (Burger and Smith 1987, NRC 1986) which indicates that 17 % of the total daily sulphur amino acids are required for the synthesis of felinine. To the knowledge

of the author, there have been no articles published presenting concentrations of isovalthine or isobuteine in the urine of the cat.

Isotopic incorporation methods have shown that mevalonic acid, leucine and acetate can serve as precursors to felinine (Avizonis and Wriston 1959, Shapiro 1962, Wang 1964). Although cysteine has been found to be a precursor to felinine by Avizonis and Wriston (1959) and Shapiro (1962), Roberts (1963) was unable to incorporate radioactively labelled cysteine and methionine into felinine. Based on isotopic incorporation studies, felinine is believed to be synthesised from the same isoprenoid unit as cholesterol and cysteine (Eggerer 1962, Shapiro 1962, Wang 1964, Borchers *et al.* 1967). Little work has been done on the biosynthesis of isovalthine and isobuteine. Leucine, however, has been shown to increase the excretion of isovalthine in the urine of cats (Fukutome 1961) which indicates that this amino acid may have a similar biosynthesis to felinine. Kuwaki *et al.* (1963) provided evidence that isovalthine can be formed in the liver in the form of a glutathione-isovaleric acid conjugate (GSIV). The same authors hypothesised that urinary isovalthine may be formed from GSIV by the liberation of isovalthine involving kidney glutathionase.

The role of felinine, isovalthine and isobuteine is still a matter for speculation. It has been suggested that felinine may be a urinary component for territorial marking or perhaps involved in the regulation of sterol metabolism (Shapiro 1962, Roberts 1963, MacDonald *et al.* 1984). The former role of felinine seems likely as male cats have been reported to have higher urinary felinine concentrations than female cats (Roberts 1963). Oomori and Mizuhara (1962) noted that isovalthine may have a yet unknown role in cholesterol metabolism.

1.3.3 Protein requirements of growing and adult cats

Animals require amino acids for maintenance and growth of all tissues in the body as well as for precursors for the synthesis of a variety of nitrogen-containing organic compounds such as creatine, purines, pyrimidines, serotonin, etc. As there is no storage of amino acids in the body (Christensen 1964), amino acids supplied above the requirement of the animal are catabolised and the nitrogen is excreted mainly as urea.

The protein requirement of the cat has been determined by various researchers over the past 40 years (Table 4). As can be seen from Table 4, the estimates of the protein requirement for growing cats have progressively decreased over time with the current requirement being 24 % of the diet when a high quality protein is given (NRC

1986). In earlier studies, requirements for individual amino acids were unknown. Therefore, the protein in these diets would only meet the requirement for the first limiting amino acid at a high dietary protein level. Miller and Allison (1958) and Jansen *et al.* (1975) used casein as the sole source of protein in their studies. It can be calculated now, that a dietary casein level of 28 % and 24 % would only supply the minimum amounts of arginine and sulphur amino acids, respectively, needed by growing cats. Smalley *et al.* (1985) fed growing cats either amino acid-based diets or casein-based diets supplemented with essential amino acids to levels exceeding the essential amino acid requirements for the growing cat. They concluded that a minimum dietary protein level of 20 % was required to maximise both weight gain and nitrogen retention. The National Research Council (NRC 1986), however, recommends a minimum dietary protein level of 24 % for growing cats when a high quality protein source is used.

TABLE 4
Estimates of the protein requirements for the growing and adult cat.

Reference	% of diet	% of dietary energy	Response criterion	Protein Source
Growing cat				
Dickinson and Scott (1956)	>37	30	Weight gain	Fish and liver
Miller and Allison (1958)	>25	20	Weight gain	Casein and liver powder
Greaves (1965)	32	29	Weight gain	Fish and liver
Jansen *et al.* (1975)	35	28	Weight gain Carcass N	Casein
Smalley *et al.* (1985)	20	16	Weight gain N-balance	Casein and amino acids
NRC (1986)	24	19		High quality protein
Adult cats				
Allison *et al.* (1956)	16	13	N-balance	Casein and liver powder
Greaves and Scott (1960)	21	19	N-balance	Fish and liver
Burger *et al.* (1984)	13	10	N-balance	Soya protein and amino acids
NRC (1986)	14	11		High quality protein

Adapted from Schaeffer *et al.* (1989)

Estimates of dietary protein requirements for adult cats have varied over the last 40 years. The first estimate of the protein requirement for adult cats (Allison *et al.* 1956) was close to the current estimate of the National Research Council (NRC 1986) (Table 4). Greaves and Scott (1960) found a protein requirement for adult cats which was several percent higher than the current estimate. The same argument applies, as for

the earlier studies involving growing cats. The individual amino acid requirements were, and still are, largely unknown for adult cats and this estimate reflects the requirement for the first-limiting amino acid in the protein. Allison *et al.* (1956), however, determined the endogenous nitrogen loss of adult cats by feeding the animals a protein-free diet and measuring faecal and urinary nitrogen output for four days. The estimate obtained by these authors is, therefore, independent of dietary protein and accounts for the lower value found in comparison to Greaves and Scott (1960). The estimate of 13 % by Burger *et al.* (1984) was determined using nitrogen balance in cats fed soya protein supplemented with amino acids sufficient to meet all the amino acid requirements of growing kittens. It is doubtful that any high quality protein included in the diet at 13 % would maintain cats as it is unlikely to find a protein source supplying the amino acid profile used by Burger *et al.* (1984). The NRC (1986), therefore, recommended a minimum dietary protein level for adult cats of 14 %.

As can be seen from Table 4, all the estimates of protein requirement for adult cats have been derived using the criterion of nitrogen balance, while many of the estimates for growing cats have relied on weight gain as the sole criterion on which the optimal inclusion of protein in the diet was determined. Estimates of minimum protein requirements using N-balance (except Allison *et al.* 1956) were determined by interpolation of a linear regression line to zero N-balance of the N-balance data of animals fed different levels of dietary protein. Nitrogen balance in these studies was determined by subtracting the losses of nitrogen in the faeces and urine from the dietary nitrogen intake. All the N-balance studies on adult cats including that of Allison *et al.* (1956) have, therefore, not taken into account the nitrogen required for the growth of hair, thereby underestimating the minimum protein requirements.

1.3.4 Amino acid requirements of growing and adult cats

The same ten amino acids deemed to be essential in the diet of other growing mammals are also found to be indispensable in the diet for growing and adult cats. Of these, arginine, which is only semi-essential for several growing mammals, is essential for growing and adult cats as it holds a critical role in preventing hyperammonaemia. The requirement for an essential amino acid in cats has generally been determined by the minimum dietary concentration that gives a specific desired response in the animal. In growing cats, weight gain and N-balance have been the response variables most often used to determine the requirements for essential amino acids. The dietary requirements

for the only two essential amino acids (lysine and methionine) in adult cats, have been determined by the N-balance technique. Weight gain and N-balance have been optimised in studies involving growing cats while zero N-balance has been used in adult cats as the criterion for response. Other response variables used for growing cats have been haematocrit and haemoglobin concentration in the blood (Quam *et al.* 1987) and orotate and citrate concentrations in the urine (Costello *et al.* 1980).

TABLE 5
Essential amino acid requirements for the growing and adult cat, dog and rat.

Amino acid	Growing			Adult		
	Cat[a]	Dog[b]	Rat[c]	Cat[d]	Dog[b]	Rat[c]
			($mg/kg^{0.75}$/day)			
Arginine	500	487	341	-	28	NE
Histidine	150	174	170	-	29	22
Isoleucine	250	349	284	-	63	87
Leucine	600	566	397	-	111	50
Lysine	400	498	397	63	66	31
Methionine+Cysteine	375	377	341	59	40	64
Phenylalanine+Tyrosine	425	694	454	-	113	53
Taurine	20	NE	NE	22	NE	NE
Threonine	350	452	284	-	58	50
Tryptophan	75	146	85	-	17	14
Valine	300	373	341	-	79	64
			($g/kg^{0.75}$/day)			
Total essential amino acids[e]	3.43	4.12	3.09	-	0.60	0.44

[a]NRC (1986) for a 1 kg growing cat.
[b]NRC (1985) for a 3 kg growing or 10 kg adult dog.
[c]NRC (1978).
[d]NRC (1986) and Burger and Smith (1987) for a 4 kg adult cat.
[e]Free base.
NE, not essential.
-, not determined.

During the past 15 years the essential amino acid requirements of the growing cat have been determined, and the National Research Council (NRC 1986) currently recommends minimum dietary levels for the essential amino acids. At present, the requirement for two essential amino acids (lysine and methionine) has been determined for adult cats (Burger and Smith 1987). Comparison of the minimum essential amino acid requirements for growing cats, dogs and rats (Table 5) shows that there are no major differences in the requirements when expressed on a per unit metabolic body weight basis. The growing dog, in general, appears to have the highest requirement for

essential amino acids followed by the growing cat and rat. Arginine and leucine requirements, however, are higher for the growing cat followed by the growing dog and rat although the differences between the three species are small. The essential amino acid requirements for maintenance between dogs and rats are also similar. The adult dog, however, requires approximately twice the amount of leucine, lysine and aromatic amino acids per unit metabolic body weight in comparison to the adult rat while the adult rat requires approximately twice the amount of sulphur amino acids in comparison to the adult dog. The requirements of adult cats for lysine and total sulphur amino acids are comparable to those for the adult dog and rat.

TABLE 6

Protein and total essential amino acid requirements for the growing and adult cat, dog and rat.

Amino acid	Growing			Adult		
	Cat[a]	Dog[b]	Rat[c]	Cat[a]	Dog[b]	Rat[c]
	($g/kg^{0.75}$/day)					
Protein	12.00	7.57	8.41	2.77	1.98	1.40
Total essential amino acids						
Free base	3.43	4.11	3.09	(0.56)	0.60	0.44
Protein-bound	2.98	3.59	2.69	(0.49)	0.52	0.38
Difference[d]	9.02	3.98	5.71	(2.28)	1.46	1.02

[a]NRC (1986) for a 1 kg growing or 4 kg adult cat. Values between brackets are calculated assuming the essential amino acid requirements are 42 % of the growing cat value as suggested by Burger and Smith (1987).
[b]NRC (1985) for a 3 kg growing or 10 kg adult dog.
[c]NRC (1978).
[d]Protein minus total protein-bound essential amino acids.

The total amount of essential amino acids per unit metabolic body weight which needs to be ingested by the growing dog appears to be higher than that for the growing cat which in turn is higher than that for the growing rat (Table 5). Again, the differences between the three species are small. As discussed previously, the protein requirement of the strictly-carnivorous cat is higher than that for the omnivores (man and rat) and the omnivore/carnivore, the dog. Table 6 presents the protein and essential amino acid requirements for growing and adult cats, dogs and rats. The values between brackets for adult cats are estimates, calculated on the basis that the other essential amino acids are approximately 42 % of the corresponding value for growing cats, like lysine and the total sulphur amino acids (Burger and Smith 1987). Subtracting the amount of protein-bound essential amino acids from the minimum protein

requirements as listed by the NRC (1986) for the three species, will give the amount of protein-bound amino acids which needs to be ingested by the animal when the essential amino acid requirements are met. This amount is often supplied by non-essential amino acids although essential amino acids can be included as they are often used for the synthesis of dispensable amino acids, other compounds containing nitrogen and to supply energy (Rogers and Morris 1983, NRC 1986). In the following discussion this fraction will be referred to as "non-essential amino acid nitrogen". It can be seen from Table 6 that the growing cat requires approximately 2.3 times the amount of non-essential amino acid nitrogen compared to the growing dog and 1.6 times the amount compared to the growing rat. The adult cat requires approximately 1.6 times more non-essential amino acid nitrogen per unit metabolic body weight compared to adult dogs and 2.2 times the amount compared to the adult rat.

The reason for the higher requirement for non-essential amino acid nitrogen is related to the non-adaptive nitrogen metabolising enzymes in the liver of the cat which are permanently set to handle a high protein diet (Rogers *et al.* 1977). The activities of the hepatic enzymes concerned with the handling of nitrogen from general amino acid catabolism and the urea cycle enzymes in the cat, have similar or greater activities than those in the liver of rats fed a high protein diet. Enzymes associated with the metabolism of essential amino acids (threonine dehydratase, histidase and tyrosine aminotransferase), have lower activities in comparison to the values for rats fed a high protein diet (Morris and Rogers 1986). It must be noted that the data on the activities of liver enzymes in rats fed high protein diets were taken from published values and that Rogers *et al.* (1977) did not compare the activity of liver enzymes of the cat and rat directly. Nevertheless, the activities of the hepatic enzymes in the liver of the cat do indicate that the cat is able to conserve individual essential amino acids but not dispensable amino acids. This is consistent with the observations (Table 6) that the cat has a comparable requirement for essential amino acids to the dog and the rat but that the cat requires more non-essential amino acid nitrogen.

In the diet of a strict carnivore only a small portion of the energy is present in the form of carbohydrates. To fulfil the obligatory glucose needs of the body considerable glucose would have to be produced by gluconeogenesis. Rogers *et al.* (1977) showed that the activity of several gluconeogenic enzymes in the liver of the cat were higher than in rats fed a high protein diet and that the activity of these enzymes is not influenced by dietary protein level. These authors also showed that the activity of

lipogenic enzymes in the liver of the cat are low in comparison to rats indicating a limited *de novo* synthesis of fatty acids. The activity of the lipogenic enzymes in the liver of the cat, furthermore, were unchanged by varying dietary protein level. All of these results indicate that the cat has a large capacity to catabolise non-essential amino acids and that the functional fate of the amino acids is directed towards synthesis of glucose and not towards fatty acid synthesis. Moreover, gluconeogenesis seems to be permanently "switched on" as indicated by the non-adaptive gluconeogenic enzymes (Rogers *et al.* 1977, Mercer and Silva 1987, Morris and Rogers 1991).

1.3.5 Energy requirements of growing and adult cats

The cat like other animals requires energy for maintenance processes and synthesis of protein, fat and carbohydrates. Several estimates of energy requirements of the growing cat at different ages have been made (Miller and Allison 1958, Waterhouse and Carver 1962, Greaves 1965, Munday and Earle 1991, Legrand-Defretin and Munday 1993) and are presented in Figure 4. As can be seen from Figure 4, the energy requirements of the growing cat decreases curvilinearly with increasing age.

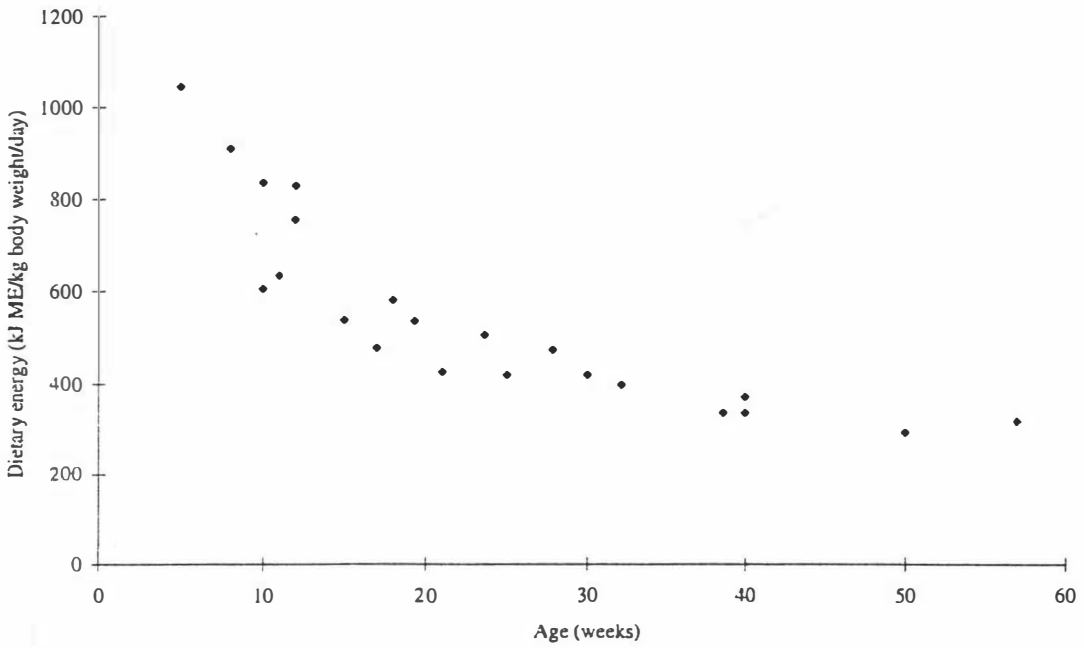

Figure 4. Estimates of the energy requirement of the growing cat (Data from Miller and Allison 1958, Waterhouse and Carver 1962, Greaves 1965, Munday and Earle 1991, Legrand-Defretin and Munday 1993).

Measurement of energy requirements of adult cats by heat production (direct calorimetry) and gaseous exchange (indirect calorimetry) has been discussed by MacDonald *et al.* (1984). These authors concluded that the adult cat requires approximately 364 kJ metabolisable energy (ME)/kg body weight/day for maintenance. Estimates of the energy requirements of adult cats have also been made based on the measurement of food intake. The NRC (1986) reviewed much of the earlier literature and concluded that inactive adult cats have a daily ME requirement of approximately 293 kJ/kg body weight and an active adult cat 334 kJ/kg body weight. Recently, however, Earle and Smith (1991b) measured the digestible energy intake of 259 adult cats of various body weights (2.5 to 6.5 kg) and found that for body weight maintenance, adult cats require a digestible energy intake of less than 293 kJ/kg body weight. The authors, furthermore, found that the digestible energy intake for body weight maintenance decreased as cats got heavier, with cats in the weight range of 2.5 to 3.0 kg needing 278 kJ DE/kg body weight/day and the heavier cats (6.0 to 6.5 kg) requiring 162 kJ DE/kg body weight/day to maintain body weight.

As a strict-carnivore, the cat has evolved on a high protein, high fat and low carbohydrate diet. To meet the energy needs of the body, the cat would be expected to have a limited ability to use carbohydrates as an energy source and to satisfies its energy needs by gluconeogenesis from amino acids and fat. The previous section has discussed some aspects of gluconeogenesis from protein and fat in the cat.

Morris *et al.* (1977) found high apparent faecal digestibility coefficients for raw corn starch (88 %), lactose (87 %) and glucose (91 %) while Kienzle (1993) found apparent faecal digestibility coefficients of nearly 100 % for glucose, lactose and sucrose in the cat. These results indicate that carbohydrates are well digested and absorbed into the body of the cat. In the body, the enzymes glucokinase and hexokinase which catalyse the first reaction of glycolysis by converting glucose to glucose-6-phosphate can be found in the liver of most omnivorous animals. Hexokinase can also be found in cells of other body tissues. This latter enzyme has a low Michaelis constant (K_m) for glucose while glucokinase has a high K_m for glucose (Lehninger 1982). Hexokinase is, therefore, constantly converting glucose to glucose-6-phosphate throughout the body while glucokinase with its high K_m comes into action when the glucose concentration in the blood becomes high e.g. after a meal high in carbohydrates. The activity of glucokinase and the incorporation of glucose into glycogen have been shown to be extremely low in the cat (Ballard 1965).

These results indicate that although glucose and other carbohydrates are well absorbed from the intestine of the cat, excess glucose may not be used in the body to yield energy as the cat may lack the ability to convert excess glucose to glucose-6-phosphate. However, the measurements of the glucokinase activities and rates of glucose incorporation into glycogen of the cat by Ballard (1965) were done on animals fed their normal diet. This is likely to have been a meat-based diet (naturally low in glucose) in the case of the cat. Therefore, the activity of the glucokinase and the rates of glucose incorporation into glycogen may have been low. A reassessment of the activity of glucokinase and the rates of glucose incorporation into glycogen in the liver of cats fed diets containing varying levels of carbohydrates, therefore, would be worthwhile to determine the extent of the adaptation of this enzyme in the cat.

1.3.6 Summary and conclusions

The domestic cat, unlike several other members of the order Carnivora, is a strict-carnivore. The constant dietary composition of the cat's diet throughout evolution has resulted in adaptations of this animal's protein metabolism. The relatively high requirement for protein and amino acids of the cat is believed to be caused by a lack of adaptation of catabolic enzymes in the liver while the high gluconeogenic capacity of the cat is believed to have evolved because of the low fraction of carbohydrates in the normal diet. Also several "unnecessary" metabolic pathways e.g. synthesis of arginine, taurine and niacin are no longer used by the cat as the diet of this strict-carnivore has always provided the end products of these pathways. Furthermore, the relative abundance of sulphur amino acids in the diet of the cat has allowed the synthesis of several unusual sulphur-containing amino acids by this animal, for which the biological roles are largely unknown.

1.4 METHODS FOR DETERMINING THE PROTEIN AND AMINO ACID REQUIREMENTS OF MAMMALS

Several methods are available for determining the protein and amino acid requirements of growing and adult animals. The following section will discuss the most commonly used approaches and where possible, the protein/amino acid requirements of the growing and adult cat will be presented according to the method of determination.

1.4.1 Dose-response method

Dose-response assays have been extensively used to determine protein and amino acid requirements of animals including the growing and adult cat. The dose-response method involves the administration of various doses of a nutrient to animals and the measurement of a particular response or particular responses.

The normal route of administration of a nutrient (e.g. protein or individual amino acids) to the animal is via the food, although other methods, such as including an amino acid in drinking water have been used (Wahlstrom *et al.* 1970). The basal diet used in dose-response studies has to be adequate in all nutrients except the nutrient under investigation and purified diets offer the possibility of accurately formulating diets with the nutrient under investigation being first limiting. When determining amino acid requirements in small animals, crystalline amino acids are often used but for larger animals the cost of crystalline amino acids may limit their use.

Response variables often used in the determination of protein and amino acid requirements are weight gain, nitrogen (N) balance, urinary urea excretion, plasma urea concentration, plasma amino acid concentration and amino acid oxidation (Lewis 1992, Zello 1995). Weight gain and N-balance have been the response variables most often used to determine protein and amino acid requirements in growing cats. Other response variables used in growing cats have been haematocrit and haemoglobin concentration in the blood and orotic acid excretion in the urine (Costello *et al.* 1980, Quam *et al.* 1987). In adult cats the response criterion of "zero nitrogen balance" has been used to determine the protein, methionine and lysine requirements.

The value obtained for the protein or amino acid requirements in a given experiment may depend on the response variable measured as not all response variables result in the same estimate of requirement, and on the pattern of the response curve (Baker 1986, Fuller and Wang 1987, Schaeffer *et al.* 1989, Lewis 1992). In cases where the response curve shows no obvious maximum, the adequate dietary inclusion level of the protein or amino acid may be difficult to determine. Response data have been examined and an arbitrary decision about an adequate dietary level of the nutrient has been made by investigators. This latter method is rather subjective and a more appropriate technique to determine the adequate dietary inclusion level of the nutrient is to fit a statistical model to the data. Statistical models used to describe response curves and determine an animal's requirement for a nutrient include the "broken line model" (Robbins *et al.* 1979), the "nutrient-response or Reading model" (Fisher *et al.*

1973) and the "saturation kinetics model" (Mercer *et al.* 1989).

Weight gain has been used in many studies to determine the minimum requirements of protein and amino acids for growing cats (Greaves 1965, Teeter *et al.* 1978, Smalley *et al.* 1985, Williams *et al.* 1987). The level of weight gain which is most satisfactory for growing cats is difficult to determine and investigators have, therefore, maximised weight gain in many experiments (Schaeffer *et al.* 1989). Another drawback of using weight gain as a response criterion is that it is often more variable than a metabolic response like N-balance (Schaeffer *et al.* 1989, Lewis 1992).

Nitrogen balance has been the only response criterion used to determine the protein and amino acid requirements of adult cats (Miller and Allison 1958, Greaves and Scott 1960, Burger *et al.* 1984, Burger and Smith 1987). Nitrogen balance is calculated by subtracting the daily nitrogen excreted in the urine, faeces, hair, skin and several other minor routes like nails and sweat from the dietary N intake. Often the losses of nitrogen in hair, sweat and other minor routes are not measured as the amount of nitrogen lost through these routes is believed to be small in comparison to the losses of nitrogen in the faeces and urine (Hegsted 1976, Robbins 1983, Manatt and Garcia 1992). When N-balance is used in dose-response studies, a regression line is fitted through the nitrogen balance data of animals fed different dietary levels of protein or an amino acid. The point on the regression line corresponding to zero N-balance is used to determine the dietary requirement of the protein or amino acid for adult animals while a defined level of positive N-balance is used to determine the protein and amino acid requirement for growing animals. The concept of N-balance and the limitations of this method have been extensively reviewed (Hegsted 1964, Hegsted 1976, Rand *et al.* 1981, Baker 1986, Manatt and Garcia 1992).

There are several additional problems in using "zero N-balance" as the response variable for determining protein and amino acid requirements in adult cats. Nitrogen is required for the growth of hair in cats and this may be a significant route of N-loss from the body which is often neglected in other animals. As the cat has been shown to have a seasonal hair growth pattern (Baker 1974, Ryder 1976), N-balance would change according to this pattern as would the cat's protein and amino acid requirements. Furthermore, the cat has been shown to ingest large amounts of its own body hair (Coman and Brunner 1972, Reed 1975, Hart 1976) which would affect the amount of nitrogen measured in the faeces. As this hair has been grown before the experimental period, the measurement of the daily amount of faecal-N would be

inaccurate.

Current estimates for amino acid requirements of growing and adult cats determined by the dose-response method are presented in Table 7.

TABLE 7
Minimum dietary levels of essential amino acids required by the growing and adult cat as determined by the dose-response method.[a]

Amino acid	Growing	Adult
	(g/kg dry matter)	
Arginine	10.0	-
Histidine	3.0	-
Isoleucine	5.0	-
Leucine	12.0	-
Lysine	8.0	3.3
Methionine+Cysteine	7.5	3.2
Phenylalanine+Tyrosine	8.5	-
Taurine	0.4	-
Threonine	0.4	-
Tryptophan	1.5	-
Valine	6.0	-

[a]NRC (1986) and Burger and Smith (1987).
-, not determined.

1.4.2 Epidemiological approach

The epidemiological approach to determining nutrient requirements involves measuring the intake of a given nutrient by a population of normal healthy individuals. The assumption is made that the individuals in this population consume food according to their requirements.

In growing animals, the intake of milk from the mother can serve as a basis for estimating the requirement of a particular nutrient. This method has been used extensively in humans (Fomon and May 1958, Räihä *et al.* 1976, Whyte *et al.* 1983, Darragh 1995). In adult animals, a detailed account of food intake by adult animals in the wild and of the digestibility of consumed food can be used to determine the requirement of a particular nutrient during this stage of life.

The epidemiological method has not been used to determine protein or amino acid requirements in growing cats. Some data, however, are available in the literature which enable estimation of the cat's requirement of these nutrients according to epidemiological approach. Remillard *et al.* (1993) and Davis *et al.* (1994) measured the amino acid composition of cat's milk while Keen *et al.* (1982) analysed the

concentration of protein, fat and carbohydrates in cat's milk and the changes in these nutrient concentrations during the course of lactation. If the daily milk intake of the kitten was known as well as the true digestibility of the milk protein, the kitten's protein and amino acid requirements could be determined. That the epidemiologically-derived protein and amino acid requirements of growing cats would give estimates similar to those made using the dose-response approach can be seen from Table 8 where the profile of amino acids required by the growing cat (NRC 1986) determined by the dose-response method and the gross amino acid profile of cat's milk are presented. It must be noted that the estimates of the amino acid requirements of growing cats, based on the composition of cat's milk, have been derived using the gross amino acid composition of milk and that requirements based on the absorbed amino acid composition of queen's milk would be more accurate.

TABLE 8
Dietary essential amino acid profile[a] required by the growing and adult cat as determined by the dose-response or epidemiological method.

Amino acid	Growing		Adult	
	Dose-response[b]	Milk composition[c]	Dose-response[d]	Mammalian tissue[e]
Lysine	100	100	100	100
Arginine	105	88	-	67
Histidine	35	45	-	35
Isoleucine	70	72	-	71
Leucine	168	201	-	103
Methionine+Cysteine	102	80	102	63
Phenylalanine+Tyrosine	99	103	-	76
Taurine	6	-	-	-
Threonine	107	96	-	72
Tryptophan	13	-	-	12
Valine	93	88	-	81

[a]Mol per 100 mol lysine.
[b]NRC (1986)
[c]Average gross amino acid composition of cat milk from Remillard *et al.* (1993) and Davis *et al.* (1994) except the value for methionine+cysteine which is from Davis *et al.* (1994).
[d]NRC (1986) and Burger and Smith (1987).
[e]Average gross amino acid composition of mammalian tissues from Albanese (1959).
-, Not determined.

The patterns of amino acids required by growing cats, estimated using the two approaches, are in good agreement. Noticeable, however, is the relatively higher amount of leucine and lower amounts of arginine and methionine plus cysteine relative to the concentration of lysine in cat's milk. The method used to determine the

methionine and cysteine content (acid hydrolysis) of cat's milk by Davis *et al.* (1994) is most likely to have resulted in low values for these two amino acids. The NRC (1986) lists the arginine requirement of growing cats as 10 g/kg based on minimal orotate excretion in the urine (Costello *et al.* 1980). This may be an overestimate as Anderson *et al.* (1979) and Costello *et al.* (1980) showed that dietary arginine levels of approximately 8.3 g/kg maximised weight gain in growing cats. If the former value of 8.3 g/kg is used instead of the 10 g/kg, an arginine value relative to lysine of 87 would be obtained.

Determining the protein and amino acid requirements of adult animals by the epidemiological approach is more difficult, given that accurate measurement of nutrient intake for adult cats in the wild are difficult to obtain. Food intakes measured for adult cats in the wild indicate that small mammals, like rabbits, rats and mice are the main prey of feral cats although insects, birds, lizards and frogs are also consumed (Coman and Brunner 1972, Jones and Coman 1981, Fitzgerald 1986, Catling 1988, Kitchener 1991). It can be argued that since the diet of cats consists mainly of soft mammalian tissues, the pattern of the amino acid requirements should be similar to the average digestible amino acid pattern of soft mammalian tissues. Table 8 also presents the average profile of amino acids relative to lysine in mammalian tissue and the profile of amino acids required by adult cats determined by the dose-response method. It must be noted that more accurate estimates would be obtained if the true amino acid digestibility of mammalian tissues was known.

The latter assumption that the profile of amino acids required by the animal is similar to the average digestible amino acid profile of soft animal tissue may not be valid, as cats may selectively eat certain tissues of their prey such as liver, kidney or muscle which may have a different amino acid composition to the hairless body. Determining absolute requirements for protein and amino acids of adult cats using the epidemiological approach would be extremely difficult as accurate food intakes per unit time and body weight of feral cats, as well as digestibility of the food consumed need to be known. Also, with this method, the assumption needs to be made that what an animal has eaten, equates with its requirement. This assumption may not be tenable.

In conclusion, the epidemiological approach can be used to determine amino acid requirements in growing and adult cats. It is questionable, however, whether this approach would yield minimum protein and amino acid requirement values. Furthermore, a large number of factors (maternal, environmental, physiological,

topographical) may influence the animal's requirements for protein. For growing cats the influence of these factors would not be great. In the adult cat, however, these factors may result in different estimates of protein and amino acid requirements. It is likely, therefore, that the epidemiological approach for determining requirements in adult cats would result in inaccurate estimates with a high degree of variation.

1.4.3 Factorial method

The factorial method has been used extensively for the estimation of protein (Greaves 1965, Calloway and Margen 1971, Glem-Hansen and Jørgensen 1973, Scrimshaw 1976, Robbins 1983, Flurer *et al.* 1988) and amino acid requirements (El Lozy and Hegsted 1975, Wiesemuller 1983, Moughan 1989, Young *et al.* 1989) in growing and adult mammals. This method consists of the summation of protein or amino acids required for growth and maintenance. Protein or amino acid requirements for growth consists of protein or amino acids required for the deposition of new tissue plus the protein or amino acids required for maintenance. The latter includes the obligatory losses of protein or amino acids in the faeces, urine, skin and other minor routes e.g. nails and sweat. As there are insufficient data in the literature to derive amino acid requirements for growing and adult cats by the factorial method, determination of the protein requirements of cats will be emphasised.

As mentioned above, the amount of protein required by growing animals consists of the protein required for growth and for maintenance. The protein requirement for growth can be estimated by body compositional analysis of normal growing animals at different stages during growth. Incremental increases in whole body protein content during growth can serve as an estimate for the amount of protein required for growth. The protein required for maintenance in growing animals can be derived in a similar way as the determination of the protein requirement of adult animals which will be discussed later. At present, there are limited data available to allow determination of the protein requirements of growing cats according to the factorial method. Body compositional changes in growing cats have been determined by Spray and Widdowson (1950) and Stratmann (1988). Greaves (1965) used the data of Spray and Widdowson (1950) to derive a factorial estimate of the protein requirements of growing cats relative to a reference protein source, which was assumed to be fully utilisable. The protein requirement for maintenance in growing cats was assumed to be similar to the maintenance protein requirement for adult cats on a unit body weight

basis. Greaves (1965) showed that the protein requirement of growing cats is approximately 19 g/kg body weight/day in the first week and rapidly falls to 10 g/kg body weight/day in the second week. After the second week requirements were shown to fall more gradually to 7, 5, and 4 g/kg body weight/day at week 3, 10 and 18, respectively. It was noted, however, that these derived estimates for the minimum protein requirement of growing cats should be regarded as extreme approximations.

In adult animals where growth has effectively ceased, the maintenance protein requirement accounts for the entire amount of protein required by the animal. Maintenance protein requirements can be determined by the summation of endogenous protein excretions in the faeces and urine, protein required for hair and nail growth, and losses of protein through other minor routes e.g. desquamated epidermis, sweat and sebaceous gland excretion. The majority of protein lost in adult mammals occurs through the catabolism of body amino acids and via losses of endogenous protein in the faeces (Calloway and Margen 1971, Robbins 1983, Flurer *et al.* 1988). The amount of protein required for the growth of hair can be expected to vary between animals depending on the quantity of hair on the animal. Although the amount of protein required for the replacement of desquamated epidermis and the production of nails, sweat and sebaceous gland excretions is believed to be small, and is not normally measured (Greaves 1965, Fuller 1991), assumptions on the amount of nitrogen lost through these minor routes are often made and used in a factorial model (Robbins 1983, Flurer *et al.* 1988, Moughan 1989)

Greaves and Scott (1960) determined endogenous urinary and faecal nitrogen excretions for cats fed different levels of dietary protein. These authors reported, after extrapolation of the nitrogen excretion data to zero protein intake, values of 0.18 and 0.12 g N/kg body weight/day for the endogenous urinary and faecal losses, respectively. Using these estimates, a crude protein requirement of 1.9 g/kg body weight/day was obtained for adult cats. This is equivalent to a dietary protein level for a 20.9 kJ ME/g diet of 13.6 % which is similar to the current minimum recommended dietary protein level (14 %) for adult cats set by the NRC (1986). It is likely, however, that the factorially-derived protein requirement for adult cats would be higher than 1.9 g/kg body weight/day as protein is required for the growth of hair which may be significant in adult cats. There are, however, no data in the literature on the amount of hair growth in adult domestic cats.

Absorbed dietary amino acids in the body of growing animals are utilised with an

efficiency of less than 100 % (Heger and Frydrych 1985, Heger and Frydrych 1989, Chung and Baker 1992). Estimation of the efficiency of utilisation of the first-limiting amino acid, therefore, needs to be included in the factorial model before an accurate recommendation of the dietary protein requirement can be made. The reason for the less than 100 % utilisation of absorbed dietary amino acids is largely unknown, although Heger and Frydrych (1989) indicated that the presence of degradative enzymes in tissues is presumably responsible for the inevitable loss of a fraction of amino acids.

1.4.4 Conclusions

The three different approaches (dose-response, epidemiological and factorial) for the determination of protein and amino acid requirements in adult animals are not without limitations. The factorial approach is preferred here for determination of the protein and amino acid requirements for the adult cat, as this method is flexible and allows requirements to be calculated according to sex, weight and season. The latter factor may be especially important in the cat in light of the seasonal hair growth pattern of this animal. The factorial method, furthermore, provides insight into the underlying biological mechanisms giving rise to the requirements and allows incorporation of data present in the literature.

1.5 INFORMATION REQUIRED FOR THE DETERMINATION OF THE PROTEIN AND AMINO ACID REQUIREMENTS OF THE ADULT DOMESTIC CAT BY THE FACTORIAL METHOD

Deriving the protein and amino acid requirements of the adult cat by the factorial method requires estimates of the amount of gut endogenous protein and amino acid excretions, the amount of catabolised body amino acids voided in the urine and the amount of protein and amino acids required for the growth of hair.

Gut endogenous protein and amino acid excretions are normally determined by feeding an animal a protein-free diet and by measurement of the amount of protein and amino acids in the faeces. This method can be criticised, however, because of the physiologically abnormal nature of the protein-free state (Low 1980). Other methods for the determination of gut endogenous protein and amino acid excretions have been developed allowing determination under more natural conditions. Among these are the

regression method (Taverner *et al.* 1981), synthetic amino acid supplementation method (Skilton *et al.* 1988), homoarginine method (Moughan and Rutherfurd 1990), enzymatically hydrolysed casein/ultrafiltration method (Moughan *et al.* 1990, Butts *et al.* 1993) and the isotope dilution method (De Lange *et al.* 1990). It has been shown, recently, that endogenous protein and amino acid excretions are increased by the presence of protein and peptides in the diet (Butts *et al.* 1993) and for this reason the homoarginine, enzymatically hydrolysed casein/ultrafiltration and isotope dilution methods are preferred. The flora of the large intestine may metabolise endogenous protein and amino acids resulting in inaccurate estimates of gut endogenous protein and amino acid excretions. Measurement of gut endogenous protein and amino acid excretions at the terminal ileum would avoid this problem but an assumption has to be made as to the relative contribution of the large intestine to total gut endogenous excretions of protein and amino acids. Elimination of the microflora in the large intestine by the use of antibiotics would also avoid this problem and give an estimate of the amount of endogenous protein and amino acids lost over the entire gastrointestinal tract. The antibiotics themselves, however, may have an influence on the endogenous excretions.

Body protein turnover is not an entirely efficient process and body amino acids which are not re-utilised are catabolised and the nitrogen excreted in the urine. Furthermore, several nitrogen containing compounds (e.g. creatine, creatinine, 3-methylhistidine, allantoin) are excreted in the urine which originate from the metabolism of amino acids. Measurement of these nitrogen losses can be accomplished by the determination of the endogenous urinary nitrogen (EUN) excretion which can be achieved by measuring the urinary nitrogen excretion following a period of zero-N intake or by linear regression to zero-N intake of the urinary nitrogen excretion data of animals fed graded levels of dietary nitrogen. The EUN excretion of many animal species has been determined after feeding a protein-free diet. Among these are the rat (Berdanier *et al.* 1967), dog (Kendall *et al.* 1982), human (Calloway and Margden 1971), pig (Moughan *et al.* 1988) and marmoset (Flurer *et al.* 1988). The regression method has been used to determine EUN excretion in rats (Berdanier *et al.* 1967), pigs (Moughan *et al.* 1988), mink (Glem-Hansen and Jørgensen 1973) and the cat (Greaves 1965). To determine the amount of metabolised body amino acids from the EUN excretion, the assumption has been made that the pattern of metabolised body amino acids occurs in proportion to the pattern or concentration of amino acids in mixed body

protein (Millward and Rivers 1988, Young *et al.* 1989, Young 1991). The amino acid composition of the body protein, therefore, needs to be determined to obtain an estimate of the amount of metabolised body amino acids. The amino acid composition of the body protein of adult cats has been determined by Dunn *et al.* (1949) and Stratmann (1988). However, the total number of animals investigated between these two studies is five and the amino acid composition data of these five animals shows a large variation. Therefore, more accurate data from a large number of animals is required.

The amount of protein and amino acids required for the replacement of hair in adult cats can be determined by measurement of the amount of hair replaced per unit of time in conjunction with the measurement of the amino acid composition of cat hair. Determination of the amount of hair replaced per unit of time can be achieved by measurement of the amount of hair growth or hair loss per unit of time. These two methods can be expected to give similar results in adult animals since it can be assumed that there is no net increase of hair in adult animals beside seasonal fluctuation. A quantitative measurement of hair growth per unit of time in animals can be made by measuring the hair growth rate on a small area of the body through clipping and relating the hair growth rate on this small area to hair growth rate over the entire body (Chapman and Young 1957, Bigham 1974, Mundt and Stafforst 1987). The assumptions made in this method are that the hair growth rate on the small area is similar to the overall hair growth rate on the body and, furthermore, that clipping of the area does not influence the hair growth rate. This method has been used in sheep where it has been shown that the hair growth rate on the mid-side area is representative of the hair growth on the whole body and that clipping *per se* does not influence hair growth (Coop 1953, Bigham 1974). No data, however, are available in cats. In sheep and humans quantitative estimates of hair growth have also been obtained by measurement of the amount of hair growth in the interval between two shavings of the entire body (Sirbu *et al.* 1967, Hawker *et al.* 1984, Hawker and Crosbie 1985). This latter method, however, relies on the assumptions that no hair is lost during the interval between shavings and that shaving does not influence hair growth. Since cats have been shown to moult (Baker 1974, Ryder 1976), this method would not give accurate estimates of hair growth in cats. A quantitative measurement of hair loss can be made by quantitatively collecting the hair lost from the body. A similar method has been used by Sirbu *et al.* (1967) to determine the integumental losses in humans.

The excretion of felinine in the urine of adult cats has been shown to be extremely high. As the sulphur in the felinine molecule originates from cysteine, although some contradictory evidence for this exists, a quantification of the excretion of felinine in adult cats needs to be made for inclusion into a factorial model. Felinine, however, is not commercially available and, therefore, has to be synthesised to enable determination of normal excretion levels of felinine in adult cats. Review of the literature on felinine indicates that there are several synthesis procedures for felinine (Trippett 1957, Eggerer 1962, Schöberl *et al.* 1966, 1968) which give satisfactory yields. These procedures could be used to obtain a standard for quantification experiments.

The amount of protein and amino acids lost through other routes e.g. sweat and nails are believed to be small in proportion to the total protein and amino acid requirements of mammals (Sirbu *et al.* 1967, Calloway and Margen 1971, Scrimshaw 1976, Robbins 1983). In cats the amount of protein and amino acids lost through these minor routes can be expected to be even smaller in comparison to other animals as sweat glands can only be found in the skin of the foot pad in cats (Baker and Thomsett 1990). Regulation of body temperature in cats is not achieved by the production of sweat but through panting and/or by behavioural changes.

1.6 CONCLUSION

From the review of the literature it is logical to infer that as a result of the strictly-carnivorous nature of the cat (i.e. the constant dietary composition throughout its evolution) several metabolic adaptations have occurred in this species. As a result of these adaptations, the cat has a relatively high requirement for protein in comparison to omnivores and other non strictly-carnivorous mammals. Although protein and amino acid requirements have been determined for growing cats, there is still a paucity of information and thus uncertainty as to the requirements of these nutrients for the adult cat.

There are several approaches (dose-response, epidemiological, factorial) available to determine the protein and amino acid requirements of animals. The factorial method involves the summation of endogenous protein and amino acids excreted in the faeces, catabolised body amino acids ultimately excreted in the urine and the amount of protein and amino acids required for the growth of hair. Several

different methods are available to determine the quantitative significance of these individual processes. The adult cat has been shown to excrete several unusual sulphur-containing amino acids in its urine. The concentration of one of these amino acids, felinine, has been reported to be extremely high and an estimate of the amount of amino acids required for the biosynthesis of felinine should be included in a factorial model.

It appears based on this review and with reference to establishing a factorial model to determine amino acid requirements for the adult cat, that worthwhile research objectives would be: (1) determination of gut endogenous protein and amino acid excretions, (2) protein and amino acids required for the growth of hair and (3) the amount of body amino acids which are catabolised by the adult cat. Furthermore, it seems worthwhile to accurately determine the excretion levels of felinine in the urine of adult cats (Chapter 5) which may be included into the factorial model for the determination of the amino acid requirements of the adult cat.

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CHAPTER 2

GUT ENDOGENOUS NITROGEN AND AMINO ACID EXCRETIONS IN THE ADULT DOMESTIC CAT (*FELIS CATUS*)

Determination of the protein and amino acid requirement of the adult domestic cat (*Felis catus*) by the factorial method requires estimates of the losses of protein and amino acids from the body. Large amounts of endogenous amino acids in animals are lost through the gastrointestinal tract during the digestion of food. The aim of the study reported in this chapter was to provide quantitative data on the excretion of endogenous nitrogen and amino acids from the gastrointestinal tract of the adult domestic cat.

GUT ENDOGENOUS NITROGEN AND AMINO ACID EXCRETIONS IN ADULT DOMESTIC CATS FED A PROTEIN-FREE DIET OR AN ENZYMATICALLY HYDROLYZED CASEIN-BASED DIET

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2.1 ABSTRACT

Ileal and fecal gut endogenous nitrogen and amino acid excretions in adult domestic cats were determined. Ileal digesta were collected (10 cm of terminal ileum) from the cats fed either a protein-free diet or an enzymatically hydrolyzed casein-based diet (free amino acids and peptides <10,000 Da) for 1 wk. Chromic oxide was included in each diet as an indigestible marker. The relative contribution of the hindgut to total endogenous gut excretion was investigated in a separate study by feeding cats a protein-free diet with or without added antibiotics for 10 d. Endogenous ileal nitrogen and amino acid nitrogen excretions of (mean ± SEM) 2.4 ± 0.27 and 1.9 ± 0.13 mg/g food dry matter intake, respectively, were found for the cats fed the protein-free diet, whereas higher excretions of 3.6 ± 0.73 (P = 0.12) and 3.6 ± 0.76 (P = 0.03) mg/g food dry matter intake were obtained in cats fed the enzymatically hydrolyzed casein. Significantly (P < 0.05) higher endogenous ileal amino acid excretions, for the enzymatically hydrolyzed casein-fed cats compared with those fed the protein-free diet, were found for methionine, aspartic acid, serine, glutamic acid, proline, valine and isoleucine, with the differences in excretions of glycine, alanine, leucine and histidine being significant at the 6 % level. Most of the endogenous fecal amino acid excretions were unaffected by the inclusion of the antibiotics in the protein-free diet, although bacterial numbers were significantly lower (69 %). Antibiotics addition led to significantly higher fecal endogenous excretions of nitrogen, taurine, threonine, serine and histidine but significantly lower excretions for methionine and lysine. Cats, like other simple-stomached mammals, excrete higher amounts of endogenous amino acids at the terminal ileum when the diet contains peptides.

2.2 INTRODUCTION

A considerable quantity of endogenous nitrogen-containing material is excreted into the lumen of the mammalian gastrointestinal tract during the digestion of food (Fauconneau and Michel 1970, Low 1982). This nitrogen originates mainly from desquamated cells, enzymes, mucoproteins, serum albumin, peptides, free amino acids, amines and urea (Fauconneau and Michel 1970). Although a large proportion of the endogenous nitrogen is digested and reabsorbed, some is unabsorbed and is excreted in the feces.

A measure of the excretion of gut endogenous nitrogen and amino acids, in addition to being of fundamental interest in feline biology, is necessary for the determination of amino acid requirements by the factorial method. There is little information available, however, on nitrogen and amino acid losses from the cat's body, and there are no data in the literature on gut amino acid losses.

In mammals, endogenous amino acid excretion from the gastrointestinal tract traditionally has been determined following the feeding of a protein-free diet. This method can be criticized, however, due to the physiologically abnormal nature of the protein-free state (Low 1980). Furthermore, attempts to feed protein-free diets to adult cats have met with limited or no success (Greaves 1965, Greaves and Scott 1960, Miller and Allison 1958). Recently, a new and more physiological method for determining endogenous amino acid losses, which may avoid food intake problems, has been developed (Butts et al. 1991, Moughan et al. 1990). With this method, an animal is fed a semipurified diet containing enzymatically hydrolyzed casein (composed of free amino acids and peptides with a molecular weight <10,000 Da) as the sole source of nitrogen. The digesta are collected from the animal, and the endogenous protein (molecular weight > 10,000 Da) is separated from unabsorbed dietary free amino acids and peptides by centrifugation and ultrafiltration.

Endogenous amino acid losses from the gastrointestinal tract can be determined by collecting ileal digesta or feces. With the fecal method, microorganisms in the large intestine may have catabolized endogenous amino acids and protein and may have synthesized amino acids, thus leading to inaccurate estimates of endogenous amino acid excretion. Determining endogenous excretion at the terminal ileum avoids this problem. When using ileal endogenous amino acid excretions in determining amino acid requirements by the factorial method, however,

assumptions need to be made as to the relative amounts of nitrogen and amino acids excreted by the large intestine itself.

The present study, which included both fecal and ileal measurements, was undertaken to obtain information on endogenous nitrogen and amino acid excretion from the gastrointestinal tract of adult domestic cats. Endogenous ileal nitrogen and amino acid excretions were determined after feeding cats a protein-free diet or an enzymatically hydrolyzed casein-based diet. Furthermore, in an attempt to determine the importance of the hindgut in endogenous nitrogen and amino acid excretion, a protein-free diet with or without an antibiotic-fungicide mixture was fed to cats.

2.3 MATERIALS AND METHODS

The two studies reported here were approved by the Massey University Animal Ethics Committee. Twenty-eight domestic short-haired cats, 1 to 6 y old and with an initial body weight range of 3035 to 4125 g (mean ± SEM; 3632 ± 76.0 g), were used.

2.3.1 Study 1

Twelve intact male cats scheduled for destruction by an animal shelter group were made available for this study. The cats had been dewormed and fed a commercially available canned cat food for 1 mo before the study. The cats were randomly allocated to two groups and were housed singly in metabolism cages, each fitted with a litter tray, at an ambient temperature of 21 ± 2°C. One group (six cats) received a protein-free (PF) diet (**Table 1**) while the other group received an enzymatically hydrolyzed casein (EHC)-based diet (Table 1), in a 7 d study. The food was given at a fixed daily intake based on the daily energy requirement of the cats. The diet contained 21 kJ metabolic energy/g dry matter and the energy requirement of inactive adult cats was taken to be 293 kJ/kg body weight (NRC 1986). On d 1, both groups received an amount of their respective diet equivalent to 50 % of energy requirement, on d 2 60 %, on d 3 70 %, and on d 4 to d 7 80 %. Each day's total food allowance was given in nine equal portions, beginning hourly at 0830 h. Water was available at all times. The frequent feeding regimen was employed in an attempt to ensure a constant flow of digesta at the terminal ileum on the day of digesta sampling.

On the final day of the study, and at 5 h after commencement of the hourly feeding, each cat was anesthetized with an intrathoracic injection (0.2 mL/kg body

wt) of sodium pentobarbitone (Pentobarb, 500 g/L, South Island Chemicals Ltd, Christchurch, New Zealand), and then killed with an intracardial injection (0.3 mL/kg body wt) of sodium pentobarbitone. The abdominal cavity of the animal was opened and the terminal 10 cm of ileum (directly anterior to the ileocecal valve) carefully removed. The outer surface of the dissected ileum was washed with distilled deionized water to remove blood and hair and carefully dried on an absorbent paper towel. The digesta were gently flushed out with 15 mL of distilled deionized water from a syringe into a 50-mL polypropylene centrifuge tube. Concentrated HCl (0.15 mL) was added to the digesta of the cats fed the EHC diet to minimize protease activity, and the centrifuge tube was then frozen (-20°C) until further processing.

TABLE 1
Ingredient compositions of the protein-free (PF) and enzymatically hydrolyzed casein (EHC)-based diets

Ingredient	Diet	
	PF	EHC
	g/kg (air-dry)	
Enzymatically hydrolyzed casein[1]	-	139.5
Beef tallow[2]	300.0	300.0
Gelatinized cornstarch[3]	350.0	210.0
Sucrose[4]	250.0	250.0
Vitamin-mineral mix[5]	53.0	53.0
Soybean oil	35.0	35.0
Cellulose[6]	10.0	10.0
Chromic oxide	2.0	2.0
Taurine	-	0.5

[1]New Zealand Pharmaceuticals Ltd. (Palmerston North, New Zealand).

[2]Weddel New Zealand Ltd. (Feilding, New Zealand).

[3]Avon pregel starch, New Zealand Starch Products Ltd. (Onehunga, Auckland, New Zealand).

[4]The antibiotic-fungicide mix (35 g) (Table 2) replaced an equal amount of sucrose in the protein-free + antibiotics (PF+) diet.

[5]Provided the following (g/kg diet): choline chloride 3.3, $CaHPO_4 \cdot 2H_2O$ 29.4, KCl 12.2, NaCl 5.2, $MgSO_4 \cdot 7H_2O$ 4.2, $FeSO_4 \cdot H_2O$ 0.25, $ZnSO_4 \cdot H_2O$ 0.21 and (mg/kg diet) nicotinic acid 61.2, all-*rac*-α-tocopherol 30.6, calcium pantothenate 5.1, thiamin hydrochloride 5.1, riboflavin 4.1, pyridoxine hydrochloride 4.1, retinyl acetate 1.8, menadione sodium bisulfide 0.18, $MnSO_4 \cdot H_2O$ 23.5, $CuSO_4 \cdot 5H_2O$ 19.9, $Ca(IO_3) \cdot H_2O$ 0.58 and (μg/kg diet) cobalamin 20.1, cholecalciferol 12.6.

[6] Ahaki Chemical Industry Co Ltd. (Osaka, Japan).

2.3.2 Study 2

Sixteen adult cats (eight male, eight female) from the BestFriend Feline Nutrition

Research Unit, Massey University (Palmerston North, New Zealand) that had been adapted previously to purified diets were used in this study. The cats were randomly allocated to two groups, with the proviso that the groups be balanced for numbers and sex. Two weeks before the study, the cats were fed a purified casein-based diet, similar in composition to the EHC diet (**Table 1**). During the study, one group received the PF diet (Table 1), and the other group received a protein-free diet plus an antibiotic-fungicide mixture (PF+ diet) (35 g of sucrose per kilogram of diet was replaced with the antibiotic-fungicide mixture) (**Table 2**).

TABLE 2
Composition of the antibiotic-fungicide mixture

Active ingredient	Composition
	g/100 g active ingredient
Nystatin[1]	29.0
Ampicillin sodium[2]	35.2
Gentamycin sulfate[3]	4.4
Oxytetracycline HCl[4]	17.6
Neomycin[5]	13.8

[1]Mycostatin®, (568182 IU/g, E. R. Squibb & Sons, Auckland, New Zealand).
[2]Ampicillin (900 g/kg, Progen Industries, Darra, Australia).
[3]Gentamycin sulfate (Serva Feinbiochemica GmbH, Heidelberg, Germany).
[4]Tetravet 100 (100 g/kg, Bomac Laboratories, Manukau City, Auckland, New Zealand).
[5]Neomix™ (715 g/kg, Upjohn Laboratories, Taoyuan, Taiwan).

The cats were housed outdoors in a semi-enclosed area in plastic metabolism cages, each fitted with a litter tray allowing total collection of uncontaminated feces. Minimum and maximum air temperatures were recorded daily during the experimental period.

Two days before the start of the study, the cats allocated to the PF+ diet received an oral dose (500,000 IU) of nystatin (Mycostatin®, E. R. Squibb & Sons Ltd, Auckland, New Zealand) daily for 2 d. The diets were fed to the cats for 10 d (free access), with food intake recorded daily. Water was available at all times. Over the last 5 d of study, the feces were collected daily and frozen separately for each cat. The bulked feces from each cat were weighed, thawed, mixed and subsampled (±5 g) for bacterial counting. The remaining feces were freeze-dried, weighed and stored (-20°C) until further processing.

2.3.3 Chemical analysis

Prior to analysis, the feces were dehaired according to the following procedure. The freeze-dried feces were crushed manually and were mixed with water (10 parts water to 1 part feces) using a magnetic stirrer until a fine suspension was obtained (approximately 20 min). The suspension was then passed through a wire sieve (0.5 mm); the suspension passed through the sieve while hairs were retained. The retained hairs were washed with a small amount of water, and the washings were added to the filtrate. The filtrate was mixed, freeze-dried and stored (-20° C) until chemical analysis. Microscopic analysis confirmed that almost all of the hairs had been removed.

The ileal digesta from the cats receiving the EHC-based diet were centrifuged at 1600 x g for 45 min at 0°C, and the supernatant liquid was decanted off and retained. The precipitate was washed with another 10 mL of deionized water and centrifuged (1600 x g, 45 min, 0°C). The second supernatant was added to the first, and the precipitate was washed with a further 10 mL of deionized water as described above. The precipitate was stored (-20°C) and the combined supernatant liquids ultrafiltered using Centriprep-10 Concentrators (Amicon, Beverly, MA) according to the manufacturer's instructions. The high-molecular-weight fraction (molecular weight >10,000 Da) was added to the thawed precipitate, mixed, freeze-dried, manually dehaired and finely ground. The ileal digesta of the cats fed the PF diet were freeze-dried, manually dehaired and finely ground. Total nitrogen, amino acids and chromic oxide were determined in the dehaired, centrifuged and ultrafiltered ileal digesta and in the dehaired ileal digesta and feces of the cats fed the PF diets. The diets were analyzed for chromic oxide.

Bacteria were counted by suspending 0.1 g of wet feces in 1 mL of PBS, whereafter the suspension was serially diluted (10, 100 and 1000 times) using a solution of 1 g of methylene blue in 100 mL of PBS. The stained bacteria were counted using light microscopy (1000x magnification).

Total nitrogen was determined in duplicate using the Kjeldahl method. Amino acids were determined in duplicate on 5-mg samples by hydrolyzing with 1 mL of 6 mol/L glass-distilled HCl (containing 0.1 g phenol/L) for 24 h at 110 ± 2° C in glass tubes, sealed under vacuum. Amino acids were detected on a Waters ion exchange HPLC system, and the chromatograms were integrated using dedicated software (Maxima 820, Waters, Millipore, Milford, MA) with amino acids identified by

retention time against a standard amino acid mixture. Cysteine and methionine were analyzed as cysteic acid and methionine sulphone by oxidation with 2 mL of performic acid (1 part 30 % H_2O_2 to 9 parts 88 % formic acid) for 16 h at 0° C and neutralization with 0.3 mL of 50 % (wt/wt) HBr prior to hydrolysis. Diaminopimelic acid (DAPA) was determined in the oxidized fecal samples. Tryptophan was not determined. No corrections were made for loss of amino acids during hydrolysis, and amino acid weights were calculated using free amino acid molecular weights. Chromic oxide concentrations in the diet and digesta samples were determined in duplicate by the method of Fenton and Fenton (1979).

2.3.4 Data analysis

Endogenous nitrogen and amino acid excretions relating to the ingestion of 1 g (dry matter) of food were calculated using the following equation (units are micrograms per gram of dry matter):

$$\textit{Excretion of component} = \textit{Component in digesta} \times \frac{\textit{Diet chromium}}{\textit{Digesta chromium}}$$

The endogenous excretion data for nitrogen and each amino acid were tested for homogeneity of variance using Levene's test (Snedecor and Cochran 1980). Where the variances were heterogeneous, the data were transformed ($\log_{10}$). The ileal endogenous nitrogen and amino acid excretion data were subjected to analysis of variance using the SAS general linear models procedure (SAS 1985). The fecal endogenous nitrogen and amino acid excretion data were subjected to ANOVA (SAS 1985) with diet, sex and diet x sex as variables. There was no effect of sex or diet x sex on the endogenous nitrogen and amino acid excretions, and the data were thus pooled across sexes. Student's *t* test was used to determine levels of significance between dietary treatments in Study 2.

2.4 RESULTS

The cats remained healthy throughout Studies 1 and 2. In Study 1, the cats fed the EHC and PF diets consumed 78 ± 2.2 % and 58 ± 1.3 % (mean ± SEM), respectively, of the food given during the first 6 d. Most of the food was eaten during the first four

meals. On the day of digesta sampling, the cats fed the EHC diet consumed 83 ± 10.1 % of the food presented, and the PF-fed cats consumed 77 ± 8.9 % of the food given. These food intakes were such as to allow an adequate collection of ileal digesta samples from all except one cat fed the PF diet. In Study 2, the average daily minimum and maximum air temperatures were 11 and 17°C, respectively. Two cats were removed from Study 2: one female cat fed the PF diet refused to eat, and a small amount of blood was found in the feces of a female cat fed the PF+ diet. The cats were observed closely during the study and coprophagy did not seem to occur. The average daily food intakes and body weights of the cats at the beginning and end of Study 2 are given in **Table 3**. Food intake was low and decreased over the 10 d period, with the cats losing on average 33 g body wt per day. The food intake of the cats fed the PF+ diet was slightly higher than the food intake of the cats fed the PF diet.

TABLE 3
Body weights at the start and end of the test period and daily food intakes in cats fed a protein-free diet (PF) and the protein-free diet containing antibiotics (PF+)[1]

	Daily food intake[2]										Pooled	Body weight	
Dietary group	1	2	3	4	5	6	7	8	9	10	SEM	start	end
	g											*kg*	
PF	16	16	14	21	21	13	13	15	10	13	0.9	3.06 ± 0.17	2.75 ± 0.16
PF+	24	18	13	23	21	13	17	18	12	14	1.3	3.21 ± 0.16	2.87 ± 0.16

[1]Values are means or means ± SEM, n=7.
[2]Daily food intake was not significantly different ($P > 0.05$) between diet groups for any day.

The mean endogenous nitrogen, amino acid nitrogen and amino acid excretions as determined at the terminal ileum in the cats fed the PF- and EHC-based diets are given in **Table 4**. A high variation in endogenous ileal amino acid excretions was found for the EHC-fed cats compared with those fed the PF diet. The data were found to be heterogeneous and were thus transformed ($\log_{10}$) before analysis of variance. Endogenous ileal amino acid excretion for the cats fed the PF diet accounted for 80 % of total nitrogen excretion, whereas a value of 99 % was found for the EHC-fed cats. Overall, endogenous ileal amino acid excretion was higher for the cats fed the EHC-based diet. Significantly higher endogenous amino acid excretions were found for aspartic acid, serine, glutamic acid, proline, valine,

isoleucine and methionine, and a significantly lower excretion of taurine was found, in EHC-compared with PF diet-fed cats. Although no significant differences were found for glycine, alanine, leucine and histidine at the 5 % probability level, these differences in ileal amino acid excretions were significant at the 6 % level.

TABLE 4

Endogenous nitrogen and amino acid excretions at the terminal ileum in cats fed a protein-free diet (PF) or an enzymatically hydrolyzed casein (EHC)-based diet[1]

Amino acid	Dietary group		Level of significance (P <)[3]
	PF	EHC[2]	
	µg/g dry matter intake		
Taurine	2091 ± 714	299 ± 177[4]	0.01
Cysteine	452 ± 37	853 ± 209	0.11
Methionine	164 ± 13	411 ± 98	0.02
Aspartic acid	1283 ± 89	2725 ± 633	0.04
Threonine	1235 ± 113	2127 ± 510	0.17
Serine	1013 ± 75	2734 ± 478	0.01
Glutamic acid	1427 ± 116	4240 ± 793	0.01
Proline	820 ± 70	1913 ± 457	0.03
Glycine	665 ± 31	1298 ± 299	0.06
Alanine	666 ± 34	1380 ± 327	0.06
Valine	696 ± 50	1687 ± 355	0.01
Isoleucine	398 ± 30	1205 ± 207	0.01
Leucine	884 ± 67	1823 ± 433	0.06
Tyrosine	599 ± 42	1046 ± 227	0.10
Phenylalanine	632 ± 46	1015 ± 251	0.26
Histidine	397 ± 23	897 ± 242	0.06
Lysine	570 ± 42	1101 ± 272	0.10
Arginine	540 ± 46	948 ± 242	0.22
	mg/g dry matter intake		
Nitrogen	2.4 ± 0.27	3.6 ± 0.73	0.12
Amino acid nitrogen	1.9 ± 0.13	3.6 ± 0.76	0.03

[1]Values are means ± SEM; the number of observations was 5 for cats fed the protein-free (PF) diet and 6 for cats fed the enzymatically hydrolyzed casein (EHC)-based diet.

[2]Centrifuged and ultrafiltered.

[3] The data were transformed (log_{10}) before analysis of variance.

[4]Underestimated due to ultrafiltration.

The mean fecal bacterial counts and fecal DAPA concentrations and excretions for the cats fed the two protein-free diets (PF and PF+) in Study 2 are given in **Table 5**. The number of bacteria was significantly lowered by the inclusion of the antibiotic-fungicide mixture in the diet. On average, the number of bacteria was 69 % lower in the feces of the cats receiving the antibiotic-fungicide mixture. The fecal

concentration and daily fecal excretion of DAPA were also significantly lowered by the inclusion of the antibiotic-fungicide mixture. The mean endogenous fecal excretions of nitrogen, amino acid nitrogen and amino acids for the cats fed the two protein-free diets in Study 2 are given in **Table 6**. The endogenous fecal nitrogen excretion was significantly higher in the cats fed the PF+ diet. The endogenous fecal amino acid nitrogen excretion accounted for 66 % of the total endogenous nitrogen excretion in both groups. The excretions of most amino acids were not significantly altered by the inclusion of the antibiotic-fungicide mixture. Significantly lower excretions of methionine and lysine and significantly higher excretions of taurine, threonine, serine and histidine were found in the PF+ group compared with the PF group.

TABLE 5
Fecal bacterial counts and fecal concentrations and daily excretions of diaminopimelic acid (DAPA) in cats fed a protein-free diet (PF) or the protein-free diet containing antibiotics (PF+)[1]

Fecal value	Dietary group PF	Dietary group PF+	Pooled SEM	Level of significance (P<)
Count ($x10^{-8}$)	25.5	8.0	3.0	0.01
DAPA				
Concent ation				
µmol/g dry matter	2.8	0.3	0.2	0.01
Excretion				
µmol/d	7.2	1.0	0.6	0.01
µmol/g dry matter intake	0.49	0.06	0.04	0.01

[1]Values are means, n=7.

2.5 DISCUSSION

The cats in the present study ate the semipurified protein-free diet, and adequately sized samples of ileal digesta were obtained. With the exception of taurine, the amino acid excretions for the cats fed the protein-free diet were only moderately variable (CV: 10.4 % for glycine to 20.4 % for threonine) and provide information on endogenous ileal amino acid excretions that can be compared with that for other species, whereby endogenous excretions have also been obtained using the traditional protein-free method. Studies using the protein-free diet method have

reported endogenous ileal nitrogen excretions of 4.4 ± 0.77, 2.2 ± 0.21 and 1.0 ± 0.05 mg/g dry matter intake in humans (Rowan et al. 1993), pigs (Wünsche et al. 1987) and rats (Butts et al. 1992), respectively. The endogenous ileal nitrogen excretion of 2.4 ± 0.27 mg/g dry matter intake found in the present study for cats fed the protein-free (PF) diet is in accordance with these values.

TABLE 6

Endogenous nitrogen and amino acid excretions determined over the entire digestive tract in cats fed a protein-free diet (PF) or the protein-free diet containing antibiotics (PF+)[1]

Amino acid	Dietary group PF	Dietary group PF+	Pooled SEM	Level of significance (P <)
	µg/g dry matter intake			
Taurine	75	470	57	0.01
Cysteine	734	1092	122	NS[2]
Methionine	365	205	40	0.05
Aspartic acid	1858	1766	149	NS
Threonine	1084	2834	146	0.01
Serine	1204	2083	119	0.01
Glutamic acid	2333	2195	289	NS
Glycine	1059	1049	78	NS
Alanine	1181	1108	85	NS
Valine	1209	1179	88	NS
Isoleucine	807	586	62	NS
Leucine	1555	1282	124	NS
Tyrosine	944	799	68	NS
Phenylalanine	927	689	75	NS
Histidine	808	4108	293	0.01
Lysine	1132	727	88	0.05
Arginine	900	945	96	NS
	mg/g dry matter intake			
Nitrogen	4.1	5.8	0.3	0.05
Amino acid nitrogen	2.7	3.8	0.3	NS

[1]Values are means, n=7.
[2]NS = not significant (P > 0.05).

The relatively low food intakes encountered in the present study for cats fed the protein-free diet have also been reported by other researchers (Greaves 1965, Greaves and Scott 1960, Miller and Allison 1958). The PF diet, in Study 1, was less readily eaten than the EHC-based diet. However, the food intakes during the first 4 h on the day of sampling were adequate to allow collection of digesta from the last 10 cm of ileum for all but one cat. The food intakes for the cats in Study 2 were low,

with a calculated metabolic energy intake of approximately only 40 % of the energy requirement for inactive adult cats (NRC 1986).

Protein-free estimates of endogenous ileal nitrogen and amino acid excretion can be criticized due to the physiologically abnormal state of an animal fed a protein-free diet. Therefore endogenous nitrogen and amino acid excretions at the terminal ileum were also determined in the present study using a physiological more normal method whereby the animal is fed a diet containing free amino acids and small (molecular weight <10,000 Da) peptides. Using this method, higher endogenous amino acid excretions were obtained than with the traditional protein-free diet method. These results are in accordance with observations made in rats (Butts et al. 1991, Darragh et al. 1990) and pigs (Butts et al. 1993a, Moughan et al. 1992). In rats (Darragh et al. 1990) and pigs (Butts et al. 1993a, De Lange et al. 1989b), negative body nitrogen retention, which is associated with feeding an animal a protein-free diet, is not the primary cause of lower endogenous nitrogen and amino acid excretions associated with protein-free diets. Higher endogenous nitrogen and amino acid excretions seem to be a direct result of the dietary peptides.

The endogenous ileal nitrogen excretion was not significantly different ($P = 0.12$) between cats fed the EHC-based and those fed the protein-free diet. A possible reason for this, and for the apparent discrepancy with the amino acid data, is that small nitrogen-containing compounds (e.g., ammonia, creatine, urea) are removed in the ultrafiltration step. These compounds will be present in the endogenous excretions from cats fed protein-free diets, reducing the expected difference between excretions of the EHC- and protein-free diet-fed cats. Endogenous amino acid nitrogen accounted for all the endogenous ileal nitrogen for the EHC-fed animals, whereas in cats fed the protein-free diet, the amino acid nitrogen accounted for only 80 % of the total endogenous nitrogen. For the EHC method to give an accurate estimate of endogenous nitrogen and amino acid excretion, the amount of endogenous free amino acids and peptides (molecular weight <10,000 Da) must be relatively low. This seems to be the case in rats (Butts et al. 1992) and pigs (Moughan and Schuttert 1991), and although no measurements have been made in cats, it is assumed that the amount of free amino acids and small peptides in the endogenous gut excretions is also low in this species.

The major endogenous amino acids excreted at the terminal ileum of cats, as in rats (Butts et al. 1991, Darragh et al. 1990, Skilton et al. 1988) and pigs (Butts et al.

1993a, Moughan and Schuttert 1991, Sauer et al. 1977, Taverner et al. 1981), were aspartic acid, glutamic acid, threonine and serine. These amino acids constitute a large proportion of the amino acids in mucus glycoproteins (Forstner and Forstner 1986). Endogenous ileal proline excretion in both pigs and rats has been found to be enhanced by protein-free feeding resulting in proline being the most abundant endogenous amino acid in the ileal digesta of these animals (Butts et al. 1993a, De Lange et al. 1989a and 1989b, Moughan et al. 1992, Skilton et al. 1988, Taverner et al. 1981). De Lange et al. (1989b) cited evidence that the high endogenous proline excretion of pigs fed a protein-free diet is the result of the induced negative nitrogen balance, resulting in muscle breakdown that leads to large quantities of glutamine being supplied to the intestine where it is metabolized to proline. Furthermore, endogenous proline excretion may be enhanced by the reduction of intestinal transport of proline and other amino acids when a protein-free diet is fed (Karasov et al. 1986). In the present study, the endogenous ileal proline excretion of cats fed a protein-free diet was in line with the endogenous ileal excretions for the other amino acids and did not seem to be elevated. Rogers and Phang (1985) showed that the pyrroline-5-carboxylate synthase activity (per kilogram of body weight) in the small intestine of cats was only 5 % of that observed in rats. Because pyrroline-5-carboxylate synthase is required for the conversion of glutamine to proline in the small intestine, the low activity of this enzyme in the cat's intestine may be the reason for the nonelevated endogenous ileal proline excretion when the protein-free diet was fed. The feeding of a protein-free diet has also been reported to lead to an elevated endogenous ileal glycine excretion in rats (Skilton et al. 1988) and pigs (De Lange et al. 1989a, Moughan et al. 1992, Sauer et al. 1977, Taverner et al. 1981). In these studies, endogenous ileal glycine excretions were higher than the endogenous excretions for aspartic and glutamic acid. In the present study, an endogenous ileal glycine excretion comparable to the other endogenous amino acid excretions was found for the cats fed the protein-free diet. Notable, however, is the relatively high endogenous ileal taurine excretion of 2091 ± 714 mg/g dry matter intake in the cats. Using a protein-containing but taurine-free diet, Hickman et al. (1992) found endogenous ileal taurine excretions of 794 ± 115 mg/(d·g diet) for cats fed the diet for 7 days and 92 ± 23 mg/(d·g diet) for cats fed the same diet for 6-8 mo. Because cats conjugate bile acids predominantly with taurine (Rabin et al. 1976) — unlike rats and pigs which use glycine predominantly — the present results indicate that

protein-free diet feeding may increase biliary secretion, decrease intestinal bile acid reabsorption, or both.

In an attempt to obtain information on the contribution of the hindgut to total gut endogenous amino acid excretion in cats, a second study was conducted in which cats were fed a protein-free diet with or without added antibiotics. The antibiotic-fungicide mixture used in this study has been shown to eliminate bacteria from the intestinal tract of dogs although the mixture was administered twice daily in this study (Amtsberg et al. 1989). In a preliminary study the antibiotic-fungicide mixture was found to completely eliminate bacteria from the intestinal tract of cats (W. Hendriks, unpublished). In the present study, however, the number of bacteria in the feces was lowered only 69 % by the inclusion of the antibiotic-fungicide mixture in the diet. The concentration and excretion of the bacteria-specific amino acid DAPA confirmed the lower bacterial numbers.

Greaves and Scott (1960) estimated the gut endogenous fecal nitrogen loss of cats by feeding diets containing different levels of protein (12.7 to 46.2 %) and extrapolating fecal nitrogen loss to zero dietary protein intake. Using this regression method, a value of 6.3 g N/kg diet dry matter consumed was obtained for the endogenous nitrogen loss. Similar values of 4.1 and 5.8 g N/kg dry matter intake were recorded in the present study for the cats fed the PF and PF+ diets, respectively. The values in the present study were obtained on dehaired feces, whereas hairs were still present in samples used for the Greaves and Scott (1960) estimates, resulting in an overestimation in the latter study. Because the antibiotic-fungicide mixture contained 1.37 % N/kg, it can be calculated that a maximum of 8.3 % of the endogenous fecal nitrogen found could have originated with the antibiotic-fungicide mixture. Ampicillin and oxytetracycline, however, are absorbed to some extent from the gastrointestinal tract.

The endogenous excretions of most amino acid were unaffected by the inclusion of the antibiotic-fungicide mixture; however, methionine and lysine excretions were significantly reduced and the excretions of taurine, serine, threonine and histidine were significantly higher. In pigs, a net synthesis of methionine and lysine by the microflora in the large intestine is found (De Lange et al. 1989a, Taverner et al. 1981, Wünsche et al. 1987). The reason for the significantly higher excretions of threonine, serine and histidine is unclear. The higher excretions could not be due to undigested antibiotic-fungicide mixture because the mixture contained

no amino acids. The higher excretions may reflect a reduced bacterial catabolism due to the significantly reduced bacterial population. The endogenous taurine excretion was significantly higher in the cats fed the PF+ diet than in those fed the PF diet. Intestinal bacteria have been shown to catabolize taurine in cats (Backus et al. 1994). In that study, when cats were fed a casein or soybean protein diet, only 50 and 20 %, respectively, of the original amount of dietary taurine was found after incubating the feces at 37° C for 24 h. However, the endogenous ileal excretions of taurine of the cats fed the protein-free diet in the present work were much higher than the fecal endogenous taurine excretions. Assuming there is no absorption of taurine in the large intestine of cats, it seems that a large amount of the taurine may also have been catabolized in the hindgut of the cats fed the PF+ diet.

The endogenous ileal nitrogen and amino acid excretions as determined using the protein-free diet method were lower than the fecal endogenous excretions except for taurine. There is an addition of endogenous nitrogenous compounds in the large intestine originating from mucus, desquamated cells, urea and other compounds. In pigs fed a protein-free diet, however, a net catabolism of amino acids by the microflora of the large intestine results in higher endogenous nitrogen excretions at the terminal ileum as compared with endogenous nitrogen excretion over the entire digestive tract (De Lange et al. 1989a, Wünsche et al. 1987). In the present study, higher endogenous nitrogen excretions were found over the entire digestive tract compared with the excretions at the ileum. Because the food intake of the cats in Study 2 was low, there may have been an effect of the low food intake on the endogenous fecal nitrogen excretions. However, in other animals the endogenous gut excretions of nitrogen and amino acids are linearly related to dry matter intake and are constant when expressed on a dry matter intake basis (Butts et al. 1993b). The effect of the low food intakes of the cats in the present study, therefore, is expected to be minor. Furthermore, the estimates of the endogenous fecal nitrogen excretion in the present study were in line with the estimate made by Greaves and Scott (1960).

An estimate of large bowl endogenous nitrogen and amino acid excretions can be made by subtracting the ileal endogenous nitrogen and amino acid excretions (determined by feeding the protein-free diet) from the endogenous fecal nitrogen and amino acid excretions when the PF+ diet was fed. It must be noted, however, that the digestive system was not totally cleared of microorganisms in cats fed the PF+ diet and that some catabolism or synthesis of amino acids may have occurred. Also, it is

not known whether the antibiotic-fungicide treatment itself may have influenced endogenous protein secretion and reabsorption.

The present study provides hitherto unavailable estimates of endogenous amino acid excretion from the gut of cats. These estimates, under the condition of peptide alimentation, can be used in calculations of the nitrogen and amino acid requirements of adult cats by the factorial method.

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CHAPTER 3

INTEGUMENTAL AMINO ACID LOSSES IN ADULT DOMESTIC CATS THROUGHOUT THE YEAR

Amino acids are constantly being lost from the body of the adult cat due to the replacement of hair. Hair replacement in the adult cat has been shown to occur in a sinusoidal pattern throughout the year. Amino acid requirements of adult cats, therefore, are not constant but can be expected to change according to this seasonal hair replacement pattern. The aim of this chapter was to provide quantitative data on hair growth (Part I) and hair loss (Part II) in adult short-haired cats and to provide accurate data on the amino acid composition of cat hair (Part III).

CHAPTER 3 PART I

SEASONAL HAIR GROWTH IN THE ADULT DOMESTIC CAT (*FELIS CATUS*)

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3.I.1 ABSTRACT

Determination of amino acid requirements by the factorial method requires an estimate of the amount of amino acids required for the replacement of hair. Hair growth rates in a total of 39 adult male and female domestic short-haired cats were determined throughout the year using the mid-side patch technique. The ratio of hair on the mid-side area to total hair on the body was also determined to allow conversion of mid-side hair growth rates to hair growth rates over the entire body. The mid-side hair growth rate showed a sinusoidal pattern throughout the year, similar to that found for day length and daily mean air temperature, with a maximum hair growth rate of 289 $\mu g/cm^2$/day in summer and a minimum hair growth rate of 62 $\mu g/cm^2$/day in winter. The peak hair growth rate for the female cats was reached earlier than that for the male cats. Sine-functions describing day length, minimum and maximum daily air temperatures and daily hair growth rates are presented. Adult domestic short-haired cats were found to grow 32.7 g of hair per kg body weight per year. Monthly amounts of hair growth per unit of body weight and body surface area are calculated.

3.I.2 INTRODUCTION

Protein is the main constituent of animal hair (Jørgensen and Eggum, 1971; Gillespie, 1983; Powell and Rogers, 1986; Mundt and Stafforst, 1987). The amino acids required for the replacement of hair in the adult cat (*Felis catus*), therefore, may make up a significant proportion of the total amino acid requirement for maintenance. To determine amino acid requirements by the factorial method, an estimate of the amino acids required for the replacement of hair is needed.

Hair growth and moulting in the cat have been shown to occur uniformly throughout the pelage in a mosaic pattern (Baker, 1974), unlike in rats and rabbits which replace hair in a wave pattern (Johnson, 1972). However, there is seasonal variation with the maximum growth occurring in summer and the minimum hair growth in winter (Baker, 1974; Ryder, 1976). The amount of amino acids required for hair growth in the cat should also reflect this seasonal pattern of growth with the highest requirement for amino acids occurring during summer and with a decreased requirement being found during the period of low hair growth rate in winter. There is little quantitative information on hair growth in the adult domestic cat.

The present study was conducted to determine hair growth throughout the year in adult domestic cats of both sexes. Hair growth was determined using the mid-side patch technique, over a total period of 416 days. To relate mid-side patch hair growth rate to hair growth rate over the entire body of the cat, the ratio of the total amount of hair on the body to the amount of hair on the mid-side area was also determined.

3.I.3 MATERIALS AND METHODS

The two studies reported here were approved by the Massey University Animal Ethics Committee. Fifty nine (39 males, 20 females) 1-6 y old short-haired domestic cats with an initial body weight range of 2255 to 6693 g (mean ± SEM; 3848 ± 96.8 g) were used.

3.I.3.1 Study 1

Hair growth in thirty nine (25 males, 14 females) adult cats from the BestFriend Feline Nutrition Research Unit, Massey University, Palmerston North, New Zealand (longitude 175°38'E, latitude 40°22'S) was determined using the mid-side patch

technique. The cats were housed outdoors in large (4.5 x 1.4 x 2.5 m) wire netting cages with the cats being exposed to natural day-light and temperature throughout the study. No artificial lighting was used during the study. The cats were divided into five pens with a distribution of males to females of pen 1, 8:0; pen 2, 5:2 and pen 3-5, 4:4. Throughout the study, the cats were fed to appetite a variety of commercially available canned cat foods of proven nutritional quality (AAFCO, 1993). Water was available at all times. Minimum and maximum daily air temperatures at the cage level were recorded throughout the study. At the start of the hair growth measurement, each cat was weighed and restrained by applying plastic clips to the nape of the neck (Tarttelin, 1991). A small patch (3 x 4 cm) of skin was shaved using electric clippers with a 1.9 cm wide clipper blade (Oster, Professional Products, McMinnville, TN) at the left lateral chest side between the 4^{th} and 7^{th} intercostal space and 5 to 10 cm ventral to the spine of the cat. At the designated collection time, the restrained cat was held in a normal standing position and the hairs, shaved off the patch with a single stroke of the clipper blade, were collected manually in a plastic container. Care was taken to collect all the hairs shaved off the patch. Any hairs remaining on the clipper blade were carefully washed into the plastic container using 20 ml of a 50 % (v/v) water-methanol solution. The length of the shaved patch was measured accurately using a ruler and the entire patch shaved again to the skin. The hair containing water-methanol solution was filtered through a preweighed sintered glass crucible and the retained hairs were washed using methanol and water, whereafter the crucible was dried until constant weight. The cats housed in pens 1, 2 and 3 were shaved at 4 week intervals over a 364 d period while the cats housed in pens 4 and 5 were shaved at consecutive intervals of 31 and 39 days, respectively, over a 381 d period. The 416 d study was started on February 21, 1994 with the start of the first hair growth measurement for the cats in the five pens spread over the first 4 weeks. The hair growth rate ($mg/cm^2/day$) calculated by dividing the hair growth (mg/cm^2) during each period by the number of days in the respective period, was taken to be the rate of hair growth for the median of the time period.

To determine if there was an effect of repeated shaving of the left mid-side patch on hair growth rate, the hair growth rate on the left as well as on the right side of every cat was determined over a 4 wk period at the end of the study using the above described procedure.

3.I.3.2 Study 2

The following study was conducted to obtain information on the relationship between the total amount of body hair and the amount of hair on the mid-side area of cats. Twenty (14 male, 6 female) adult cats which had been put down by an Animal Shelter Group, using carbon dioxide as the method of euthanasia, were made available for this study. The animals had been killed at different times of the year and their nutritional history was unknown. After death the cats were weighed, and the skin was removed and spread flesh-side upwards on a plastic sheet. A small patch (5 x 5 cm) was dissected from the skin at the position where the hair growth measurement was made in study 1. The flesh-side of the skin and the patch were then treated with approximately 2.3 ml/dm^2 of a depilatory solution (1.0 M NaOH and 2.0 M $Na_2S \cdot 9H_2O$) and the skin was stored at 4° C at a relative humidity of 100 %, for 16 h. On removal from the chiller the skin was placed with the hair-side up and the hair was plucked off manually. The hair was then washed for 5 h in a detergent-water solution using a laboratory stirrer. After washing, the hairs were strained through a wire sieve (1.5 mm) and the detergent was removed by rinsing with excess water and the hairs were dried until constant weight. The hair on the 25 cm^2 mid-side patch was collected in a beaker, was washed for 5 h in a detergent-water solution using a magnetic stirrer and was then filtered onto a preweighed glass sintered crucible. The detergent was removed using excess water and the crucible was dried until constant weight. The amount of hair (g) per cat was calculated on a body weight basis and a surface area (cm^2) basis using the equation of Greaves (1957). The amount of hair (mg/cm^2) on the mid-side patch was calculated by dividing the amount of hair on the patch (mg) by the area of the patch (25 cm^2).

3.I.3.3 Data analysis

Ryder (1976) showed that hair growth in cats throughout the year occurs in a sinusoidal pattern and, therefore, the following sine-function was fitted to the daily hair growth (mg/cm^2/day) data for each cat, using the SAS non-linear regression procedure and the Marquardt method (SAS, 1985).

$$Y = a + b * \sin(c * (X - d))$$

where:

Y = dependent variable

X = time in days

a = horizontal shift

b = half amplitude

c = frequency

d = phase start

The parameter ***c*** was set to 0.0172 ($2\pi/365.25$ days) when fitting the sine-function. The estimates of the parameters ***a***, ***b*** and ***d*** were subjected to analysis of variance (GLM procedure, SAS) with gender, shaving interval and the interaction of gender and shaving interval as variables. There was no effect of gender, shaving interval or the interaction between gender and shaving interval on the estimates of ***a*** and ***b*** and the data were thus pooled across sexes and shaving intervals when fitting the sine-functions. There was an effect of gender on the estimates for **d** and separate functions were fitted to the daily hair growth data for the male and female cats with the parameters ***a*** and ***b*** set to 175.5 and 133.9, respectively. The sine-function was also fitted to the body weight data for every cat throughout the study with the values for the parameters *a*, ***b*** and ***d*** being subjected to analysis of variance (GLM procedure, SAS) with gender as a variable. There was no effect of gender on the estimates of ***b*** and ***d*** and the data were thus pooled across sexes. There was an effect of gender on the estimates of ***a*** and separate functions were obtained for the male and female cats with the parameters ***b*** and ***d*** being set to 35 and 299, respectively. The sine-function was also fitted to day length data (time between sun rise and sun set) at longitude 175°38'E, latitude 40°22'S (Lamount, 1993, 1994), and the minimum and maximum daily air temperatures occurring during the study. A paired *t* test was used to determine the level of significance for differences between the hair growth rates ($mg/cm^2/day$) on the left and right mid-side patches of the cats during the 4 wk period at the end of the study.

Student's *t* test was used to determine the effect of gender on the total amount of hair on the body per unit of body weight and surface area, and amount of hair on the mid-side area of the cats in study 2.

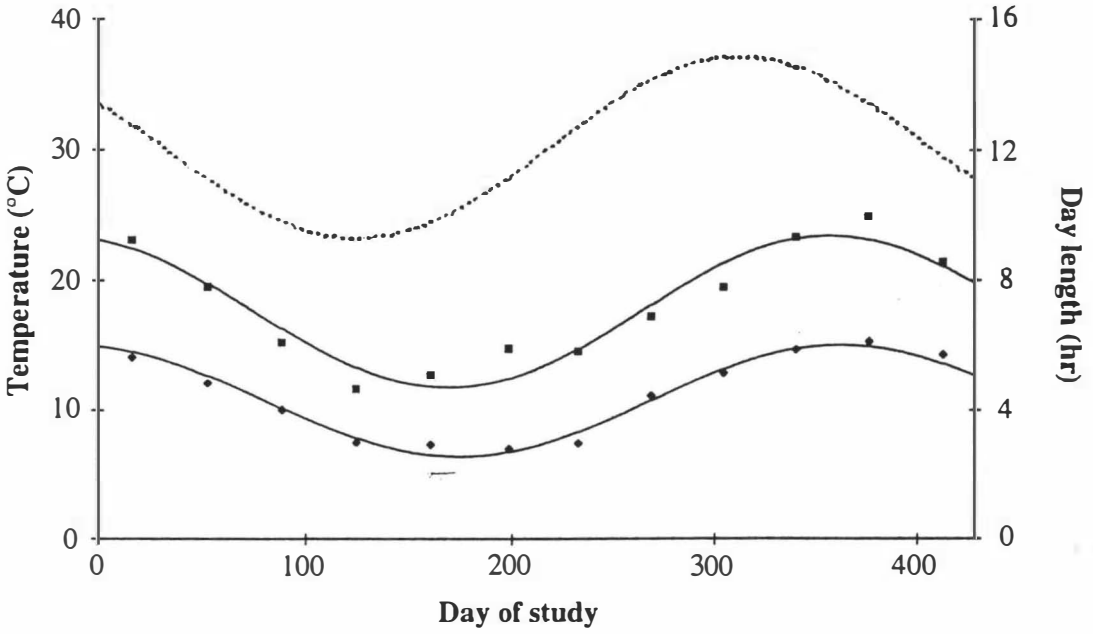

Fig. 1. Day length at long. 175°38'E, lat. 40°22'S (---), average minimum (♦) and maximum (■) monthly temperatures and the sine-functions fitted to the day length (---) and minimum and maximum daily temperature (—) data.

3.I.4 RESULTS

The cats used in study 1 remained healthy throughout the experimental period. There was no significant difference (P = 0.13) in hair growth rate between the left or right mid-side patches of the cats. A mean ± SEM hair growth rate of 0.28 ± 0.01 mg/cm^2/day was found for the left mid-side while a value of 0.27 ± 0.01 mg/cm^2/day was obtained for the right mid-side.

The day length, average minimum and maximum monthly air temperatures during the study and the fitted sine-functions are shown in Figure 1. Day length showed a sinusoidal pattern with the shortest and longest days at 120 and 303 days after the start of the study, respectively. The minimum and maximum daily air temperatures showed a similar sinusoidal pattern, but out of phase with day length, with the coldest and warmest day being at around days 167 and 350, respectively.

The average daily hair growth rate expressed per unit of body surface area for the male and female cats as measured using the mid-side patch technique throughout the 416 day study and the sine-functions fitted to the male and female hair growth rate data are shown in Figure 2. There was a distinct sinusoidal pattern in the hair

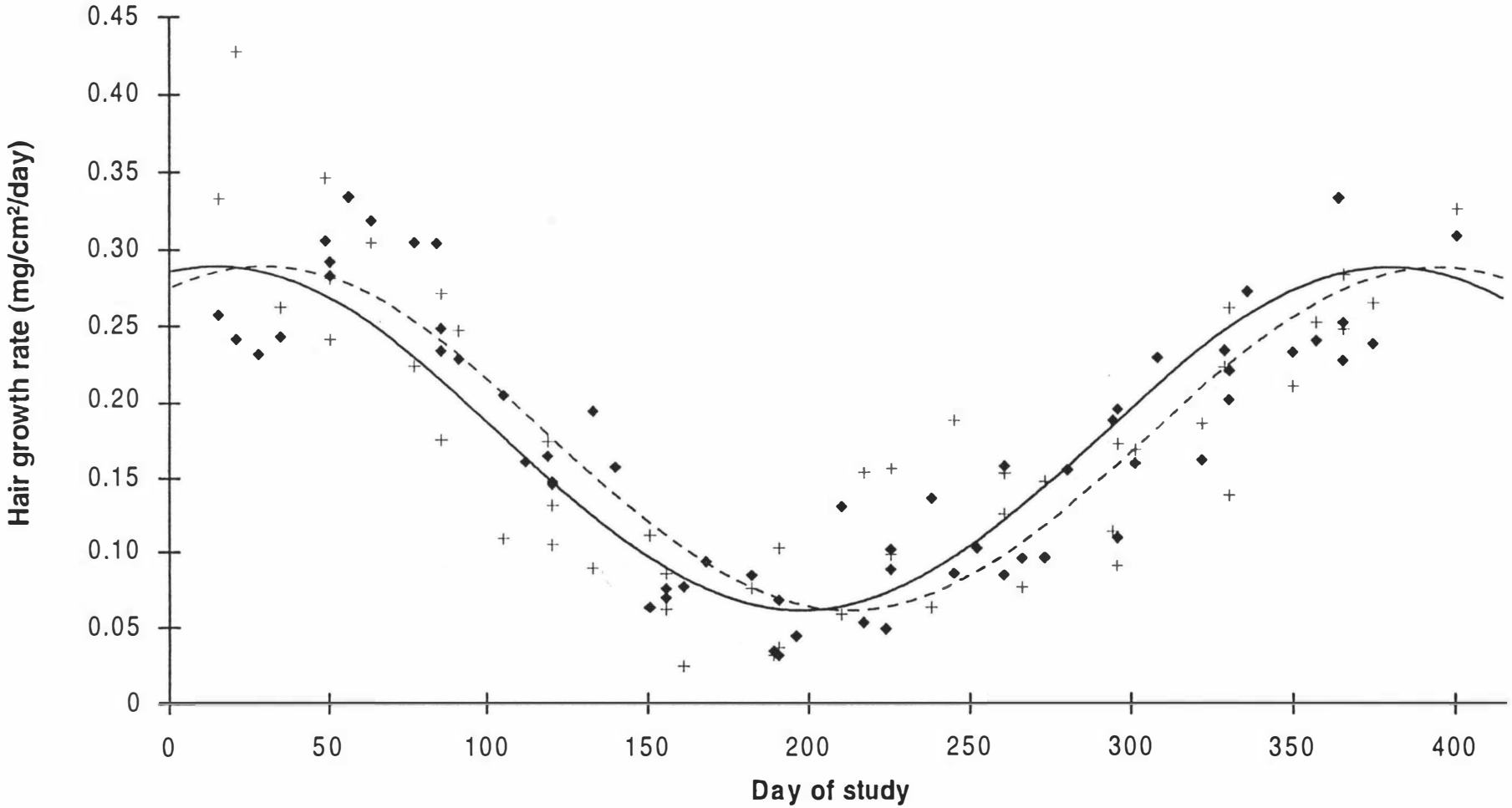

Fig. 2. Daily hair growth rate as measured on the mid-side area in adult domestic short-haired cats of both sexes (◆ = male, average of 8, 5 or 4 animals, + = female, average of 4 or 2 animals) and the sine-functions fitted to the data (--- = male, — = female).

growth rate data with maximum and minimum daily hair growth rates occurring at approximately 20 and 210 days, respectively. A coefficient of variation between cats of 17 % was found for the estimates of ***a*** for the sine-function fitted to the daily hair growth rate data, while a coefficient of variation of 31 % was found for the estimates of ***b***. The estimates of ***d*** had a coefficient of variation of 23 % for the male cats and a coefficient of variation of 25 % for the female cats. There was no significant effect ($P > 0.05$) of gender, shaving interval or the interaction between gender and shaving interval on the estimates of ***a*** and ***b***. Furthermore, there was no effect ($P > 0.05$) of shaving interval or the interaction between gender and shaving interval on the estimates of ***d***. There was, however, an effect ($P < 0.05$) of gender on the estimates of ***d***. Maximum and minimum daily hair growth rates, as estimated using the sine-function fitted to the hair growth rate data, of 289 and 62 $\mu g/cm^2/day$, respectively were found.

Table 1. Parameters (***a***, ***b***, ***d***), coefficient of determination (R^2), minima and maxima of the sine-functions[a] fitted to day length (long. 174°38'E, lat. 40°22'S), minimum and maximum daily air temperature, and mid-side hair growth rate data for adult male and female short-haired cats.

	parameter					
	a	***b***	***d***	R^2	min. (days)	max. (days)
Day length (h)	12.11	2.77	211	1.00	120	303
Temperature (°C)						
min.	10.73	4.32	260	0.96	169	352
max.	17.57	5.80	255	0.96	164	346
Hair growth ($\mu g/cm^2/day$)						
male	175.5	113.9	304	0.91	212	30
female	175.5	113.9	289	0.86	197	15

[a] $f(x)=a+b*\sin(0.0172*(x-d))$ where ***a***=horizontal shift, ***b***=half amplitude and ***d***=phase shift of the sine.

The maximum and minimum hair growth rates for the female cats was reached on days 15 and 197, respectively while the maximum and minimum hair growth rates for the male cats were reached on days 30 and 212, respectively. Table 1 shows the parameters for the sine-function fitted to day length, minimum and maximum daily air temperatures and the daily hair growth rates for the mid-side patch of the male and female cats. There was an effect ($P < 0.05$) of gender on the estimates of ***a*** for the sine-function fitted to the body weight data but no effect ($P > 0.05$) of gender was found for the estimates of ***b*** and ***d***. The male cats were significantly heavier than the

female cats with a mean body weight of 4.35 kg for the males and 3.17 kg for the female cats.

The depilation of the skin by the procedure described in the present study allowed easy removal of most (>99 %) hair. There was no effect ($P > 0.05$) of gender on the total amount of hair on the body per unit of body weight and per unit of surface area, or the amount of hair on the mid-side area. The data were, therefore, pooled across sexes. Table 2 shows the mean, standard error and coefficient of variation for body weight, total amount of hair on the body and the amount of hair on the mid-side area for the 20 cats. There was approximately 20 g of hair per kg of body weight on the body of the cats although there was a large variation (CV 23 %) between animals. The amount of hair collected from the mid-side area had a high variability (CV 26 %) with an average amount of 39.7 mg of hair per cm^2 surface area. The ratio of hair on the whole body to hair on the mid-side area was less variable with a coefficient of variation between animals of 12 % when the total amount of hair on the body was expressed on a body weight basis (ratio 1) and a value of 11 % when the total amount of hair on the body was expressed relative to surface area (ratio 2).

Table 2. Mean, standard error (SEM) and coefficient of variation (CV) for body weight (BW), total body hair and hair on the mid-side patch of adult domestic short-haired cats.

	Mean (n=20)	SEM	CV (%)
Body weight (g)	3819	135	15.8
Total body hair			
(g/kg BW)	19.9	1.0	22.6
(mg/cm^2 SA[a])	23.7	1.2	22.3
Patch hair (mg/cm^2 SA)	39.7	2.3	26.3
Ratio 1 (cm^2 SA/g BW)[b]	0.51	0.01	12.3
Ratio 2[c]	0.61	0.02	11.0

[a]Calculated using surface area (SA)=$14.15*BW^{0.657}$ from Greaves (1957).
[b]Total body hair (mg/g BW)/patch hair (mg/cm^2 SA).
[c]Total body hair (mg/cm^2 SA)/patch hair (mg/cm^2 SA).

3.I.5 DISCUSSION

No significant difference was found for hair growth rates on the left and right mid-side areas over the 4 wk period at the end of study 1, indicating that there was no

effect of the repeated clipping on hair growth rate. Observations made in sheep have shown that there is no effect of clipping *per se* on the growth rate of wool as measured on mid-side patches (Coop, 1953; Downes and Lyne, 1961; Bigham, 1974). In humans, Saitoh *et al.* (1969) measured growth of chest hairs using a sensitive capillary tube method and also found no effect of shaving on hair growth rates. However, a reduction in wool growth has been observed in sheep when the temperature of the clipped area was lowered (Bottomley, 1978). In the present study, there may have been an effect of the reduced insulation of the mid-side patch on the hair growth rate due to the clipping. This effect, however, is expected to be minor as the clipped area was small (3 x 4 cm) and was partially covered by the surrounding hairs throughout the experiment. Also there was no influence of the interval (frequency) of clipping on the mid-side patch hair growth rate, in the present study. This is in accordance with observations made in sheep where the frequency of clipping a patch of skin has been found to have no effect on wool growth (Coop, 1953; Bigham, 1974).

The body weight of the cats throughout the study showed no obvious sinusoidal pattern. A half amplitude (parameter ***b***) of the sine-function fitted to the body weight data of 35 g was found indicating that the average variation in body weight throughout the year was small. This was also observed by Ryder (1976) who reported a slight tendency for an increased body weight of cats during summer and a decrease during winter.

The daily hair growth rate on the mid-side patch of cats as measured over the experimental period (Fig. 2) showed a distinct sinusoidal pattern similar to the pattern for day length. Hair growth rates in many animals including cattle (*Bos taurus*), ferrets (*Mustela furo*), dogs (*Canis familiaris*) and sheep (*Ovis aries*), have been shown to be regulated by photoperiod (Yeates, 1957; Harvey and Macfalane, 1958; Nagorcka, 1978; Rougeot, 1981). Although no measurements have been made in cats, Baker (1974) implied that the increased photoperiod is a stimulus for the replacement of hair in cats and the present data are in line with this hypothesis. The minimum and maximum daily temperatures also showed a sinusoidal pattern out of phase with the day length and, although unlikely, it cannot be excluded that temperature may regulate hair growth in cats.

The sine-function fitted to the day length data explained more than 99.9 % of the variation in the data. Baker (1974) showed that hair growth in adult cats follows a

sinusoidal patterns throughout the year and, therefore, a sine-function was fitted to the daily hair growth rate data of the cats in the present study. The sine-function fitted to the daily mid-side hair growth rate, in the present study, explained 91 % of the variation in the data for male cats and 86 % of the variation in the data for female cats. Nagorcka (1979) found slightly lower coefficients of determination (0.77 and 0.78, respectively) when fitting a sine-function to wool growth data from Ferguson *et al.* (1949) and Hart *et al.* (1963). The sinusoidal hair growth pattern in cats has also been observed by Baker (1974) who measured hair follicle inactivity in different cats killed during the year in the northern hemisphere. Maximum follicle inactivity was observed during February and March which corresponds to maximum daily hair growth rates found in the present study. Ryder (1976) measured the follicle inactivity of primary and secondary hairs for two male cats and one female cat for a 19 month period, in the southern hemisphere, and found the same sinusoidal pattern in follicle inactivity as the sinusoidal pattern in daily hair growth rates found in the present study. The minimum follicle inactivity (40 %) of the primary and secondary hairs was found in mid-summer while the maximum follicle inactivity (85 %) was found in winter (Ryder, 1976). These percentages agree closely with the estimates for minimum and maximum hair growth rates on the mid-side area found in the present study. Assuming that 40 % of the follicles were inactive during the period relating to the highest daily hair growth rate (289 $\mu g/cm^2$/day, Table 1) in the present study, an estimate of the minimum daily hair growth rate (85 % of the follicles inactive) of 72 $\mu g/cm^2$ would be obtained. This estimate is similar to the minimum daily hair growth rate of 62 $\mu g/cm^2$ (Table 2) found here.

There was no difference in the average daily hair growth rate (parameter *a*) and the half amplitude of the variation in the daily hair growth rate (parameter *b*) between the male and female cats. Baker (1974) measured daily growth of hair length for a male and two female cats during an eleven month period and concluded that the average daily hair growth rates over the eleven month period were not different for the two sexes. The relatively small coefficient of variation found for parameter *a* (17 %) in comparison to parameter *b* (31 %) in the present study, indicates that the average daily hair growth rate between cats is similar but that there is a large variation in the amplitude of the daily hair growth rate. A significant difference in the timing of the hair growth (parameter *d*) was found between the male and female cats in the present study. Ryder's (1976) data shows that the maximum follicle inactivity

of the female cat is reached earlier than that of the adult male cats which is consistent with the results found in the present study. Reproductive hormones have been shown to have an effect on the timing of hair growth in the rat (*Rattus rattus*) and guinea-pig (*Cavia porcellus*). Johnson (1958) and Jackson and Ebling (1972) showed that oestradiol delays the initiation of hair growth in the female of these species. Johnson (1958) also showed that testosterone had no effect on the initiation of hair growth in the male rat. As all the cats in study 1 were castrated, it is possible that the reduced levels of body oestrogen in the female cats may have resulted in hair growth being initiated earlier in comparison to the hair growth of the male cats which were unaffected by the reduced levels of body testosterone

The mid-side patch technique has been used to measure wool growth in sheep (Coop, 1953; Chapman and Young, 1957; Bigham, 1974) and hair growth in dogs (Mundt and Stafforst, 1987). In sheep, it has been shown that the wool growth rate on the mid-side area is close to the average wool growth rate over the entire body (Chapman and Young, 1957). Wool production can, therefore, be estimated by using the mid-side patch hair growth rate and an estimate of the total wool-bearing skin surface (Chapman and Young, 1957). Another method to estimate wool production in sheep was used by Hawker *et al.* (1984) and Hawker and Crosbie (1985), who partitioned the fleece weight of sheep according to the relative weights of wool clipped from the mid-side patch at different times. No measurement of the relationship between the hair growth rate on the mid-side area and the hair growth rate on other areas of the body has been made in cats and it is unknown whether the mid-side hair growth rate is representative of the hair growth rate over the entire body. Furthermore, as cats loose significant amounts of hair throughout the year, the latter method used by Hawker *et al.* (1984) and Hawker and Crosbie (1985) cannot be used. In the present study and in order to convert the mid-side patch hair growth rate to hair growth rate over the entire body, the assumption was made that hair growth rate per unit of hair weight on the mid-side patch is representative of the hair growth rate per unit of hair weight over the entire body (i.e. the turn-over of hair over the entire body of the cat is similar). Evidence to support this assumption is given by Baker (1974) who states that hair replacement occurs uniformly throughout the pelage in adult cats. This indicates that the turn-over of hair is similar on any area of the cats body, otherwise uniform hair replacement could not occur. Therefore, to convert hair growth rate on the mid-side patch to hair growth rate over the entire

body, in the present study, the ratio of the amount of total body hair (in mg/g body weight and mg/cm^2 surface area) to the amount of hair on the mid-side area (mg/cm^2) was determined. Ratio 1 (Table 2) can be used to convert mid-side patch hair growth rates to hair growth rates over the entire body expressed on a body weight basis while ratio 2 (Table 2) can be used to convert mid-side patch hair growth rates to hair growth rates over the entire body expressed on a surface area basis. The amount of hair grown by domestic short-haired cats over a period of time can now be estimated by integrating the sine-function over a designated period and multiplying this value by the body weight of the cat and ratio 1 (Table 2) or the surface area of the cat and ratio 2 (Table 2). Relationships between the surface area and the body weight of cats have been published by Greaves (1957) and Bartorelli and Gerola (1963). The monthly hair growth in male and female adult domestic short-haired cats at longitude 175°38'E and latitude 40°22'S calculated using the above described method is shown in Table 3.

Table 3. Monthly hair growth rate[a] per unit body weight (BW) and surface area (SA) for adult male and female domestic short-haired cats (long. 174°38'E, lat. 40°22'S).

	Hair growth rate			
Month	Male		Female	
	(g/kg BW/month)[b]	(mg/cm^{2}SA/month)[c]	(g/kg BW/month)[b]	(mg/cm^{2}SA/month)[c]
January	3.54	4.21	3.93	4.67
February	3.86	4.59	4.05	4.82
March	4.55	5.41	4.54	5.40
April	4.26	5.07	4.03	4.80
May	3.82	4.55	3.42	4.07
June	2.86	3.41	2.42	2.88
July	2.05	2.43	1.66	1.97
August	1.32	1.57	1.11	1.32
September	0.97	1.15	0.99	1.17
October	1.16	1.38	1.40	1.67
November	1.70	2.02	2.09	2.48
December	2.62	3.12	3.07	3.66

[a]Calculated using integrated function $F(x)=a*x-(b/0.0172)*\cos(0.0172*(x-d))$ and estimates of a, b and d (Table 1).
[b]Calculated using ratio 1 (Table 2).
[c]Calculated using ratio 2 (Table 2).

The calculated turn-over of hair on the mid-side area for cats in the present study was 226 days. This, however, may not be an entirely accurate estimate as the amount of hair found on the mid-side area (39.7 mg/cm^2) may not have been the average amount of hair present on the mid-side area throughout the year because 8

cats used in study 2 were obtained in summer and 11 cats were obtained in early autumn. Moulting starts in October (Baker, 1974; Ryder, 1976) and the cats in study 2 would have had a relatively "light" coat at the time of measurement as compared to winter. However, this should not affect the ratio of the amount of hair on the entire body to the amount of hair on the mid-side area (ratio 1 and 2), as this may be expected to be constant throughout the year.

Mundt and Stafforst (1987) measured hair growth in various breeds of another carnivore, the dog (*Canis familiaris*), and reported yearly amounts of hair growth of 60-186 g per kg body weight, depending on the breed of dog. In the present study, a yearly amount of hair growth of 32.7 g per kg body weight was found for adult short-haired cats which is about half of the lowest value of hair growth reported by Mundt and Stafforst (1987) for dogs. The total amount of body hair of cats, however, is comparable to that of dogs with a high weight of body hair. Mundt and Stafforst (1987) found an average amount of body hair of 20.5 g per kg body weight for hairy breeds of dog (pekinese, collie, pomeranian, German shepherd dog) which is comparable to the 19.9 g of hair per kg body weight found in the present study for cats.

The present study provides estimates of hair growth in adult male and female short-haired domestic cats. These can be used in the determination of the nitrogen and amino acid requirements of the adult domestic cat by the factorial method.

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CHAPTER 3 PART II

SEASONAL HAIR LOSS IN THE ADULT DOMESTIC CAT (*FELIS CATUS*)

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Comparative Biochemistry and Physiology (submitted)

3.II.1 ABSTRACT

The amount of hair loss in adult domestic short-haired cats throughout the year was determined. Ingested and non-ingested hair loss from a total of 16 adult cats was collected during 12 periods of 31 days, covering a period of 416 days in total. The ingested, non-ingested and total hair loss rates showed sinusoidal patterns throughout the year with the maximum and minimum hair loss rates occurring in summer and winter, respectively. There was no significant difference in hair loss rate between male and female cats. Sine-functions fitted to day length, minimum and maximum daily air temperature, and the ingested, non-ingested and total hair loss rate data in adult cats throughout the year are given. Adult cats were found to lose 28.1 g hair per kg body weight per year with two-thirds of the total hair loss found in the feces. Monthly ingested, non-ingested and total hair loss rates are presented. Combining hair loss and hair growth data, it is shown that the changes in the pelage of the cat throughout the year are optimally regulated to obtain the densest coat at the coldest period of the year and the sparsest coat at the warmest period of the year.

3.II.2 INTRODUCTION

Moulting in mammals is a cyclic phenomenon and involves the growth and replacement of hairs (Johnson, 1972 and 1984). The well known seasonal hair replacement pattern in cats is achieved by the timing of the activity of the hair follicles. As more hair follicles become active in early spring, more hairs are lost (Ryder, 1976). The individual hair follicles, furthermore, behave independently from each other in the cat resulting in a mosaical hair replacement pattern (Baker, 1974; Ryder 1976). There is little information in the literature on the quantitative aspects of hair loss in adult domestic short-haired cats.

Recently Hendriks *et al.* (1996) determined daily hair growth rates on the mid-side area of adult short-haired cats of both sexes. The latter authors converted the daily hair growth rates on the mid-side area to daily hair growth rates over the entire body, by assuming that the hair growth rate per unit of hair weight on the mid-side area was representative of the hair growth rate per unit of hair weight over the entire body. This method yielded an average value for hair growth rate over the entire body of cats of 32.7 g per kg body weight per year. As the amount of hair grown should equal the amount of hair lost over the year in adult cats, the hair loss in adult domestic short-haired cats should be approximately 33 g per kg body weight.

The present study was conducted to generate information on the amount and pattern of hair loss throughout the year in adult domestic short-haired cats. Hair loss was measured in cats of both sexes during a 416 day study in which the amounts of ingested and non-ingested hair loss were measured.

3.II.3 MATERIALS AND METHODS

The study reported here was approved by the Massey University Animal Ethics Committee.

3.II.3.1 Animals and diet

Sixteen (8 male, 8 female) 2-6 y old short-haired domestic cats from the BestFriend Feline Nutrition Research Unit, Massey University, Palmerston North, New Zealand (longitude 175°38'E, latitude 40°22'S) with an initial body weight range of 3018 to 4626 g (mean ± SEM: 3798 ± 134 g) were used. Prior to the hair collection period, the

cats were housed in two large wire netting outdoor pens (4.5 x 1.4 x 2.5 m) with 4 males and 4 females in each pen. Throughout the study the cats were fed, to appetite, a commercially available canned cat food (g/100g dry matter: protein 52.0, fat 28.5, carbohydrates 10.5, ash 9.0) of proven nutritional quality (AAFCO, 1993). Water was available at all times.

3.II.3.2 Experimental design

Hair from each cat was collected for six non-consecutive periods of 31 days each, with collection alternating between the two pens. The design was such that after collection of hair from the cats in a pen, there was a 4 day period in which no hair was collected before collection of hair from the cats in the other pen. The experiment was started on February 21, 1994 and ended on April 13, 1995. The cats were exposed to normal day length and natural lighting.

3.II.3.3 Hair collection

At the start of a hair collection period the cat was removed from its pen, weighed and individually housed in a metabolism cage fitted with a litter tray allowing total collection of uncontaminated feces. The metabolism cage was made from a polyethylene plastic bin (0.8 x 1.1 x 0.8 m) with a partially covered galvanized steel barred door fitted to the front. The metabolism cage was positioned outdoors, in a semi-enclosed area shielded from wind and rain. All hair in the cage, water bowl, food bowl, and litter tray was collected daily in water using a damp cloth. Urine was passed through a wire sieve (mesh size: 0.5 mm) and the hairs retained by the sieve were added to the water. During the cleaning of the metabolism cage the cat was housed in a closed plastic bin from which hairs could be collected. The hair containing water solution was passed through the 0.5 mm mesh size sieve and the hairs retained by the sieve were stored in a plastic bag. Feces were collected and weighed each day, and total feces collected on day 4, 8, 12, 16, 20, 24 and 28 frozen (-20°C) until cleaning. On three days (Mon., Wed., Fri.) of each week during the 31 day collection period, each cat was gently brushed and the hairs collected during brushing were bulked and stored in a plastic bag. The brushing of the cat was employed to reduce the amount of hair lost in the cage and to mimic environmental abrasive factors influencing hair loss. At the end of the 31 day collection period each cat was gently brushed, weighed and returned to its outdoor group pen. Minimum

and maximum daily air temperatures at the cage level were recorded throughout the study.

3.II.3.4 Hair cleaning

The bulked hair, collected from the metabolism cage and from brushing of the cat, was manually freed of major contaminants and washed for 24 h in a detergent/water solution. The washed hair was strained through a wire sieve (mesh size: 0.5 mm), rinsed with excess water to remove detergent and was dried to a constant weight. This procedure ensured that a clean, uncontaminated fraction was obtained. The frozen feces were weighed, freeze-dried, manually crushed and passed through a wire sieve (mesh size: 0.5 mm). The fraction passing through the sieve contained no hair, as examined by microscopy, and was discarded. The fraction retained by the sieve was suspended in a detergent/water solution and mixed on a magnetic stirrer. After an homogenous suspension was obtained (24 h) the liquid was carefully passed through the 0.5 mm mesh size sieve and the retained hair was washed again. Care was taken to exclude any solid particles at the bottom of the beaker. The above cleaning procedure was repeated twice more, whereafter the clear hair-containing liquid was filtered through a pre-weighed sintered glass crucible. Detergent was removed by rinsing with excess water and the crucible was dried to constant weight.

The total amount of ingested hair loss per collection period was calculated by multiplying the amount of cleaned fecal hair from the collected feces by the ratio of the total amount of feces produced per period and the amount of feces sampled per period. The daily ingested hair (IH) loss rate and non-ingested hair (NIH) loss rate (hair collected from the metabolism cage plus brushing) were calculated by dividing the amount of IH and NIH loss per collection period by 31. The total hair (TH) loss rate per period was calculated by addition of the IH and NIH loss per period and dividing by 31. The daily IH, NIH and TH loss rates were expressed on a body weight basis using the average body weight of the cat throughout the study and a surface area basis using the equation of Greaves (1957). The hair loss rate calculated in this manner was taken to be the rate of hair loss at the median of the time period.

3.II.3.5 Data analysis

Ryder (1976) and Hendriks *et al.* (1996) showed that hair growth in cats throughout the year follows a sinusoidal pattern. Therefore, the following sine-function was

fitted to the body weight and the IH, NIH and TH loss rate data for each cat using a non-linear regression procedure and the DUD method (SAS, 1985).

$$Y=a+b*\sin(c*(X-d))$$

where:

Y = dependent variable

X = time in days

a = horizontal shift

b = half amplitude

c = frequency

d = phase start

The parameter c was set to 0.0172 ($2\pi/365.25$ days) when fitting the sine-function. The estimates of the parameters a, b and d for each data set were subjected to ANOVA (SAS 1985) with gender, pen and the interaction between gender and pen as variables. Differences were considered significant at $P < 0.05$.

There was no effect of gender, pen or the interaction between gender and pen on the estimates of b and d of the sine-function fitted to the body weight data of the cats. There was an effect of gender on the estimates of a of the sine-function fitted to the body weight data of the cats with no effect of pen or the interaction between gender and pen. The data were thus pooled across sexes and pens to obtain estimates for b and d and the data were pooled across pens to obtain an estimate of a for the male and female cats with the parameters b and d set to 57.9 and 34, respectively.

There was no effect of gender, pen or the interaction between gender and pen on the estimates of the parameters a, b and d of the sine-function fitted to the IH, NIH and TH loss rate data and the data were thus pooled across sexes and pens when fitting the sine-functions.

The sine-function was also fitted to day length data (time between sunrise and sunset) at longitude 175°38'E, latitude 40°22'S (Lamont, 1993 and 1994) and the minimum and maximum daily air temperature data.

3.II.4 RESULTS

The cats remained healthy throughout the study. There was an effect of gender on the estimates of ***a*** for the sine-functions fitted to the body weight data with no effect of pen or the interaction between gender and pen on the estimates of ***a***. There was no significant effect of gender, pen or the interaction between gender and pen on the estimates of ***b*** and ***d***. An average body weight (parameter ***a***) throughout the study of 4235 g was found for the male cats with the female cats weighing on average 3378 g. Estimates of 57.9 and 34 were obtained for the parameters ***b*** and ***d***, respectively, when the sine-function was fitted to pooled body weight data.

Day length showed a sinusoidal pattern with the shortest and longest days at 120 and 303, respectively, after the start of the study. The minimum and maximum daily air temperatures showed a similar sinusoidal pattern with the coldest and warmest days at approximately 167 and 350, respectively.

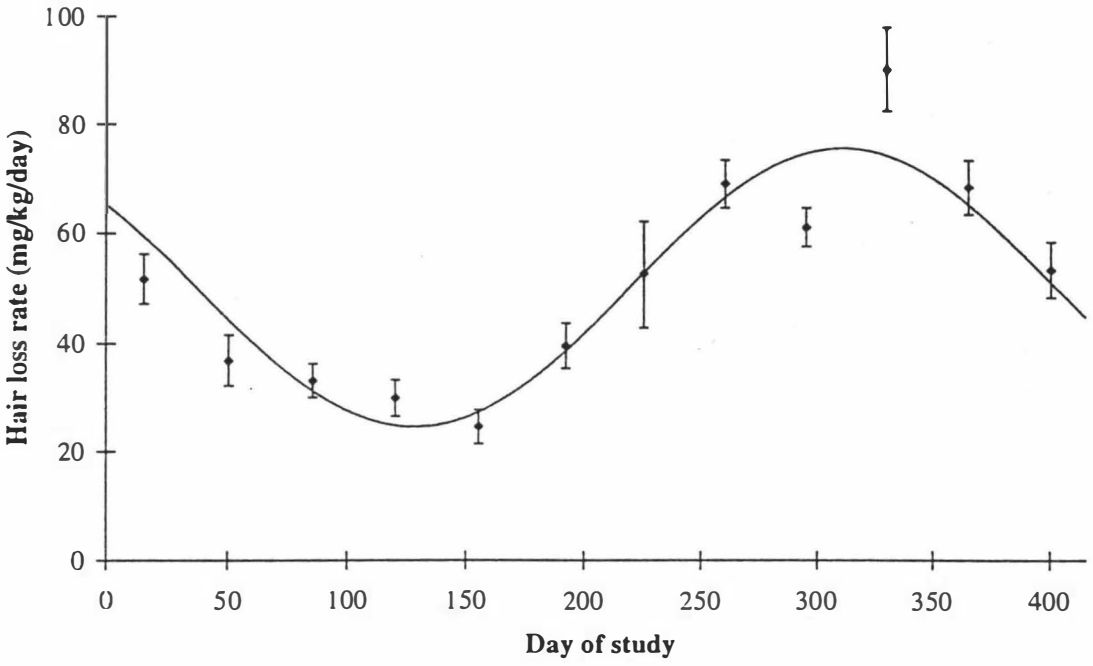

Fig 1. Mean (± SEM) ingested daily hair loss rate in adult domestic short-haired cats and the sine-function fitted to the data (♦ = average of 4 male and 4 female cats).

There was no statistically significant effect of gender, pen or the interaction between gender and pen on the estimates of ***a***, ***b*** and ***d*** of the sine-functions fitted to the IH, NIH and TH loss rate data. The daily IH loss over the 12 periods and the sine-

function fitted to the data are shown in Figure 1. The IH loss data showed a distinct sinusoidal pattern similar to that found for day length. The minimum amount of hair ingested (25 mg/kg body weight/day) was found at 130 days after the start of the study and the maximum amount of hair ingested (75 mg/kg body weight/day) was found at approximately 310 days. The daily NIH loss throughout the study and the sine-function fitted to the data are shown in Figure 2. The NIH loss rate data also showed a sinusoidal pattern with the minimum amount of NIH loss of 10 mg/kg body weight/day at 135 days and the maximum of 33 mg/kg body weight/day at 317 days after the start of the study. The amount of hair collected from the metabolism cage and by brushing was approximately half of the amount of hair found in the feces.

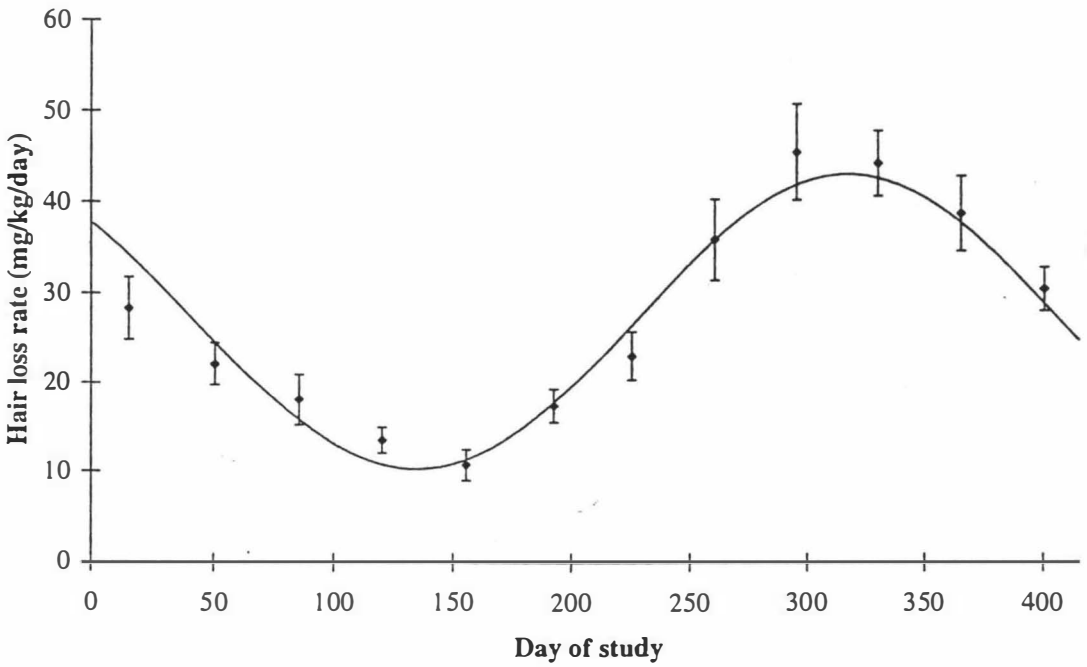

Fig 2. Mean (± SEM) non-ingested daily hair loss rate in adult domestic short-haired cats and the sine-function fitted to the data (♦ = average of 4 male and 4 female cats).

Figure 3 shows the daily TH loss for the cats throughout the study and the sine-function fitted to the data. A maximum amount of 118 mg of hair per kg body weight per day was lost around day 310 of the study and the least hair (35 mg/kg body weight/day) was lost around 130 days after the start of the study.

The estimates of ***a***, ***b*** and ***d*** of the sine-function fitted to day length, minimum and maximum daily air temperatures and the daily IH, NIH and TH loss expressed on

a body weight and body surface area basis are shown in Table 1.

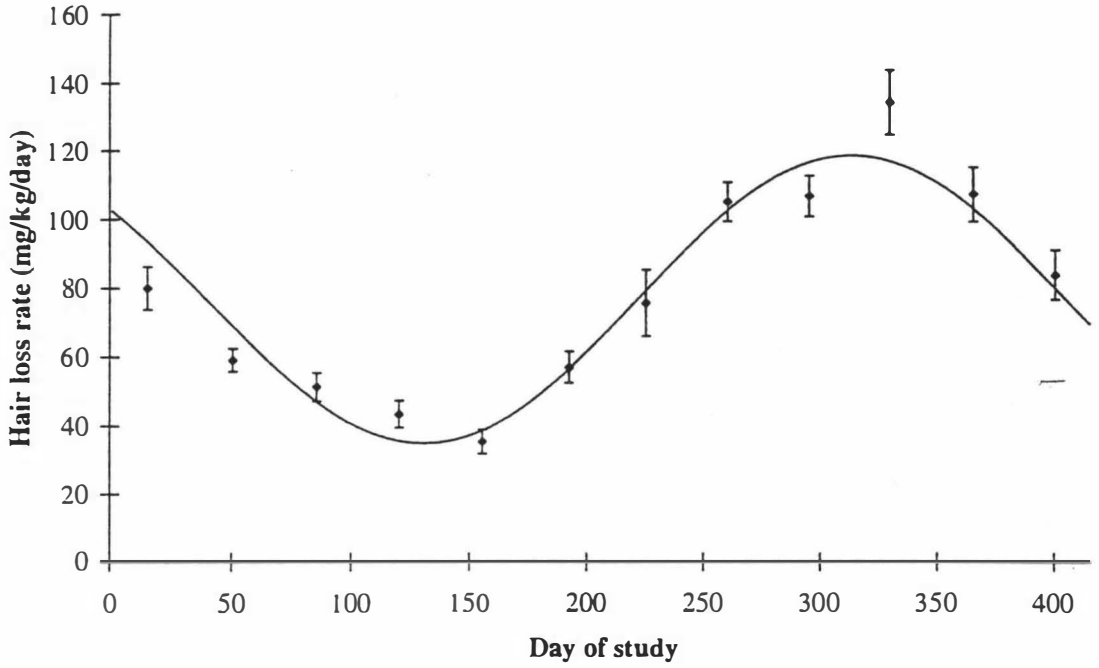

Fig 3. Mean (± SEM) total daily hair loss rate in adult domestic short-haired cats and the sine-function fitted to the data (♦ = average of 4 male and 4 female cats).

Table 1. Parameters (*a*, *b*, *d*), coefficient of determination (R^2), minima and maxima for the sine-function[a] fitted to day length (long. 174°38'E, lat. 40°22'S), minimum and maximum daily air temperature, ingested hair loss, non-ingested hair loss and total hair loss data for adult domestic short-haired cats throughout the year.

	parameter					
	a	*b*	*d*	R^2	min. (days)	max. (days)
Day length (h)	12.11	2.77	211	1.00	120	303
Temperature (°C)						
min.	10.73	4.32	260	0.96	169	352
max.	17.57	5.80	255	0.96	164	346
Ingested hair loss						
(mg/kg BW/day)	50.15	25.46	220	0.92	128	311
(µg/cm^2 SA/day)	63.95	32.49	221	0.93	130	313
Non-ingested hair loss						
(mg/kg BW/day)	26.65	16.41	226	0.92	135	317
(µg/cm^2 SA/day)	34.56	21.61	226	0.89	135	318
Total hair loss						
(mg/kg BW/day)	76.80	41.81	222	0.95	131	313
(µg/cm^2 SA/day)	98.51	54.04	223	0.94	132	315

[a] $f(x)=a+b*\sin(0.0172*(x-d))$ where *a*=horizontal shift, *b*=half amplitude and *d*=phase shift of the sine.

BW, body weight; SA, surface area.

3.II.5 DISCUSSION

The body weight of the cats in the present study showed only a small sinusoidal pattern throughout the year with an average change in body weight for the male and female cats of only 1.4 and 1.7 %, respectively. There was an increased body weight during summer and a decreased body weight during winter as found by Ryder (1976). Hendriks *et al.* (1996) also fitted the sine-function to the body weight data of 39 cats and found a small change in body weight (parameter b=35.0) throughout the year. This is unlike ferrets (*Mustela furo*), mink (*Mustela vison*) and polecats (*Mustela putorius*) which exhibit marked seasonal changes in their body weight of up to 15 % (Harvey and Macfarlane, 1957; Korhonen, 1988). It can be concluded that adult cats fed to appetite keep a relatively constant body weight throughout the year.

In a preliminary study (W Hendriks, unpublished), more than 99 % of a preweighed amount of cat hair suspended in water was retained by the 0.5 mm mesh size sieve, used for the collection of hair in the present study. It was concluded, therefore, that the method described here was satisfactory for the collection of cat hair from solutions. The hair cleaning procedure employed in the present study resulted in relatively clean uncontaminated samples of hair. Although no direct measurements of the purity of the cleaned hair fractions could be made, the fractions of hair obtained after cleaning the ingested and non-ingested hair were judged, using microscopy, to contain minimal contamination. Loss of hair could have occurred through the door of the metabolism cage which was only partially covered. However, this loss was expected to be small as hairs lost in the metabolism cage tended to adhere to the plastic surface of the cage.

The daily IH, NIH and TH loss rate data showed distinct sinusoidal patterns with minimum daily hair loss rates occurring during mid winter and maximum daily hair loss rates occurring during the middle of summer. This hair loss pattern has also been observed by Baker (1974). Ryder (1976) noted that cats have no marked and obvious moult and that hair replacement occurs gradually. The present study supports this view as there was a non-abrupt decrease in the amount of daily hair loss rate until day 131, whereafter a non-abrupt increase occurred until day 314.

The amount of hair found in the feces was approximately two-thirds of the total amount of hair lost by the cats in the present study. This is surprising, but not totally unexpected, as problems relating to the ingestion of large amounts of hair have been

documented in cats (Reed, 1975; Hart, 1976). The large amounts of hair normally present in the feces of the cat may influence the determination of protein and amino acid digestibilities in this species. As hair has been shown to mainly consist of amino acids (Gold and Scriver, 1971; Jørgensen and Eggum, 1971; Mundt and Stafforst, 1987), the digestibility of amino acids may be underestimated. This may be particularly significant for the amino acid cysteine which is found in high concentrations in hair (Gold and Scriver, 1971; Jørgensen and Eggum, 1971; Mundt and Stafforst, 1987).

There was no difference in the average amount of hair loss (parameter ***a***), half amplitude of the variation in hair loss (parameter ***b***) or timing of the hair loss (parameter ***d***) between the male and female cats. Hendriks *et al.* (1996) measured hair growth in adult cats of both sexes and also found no difference in the average amount of hair growth or half amplitude of the hair growth between male and female cats. In the latter study, however, a difference in the timing of the hair growth between male and female cats was found with the female cats growing hair significantly earlier than males. The number of measurements of hair loss in the present study may be the reason for this apparent discrepancy as there were only 12 collection periods of 31 days duration each. The difference in hair growth between male and female cats as found by Hendriks *et al.* (1996) was 15 days and this difference in hair growth may have been too small to detect in the present study.

The sine-functions describing daily hair loss rates (Table 1) in adult domestic short-haired cats throughout the year can generate estimates on the amount of hair loss during a designated period of time by using the integral of the sine-functions. The monthly amount of IH, NIH and TH loss in adult domestic cats expressed per unit of body weight and body surface area, calculated using the respective integrated sine-function and estimates of ***a***, ***b*** and ***d*** are shown in Table 2. The cats lost the most hair during spring and summer with relatively small amounts of hair being lost during autumn and winter. A total hair loss of 28.1 g per kg body weight per year was found for adult domestic short-haired cats in the present study.

Hendriks *et al.* (1996) determined hair growth in adult domestic short-haired cats and found a total amount of hair growth of 32.7 g per kg body weight per year. To derive this latter value Hendriks *et al.* (1996) made the assumption that the hair growth rate per unit of hair weight on the mid-side area was representative of the hair growth rate per unit of hair weight over the entire body (i.e. the turn-over of hair

throughout the pelage is the same). Evidence to support the latter assumption was published by Baker (1974) who noted that hair growth occurs uniformly throughout the pelage of the cat, indicating that there are no areas on the cat's body which have a higher or lower turn-over. Furthermore, that the two values for the amount of hair loss (28.1 g/kg body weight/year) and hair growth (32.7 g/kg body weight/year) agree closely, supports the assumption made by Hendriks *et al.* (1996).

Table 2. Monthly ingested, non-ingested and total hair loss rate[a] per unit body weight (BW) and surface area (SA) for adult domestic short-haired cats (long. 174°38'E, lat. 40°22'S).

	Hair loss rate					
Month	Ingested	Non-ingested	Total	Ingested	Non-ingested	Total
	(g/kg BW/month)			(mg/cm^2 SA/month)		
January	2.30	1.32	3.61	2.94	1.72	4.66
February	1.90	1.10	3.00	2.44	1.43	3.87
March	1.74	0.99	2.73	2.24	1.30	3.54
April	1.29	0.71	2.00	1.67	0.93	2.59
May	0.99	0.50	1.49	1.28	0.65	1.92
June	0.77	0.34	1.11	0.99	0.43	1.42
July	0.81	0.33	1.14	1.02	0.42	1.44
August	1.03	0.45	1.47	1.29	0.57	1.86
September	1.34	0.64	1.98	1.68	0.83	2.51
October	1.79	0.92	2.71	2.26	1.20	3.45
November	2.06	1.12	3.18	2.61	1.46	4.07
December	2.32	1.30	3.62	2.95	1.70	4.65

[a]Calculated using the integrated function $F(x)=a*x-(b/0.0172)*\cos(0.0172*(x-d))$ and estimates of ***a***, ***b*** and ***d*** (Table 1).

The processes of hair growth and hair loss occur simultaneously throughout the year in adult cats. Using the data of Hendriks *et al.* (1996) for daily hair growth, and the daily hair loss data in the present study, the change (increase or decrease) in the amount of hair on the body of the cat throughout the year can be determined by subtracting daily hair loss from the daily hair growth. Figure 4 shows the sine-functions describing daily hair growth and hair loss rates in adult cats and the change in hair weight on the body of the cats throughout the year. The total amount of hair loss per year was assumed to equal the total amount of hair growth and, therefore, an average value for parameter *a* of 83.2 and parameter ***b*** of 50.0 was used from the hair growth data of Hendriks *et al.* (1996) and the hair loss data from the present study. The estimate for the parameter ***d*** for hair growth was taken from Hendriks *et al.* (1996) and for hair loss from the present study. As can be seen from Figure 4, hair loss in the cat was found to be approximately 75 days out of phase with hair growth

resulting in the minimum and maximum hair growth rates occurring approximately 75 days after minimum and maximum hair loss rates had occurred, respectively. The sine-function describing the rate of increase or decrease of body hair shows that during summer and autumn there was a net build up of hair on the body of the cat with a net loss of hair during winter and autumn. At days 168 and 351 there was no increase or decrease in the amount of hair on the body of the cat and at these days the cats had the highest and lowest amounts of hair on their body, respectively. The coldest and warmest days during the studies, as determined using the sine-function fitted to the minimum and maximum daily air temperatures, were day 167 and 350, respectively (Table 1).

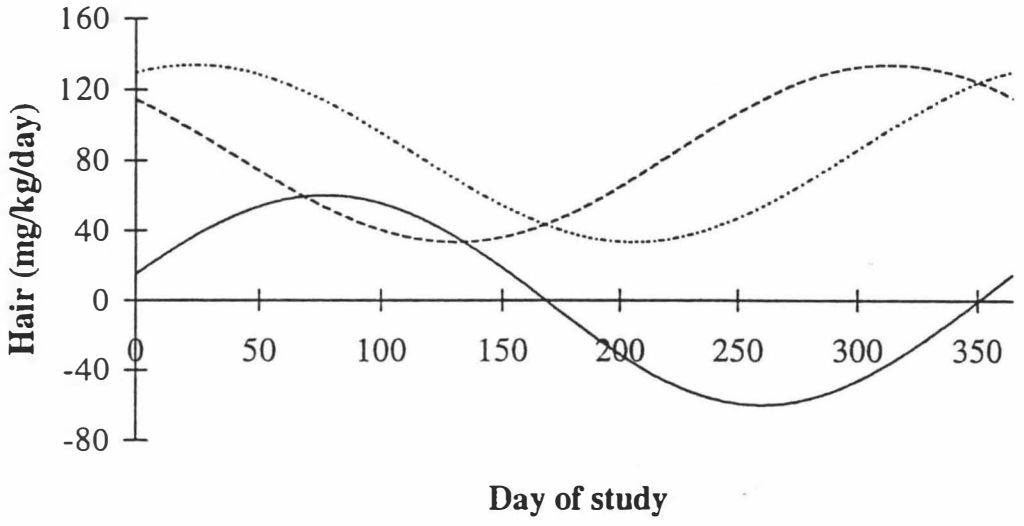

Fig 4. Sine-functions[a] describing daily hair growth (···) and hair loss (----) rate[b] and the daily change in body hair[c] (—) throughout the year in adult domestic short-haired cats (longitude 174°38'E, latitude 40°22'S). [a]$f(x)=a+b*\sin(0.0172*(x-d))$ where a=horizontal shift, b=half amplitude and d=phase shift of the sine. [b]Average value of 83.2 and 50.0 for parameter a and b taken from Hendriks *et al.* (1996) and the present study. Values for parameter d for hair growth taken from Hendriks *et al.* (1996) and for hair loss from the present study. [c]$y=58.6*\sin(0.0172*x)+14.9*\cos(0.0172*x)$.

These results indicate that the processes of hair growth and hair loss in adult cats are regulated so that this animal obtains the densest coat at the coldest period of the year while the sparsest coat is obtained at the warmest period of the year. It, furthermore, seems likely from the similar hair growth, hair loss and day length

patterns, that photoperiod, besides controlling the reproduction cycle in female cats (Scott and Lloyd-Jacob, 1959; Hurni, 1981), regulates the processes of hair growth and hair loss. Integrating the function describing the rate of change in body hair of the cat throughout the year over the period between 167 and 351 days, it can be calculated that the difference between the densest and sparsest coat of adult short-haired cats is 7.0 g of hair per kg body weight.

The present study provides estimates of ingested, non-ingested and total hair loss throughout the year in short-haired adult domestic cats. In the cat, the processes of hair loss and hair growth appear to be optimally timed to give the densest coat during the coldest days of the year and the sparsest coat during the warmest days of the year. The amino acid requirements of the adult cat should follow the change in the amino acid requirements for the replacement of hair.

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CHAPTER 3 PART III

THE AMINO ACID COMPOSITION OF CAT (*FELIS CATUS*) HAIR

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3.III.1 ABSTRACT

The amino acid composition of cat hair was determined by conventional 24 h acid hydrolysis and non-linear least squares extrapolation to time zero of the amino acid composition data from a series of hydrolysis intervals. Twenty-five individual samples of cat hair, consisting of four colors, were also analyzed (24 h hydrolysis) to determine if there was an effect of hair color on amino acid composition. Amino acids were determined following 6M HCl hydrolysis with cysteine and methionine determined by performic acid oxidation of the sample prior to hydrolysis. Tryptophan was not determined. There was no significant effect of hair color on the amino acid composition of cat hair. The non-linear compartmental model used to determine the amino acid composition of cat hair took into account the simultaneous occurring processes of hydrolysis and degradation of amino acids over time. The amino acids cysteic acid, methionine-sulphone, threonine and serine exhibited high loss rates during 6M HCL hydrolysis while the peptide bonds involving valine and leucine were slowly hydrolyzed. Amino acid nitrogen accounted for 94 % of the total nitrogen in cat hair when determined by conventional 24 h hydrolysis and 99 % of the total nitrogen when the compartmental model was applied. An average nitrogen content in cat hair protein of 17.5 % was found. The amino acid composition of cat hair protein is comparable to that of dog, horse, sheep and human hair although the proline content of cat hair protein appears to be lower than that in the other species.

3.III.2 INTRODUCTION

In recent studies (Hendriks *et al.*, 1996a and 1996b), hair growth and hair loss were determined in the adult domestic cat (*Felis catus*) and were shown to occur in sinusoidal patterns throughout the year, with maximum hair growth in summer and minimum hair growth in winter. Since hair consists mainly of amino acids (Jørgensen and Eggum, 1971; Gillespie, 1983; Powell and Rogers, 1986; Mundt and Stafforst, 1987), the amino acid requirements for hair growth in the adult cat should also follow this sinusoidal pattern.

There is little information in the literature, however, on the amino acid composition of cat hair.

The present study was undertaken to accurately determine the amino acid composition of cat hair. A representative sample of cat hair was subjected to multiple acid hydrolysis time intervals and the amino acid composition of the sample was determined by extrapolation using a non-linear least squares model (Robel and Crane, 1972; Darragh *et al.*, 1996). Twenty-five individual samples of cat hair, consisting of four colors were also analyzed using conventional amino acid analysis (24 h hydrolysis) to determine if there was an effect of hair color on amino acid composition.

3.III.3 MATERIAL AND METHODS

3.III.3.1 Hair samples

Samples of hair representing four colors (black, white, gray and ginger) were obtained by brushing the coat of adult (1-7 y) male and female cats housed at the BestFriend Feline Nutrition Research Unit, Massey University (Palmerston North, New Zealand). The numbers and sexes (M=male, F=female) of cats in each hair color group were as follows: black 4M, 3F; white 3M, 2F; grey 4M, 2F; ginger 6M, 1F. The hair samples were cleaned using methanol and boiling demineralised water and were dried at 70° C and finely cut using scissors. For the study which included different hydrolysis times, 0.5 g samples of black, white, gray and ginger colored hair, obtained from four different cats, were cleaned, finely cut and thoroughly mixed.

3.III.3.2 Chemical analysis

Samples (5 mg) of hair were accurately weighed into 10 ml pyrolysed glass hydrolysis

tubes and 1 mL of glass distilled 6M HCl, containing 0.1 % phenol (w/v), was added. The hydrolysis tubes were sealed under vacuum and the samples were hydrolyzed at 110 ± 2° C. The mixed hair sample was hydrolyzed in triplicate for either 3, 6, 9, 12, 16, 20, 24, 32, 48, 72, 96 or 144 h. In the study to determine the amino acid composition of different colored hair, the samples were hydrolyzed in triplicate for 24 h. After hydrolysis, 50 µL of 40 mM norleucine was added to each tube as an internal standard and the tubes were dried under vacuum (Savant Speedvac Concentrator AS 290, Savant Instruments Inc, Farmingdale, NY). The amino acids were dissolved in 2 mL of loading buffer (0.2 M sodium citrate, pH 2.2) and the solution filtered through a 0.45 µm filter prior to loading onto a Waters ion-exchange HPLC system (Millipore, Milford, MA). Detection was by postcolumn derivatisation with *O*-pthalaldehyde and fluorescence spectrometry. The chromatograms were integrated using dedicated software (Waters, Maxima 820, Millipore, Milford, MA) with the amino acids identified and quantified using a standard amino acid solution (Pierce, Rockford, Illinois).

Cysteine and methionine in the hair samples were determined following performic acid oxidation of the samples prior to hydrolysis. Samples (± 5 mg) of hair were accurately weighed into 10 mL pyrolysed glass hydrolysis tubes and 2 mL of freshly prepared performic acid (1 part of 30 % H_2O_2 to 8 parts of 88 % formic acid) was added. The tubes were kept at 0° C for 16 h after which time the reaction was terminated using 0.3 mL of 48 % HBr. In a preliminary study (W. Hendriks, unpublished), it was shown that 16 h was sufficient to allow maximal oxidation of cysteine and methionine. The hydrolysis tubes were dried under vacuum and the oxidised samples were hydrolyzed and quantitated using the procedure and equipment described above. Cysteine and methionine were detected as cysteic acid and methionine-sulphone, respectively. Proline was determined in one mixed hair sample in triplicate by 6M HCl hydrolysis for 24 h as described previously and loading on a Waters ion-exchange HPLC system (Millipore, Milford, MA) employing postcolumn derivatisation with ninhydrin and absorbance detection at 440 nm. Tryptophan was not determined. The amino acid concentrations were corrected for the recoveries of norleucine.

Total nitrogen in the mixed hair sample was determined (8 subsamples) using the Kjeldahl method and dry matter was determined by oven drying at 100° C for 16 h. Ash was determined by heating the samples at 550° C for 16 h.

3.III.3.3 Data analysis

The following non-linear compartmental model (Robel and Crane, 1972) was fitted to the hydrolysis time interval data for each amino acid which consisted of 12 triplicate analyses, using the SAS non-linear regression procedure and the Marquardt method (SAS, 1985).

$$B(t) = \frac{Ao\ h}{h - l}(e^{-lt} - e^{-ht}) + \varepsilon$$

constraints: $Ao \geq 0, h \geq 0, l \geq 0$

where,

B(t) = amino acid concentration measured at time t.

Ao = original level of the amino acid in the hair prior to hydrolysis.

h = rate at which the protein bound amino acid is hydrolyzed to a free form.

l = rate at which the amino acid is destroyed.

ε = random residual error.

The amino acid concentration data for the different colored hair samples were subjected to analysis of variance using the SAS General Linear Models (GLM) procedure (SAS 1985), with color and sex as fitted variables.

3.III.4 RESULTS AND DISCUSSION

The overall mean coefficient of variation (within samples) for the triplicate amino acid analyses determined over all the samples, amino acids and hydrolysis times, was 3.3 %. The overall mean recovery for norleucine, over all the samples and hydrolysis times was 96 %.

There was no significant effect ($P > 0.05$) of sex on the amino acid composition of cat hair and, therefore, the data for the two sexes were combined to statistically test the effect of hair color. There was no significant ($P > 0.05$) effect of color on the amino acid composition of cat hair as determined using conventional 24 h hydrolysis of the sample (Table 1). These results are in accordance with measurements made in sheep (Aitken *et al.*, 1994) where the amino acid composition has been found to be independent of the color of the hair. Kuttin *et al.* (1986), however, analyzed black and

white hair obtained from a single cow and found differences in the concentrations of alanine, cystine, methionine and glycine. In sheep (Aitken *et al.*, 1994), dogs (Mundt and Stafforst, 1987) and mink (Lohi *et al.*, 1991), the mineral content of hair has been shown to be different between different colored hair. As there was no significant difference in the amino acid composition of the different colored cat hair samples in the present study, the color of cat hair may be associated with the mineral composition of the hair.

Table 1. Amino acid compositions of black, white, gray and ginger colored cat hair determined by 24 h hydrolysis.[1]

Amino acid	Hair color[2]			
	Black	White	Gray	Ginger
	(μmol/g LFDM)			
Cysteine[3]	1079 ± 23.9	1098 ± 35.6	1116 ± 16.6	1101 ± 29.5
Methionine[3]	74 ± 3.0	77 ± 0.8	74 ± 1.4	68 ± 2.5
Aspartic acid	460 ± 2.3	466 ± 6.1	462 ± 5.8	469 ± 4.0
Threonine	474 ± 5.2	487 ± 9.9	488 ± 8.2	481 ± 5.3
Serine	762 ± 7.0	783 ± 17.8	775 ± 6.0	777 ± 5.5
Glutamic acid	884 ± 5.3	905 ± 11.5	875 ± 9.9	897 ± 6.2
Glycine	771 ± 15.5	787 ± 28.7	794 ± 16.0	755 ± 11.6
Alanine	383 ± 2.6	393 ± 8.2	382 ± 4.0	392 ± 2.7
Valine	358 ± 5.3	371 ± 7.2	363 ± 4.5	364 ± 3.6
Isoleucine	187 ± 1.7	194 ± 3.1	188 ± 2.1	192 ± 1.8
Leucine	509 ± 4.1	527 ± 6.3	518 ± 5.7	519 ± 4.2
Tyrosine	247 ± 7.2	231 ± 8.7	241 ± 7.2	238 ± 7.5
Phenylalanine	184 ± 2.4	185 ± 3.0	180 ± 13.7	185 ± 1.4
Histidine	97 ± 1.6	95 ± 2.4	94 ± 0.9	93 ± 3.0
Lysine	222 ± 5.4	234 ± 7.1	218 ± 6.6	237 ± 4.0
Arginine	477 ± 8.1	503 ± 10.5	484 ± 7.2	484 ± 5.8

[1]Values are means ± SEM. The number of observations for hair color black = 7, white = 5, gray = 6, and ginger = 7.
[2]Means were not significantly ($P > 0.05$) different.
[3]Determined by performic acid oxidation of the samples prior to hydrolysis.
LFDM, lipid free dry-matter.

Amino acids are normally determined after 6M HCl hydrolysis of the sample for 24 h with subsequent detection of the free amino acids (Finley, 1985). However, during hydrolysis of the sample, amino acids may be degraded to an unmeasurable form or the hydrolysis of the protein may not be complete, resulting in an underestimation of the amino acid content of the sample. A compartmental model which takes into account the processes of incomplete hydrolysis and degradation of amino acids was published by Robel and Crane (1972) and recently evaluated by Darragh *et al.* (1996). The model

consists of three distinct compartments (states). State A represents the amount of an amino acid in a protein-bound form. Upon hydrolysis of the protein, the peptide linkages are broken and the amino acid moves to a form where it is detectable (state B). As hydrolysis proceeds the amino acid in state B can be destroyed making it undetectable (state C). The Robel and Crane (1972) model takes into account the simultaneously occurring processes of hydrolysis and degradation of an amino acid over time. The amino acid composition of a sample is determined by non-linear least squares extrapolation to time zero of the amino acid composition data from a series of hydrolysis intervals. The model determines the original level of an amino acid in the protein prior to hydrolysis (A_0), the average rate at which the peptide bonds of an amino acid are hydrolyzed (h) and the average rate at which a free amino acid is destroyed (l). Robel and Crane (1972) used the compartmental model to determine the amino acid composition of lysozyme for which sequencing data are available and found recoveries of 92-110 % (mean 99 %). Darragh *et al.* (1996) also determined the amino acid composition of lysozyme using the compartmental model and reported recoveries of 94-110 % (mean 100 %). Conventional 24 h hydrolysis yielded lower recoveries with a range of 84-109 % (mean 97 %) (Robel and Crane 1972), and 81-110 % (mean 93 %) (Darragh *et al.*, 1996).

In general, the hydrolysis of cat hair over time showed a sharp increase in the concentration of an amino acid up to 14 h whereafter the concentration either remained constant, increased slightly or decreased. For most amino acids, the maximum point on the hydrolysis time curve, in the present study, occurred before 14 h except for valine and isoleucine for which no maxima were found between 3 and 144 h. Table 2 shows the estimates of Ao, h and for the non-linear compartmental model fitted to the 12 hydrolysis time intervals for each amino acid. High loss rates were found for cysteic acid, methionine-sulphone, threonine and serine with lower loss rates for alanine, isoleucine, tyrosine, phenylalanine and arginine. Robel and Crane (1972) and Darragh *et al.* (1996) also found high loss rates for threonine and serine when using the compartment model to determine the amino acid composition of lysozyme. Darragh *et al.* (1996), furthermore, found a high loss rate for cysteic acid while for methionine-sulphone there was no loss rate. Cysteic acid, threonine and serine seem to be acid labile amino acids and relatively easily destroyed under the conditions of 6M HCl hydrolysis. Low hydrolysis rates were observed for valine and leucine indicating that peptide bonds in hair protein involving these amino acids are difficult to cleave.

Table 2. Estimates (*Ao*, *h*, *l*) and coefficients of determination (R^2) for a compartmental model[1] used to determine the amino acid composition of cat hair.

Amino acid	*Ao*	*h*	*l* (x10^3)	R^2
	(μmol/g LFDM)			
Cysteic acid[2]	1271	0.7371	3.127	0.90
Methionine-sulphone[2]	75	0.5856	1.043	0.74
Aspartic acid	453	0.7105	0	0.31
Threonine	516	0.3863	1.146	0.84
Serine	854	0.8146	3.573	0.97
Glutamic acid	916	0.5504	0	0.78
Glycine	765	1.0527	0	0.48
Alanine	406	0.7498	0.638	0.95
Valine	393	0.1702	0	0.97
Isoleucine	203	0.3774	0.122	0.97
Leucine	542	0.1742	0	0.98
Tyrosine	242	0.4426	0.626	0.91
Phenylalanine	187	0.3722	0.129	0.91
Histidine	99	0.4794	0	0.52
Lysine	235	0.3059	0	0.86
Arginine	486	0.3648	0.002	0.95

[1]Robel and Crane (1972).
[2]Determined by performic acid oxidation of the samples prior to hydrolysis.
Ao, amount of the amino acid in the protein; *h*, hydrolysis rate of the protein bound amino acid; *l*, loss rate of the free amino acid; LFDM, lipid free dry-matter.

As the processes of hydrolysis and loss of amino acids occur simultaneously, 24 h hydrolysis may underestimate the concentration of an amino acid in a protein due either to a slow hydrolysis rate or high loss rate of the amino acid, or a combination of these two processes. Table 3 shows the amino acid composition of cat hair as determined using the compartmental model and conventional 24 h hydrolysis. The values obtained from a single 24 h hydrolysis time were generally lower than those obtained using the compartmental model with cysteine, serine, valine, threonine and isoleucine being 12, 9, 9, 7 and 7 % units lower, respectively. Twenty-four h aspartic acid and lysine values were higher than the values obtained by the model.

Asparagine (ASN) and glutamine (GLN) are converted to aspartic acid (ASP) and glutamic acid (GLU), respectively, under the conditions of acid hydrolysis. Holt *et al.* (1971) determined the concentration of ASN and GLN in wool and found that 65 % the total ASP originated from ASN while 43 % of the GLN originated from GLU. Using these estimates of Holt *et al.* (1971), amino acid nitrogen accounted for 94 % of the total nitrogen with the conventional 24 h acid hydrolysis method while amino acid nitrogen accounted for 99 % of the total nitrogen with the compartmental model (Table 3). If the concentration of tryptophan in hair is assumed to be 4.0 μmol/g N (Block and

Bolling, 1947), only 0.12 % of the total N was unaccounted for using the compartmental model in the present study.

Table 3. Amino acid composition of cat hair determined using a compartmental model[1] and conventional 24 h hydrolysis.

Amino acid	Model[2]	24 h[3]
	(μmol/g LFDM)	
Cysteine	1271 ± 17.5	1139 ± 30.2
Methionine	75 ± 1.0	71 ± 1.5
Aspartic acid	453 ± 13.1	462 ± 3.0
Threonine	516 ± 8.3	481± 5.4
Serine	854 ± 6.7	784 ± 15.7
Glutamic acid	916 ± 11.1	868 ± 8.5
Glycine	765 ± 3.2	757 ± 11.0
Alanine	406 ± 4.4	394 ± 5.2
Valine	393 ± 7.4	361 ± 3.8
Isoleucine	203 ± 3.0	189 ± 1.6
Leucine	542 ± 3.7	538 ± 4.0
Tyrosine	242 ± 8.3	233 ± 1.1
Phenylalanine	187 ± 2.4	184 ± 1.3
Histidine	99 ± 2.0	95 ± 1.7
Lysine	235 ± 4.3	237 ± 4.0
Arginine	486 ± 4.3	487 ± 6.5
Proline	ND	397 ± 1.2
	(mmol/g LFDM)	
Total nitrogen	10.75 ± 0.08	10.75 ± 0.08
Amino acid nitrogen	10.62[4,5]	10.16[4] ± 0.03

[1]Robel and Crane (1972).
[2]Amino acid values are estimates ± asymptotic standard error, nitrogen values are means ± SEM.
[3]Amino acid values are means ± SEM, nitrogen values are a mean ± SEM or mean ± pooled SE.
[4]Assuming 65 % of ASP was ASN and 43 % of GLU was GLN (Holt *et al.* 1971).
[5]Including 24 h proline value.
LFDM, lipid free dry-matter; ND, not determined.

From the amino acid compositional data of cat hair determined by the compartmental model (Table 2) and the molecular weights of the individual amino acids bound in protein (free molecular weight minus 18) it can be calculated that amino acids make up 86 % of the total weight of cat hair. The 14 % of the weight of cat hair unaccounted for in the present study is partly tryptophan, proline and other non-nitrogen containing compounds such as minerals. Cat hair, in the present study, was found to contain 0.6 % ash. Other non-nitrogen compounds likely to be present in cat

hair are fats, sterols and complex lipoids. These latter non-nitrogen compounds, however, have been shown to represent a maximum of up to 1 % of the weight of wool (Anderson and Leeder, 1965; Baumann, 1979). In total, all these compounds should not make up more than 3 % of the weight of hair and it is, at this stage, unknown what constitutes the remaining 11 % of the weight of cat hair.

Table 4. Published amino acid composition of cat, dog, horse, sheep and human hair protein.

Amino acid	Cat[1]	Dog[2]	Horse[3]	Sheep[4]	Human[5]
			(mol/100 mol of residue)		
Cysteine	15.8	16.7	14.4	13.1	17.8
Methionine	0.9	0.9	0.2	0.5	0.6
Aspartic acid	5.6	5.3	6.0	5.9	4.9
Threonine	6.4	6.2	5.5	6.5	6.8
Serine	10.6	10.5	9.6	10.8	11.7
Glutamic acid	11.4	11.1	11.3	11.1	11.4
Glycine	9.5	7.8	6.4	8.6	6.4
Alanine	5.1	5.1	5.5	5.2	4.6
Valine	4.9	4.9	5.9	5.7	5.8
Isoleucine	2.5	2.5	3.6	3.0	2.6
Leucine	6.7	6.1	7.5	7.2	5.8
Tyrosine	3.0	2.7	1.9	3.8	2.0
Phenylalanine	2.3	1.7	2.5	2.5	1.6
Histidine	1.2	0.9	1.1	0.8	0.9
Lysine	2.9	3.9	2.9	2.7	2.7
Arginine	6.1	6.3	7.9	6.2	5.8
Proline	4.9	7.3	7.8	6.6	8.4

[1]Present study.
[2]Marshall (1986).
[3]Samata and Matsuda (1988).
[4]Marshall and Gillespie (1977).
[5]Gillespie (1983).

The most abundant amino acids in cat hair protein are cysteine, serine, glutamic acid and glycine while only small amounts of methionine and histidine were found. This pattern of amino acids is generally found in hair protein from other animals (Table 4). The amino acid composition of hair protein is similar between the cat (*Felis catus*), dog (*Canis familiaris*), horse (*Equus caballus*), sheep (*Ovis aries*) and human (*Homo sapiens*) although it is notable that the proline content of cat hair appears to be lower than the other species. Mundt and Stafforst (1987) determined the amino acid composition and nitrogen content of dog hair and found that hair from this species contains 16.0 % nitrogen. This value is higher than the 15.1 % found in the present study for cat hair. Using the relative weight of the nitrogen in the individual amino

acids, the amino acid composition of cat hair (Table 2) and a tabulated value of 4.0 μmol tryptophan/g N (Block and Bolling, 1947), it can be calculated that the average percentage of nitrogen in cat hair protein is 17.5. Therefore, to calculate the crude protein content from the total nitrogen content of cat hair, a conversion factor of 5.78 should be used instead of the commonly used factor of 6.25 for protein or the factor of 6.37 for milk protein (Kirchgessner *et al.*, 1967). The lower conversion factor for cat hair is mainly due to the relatively high concentrations of arginine, histidine and lysine present in this type of protein. Because of the similar amino acid compositions of hair proteins between different species (Table 4), the conversion factor of 5.78 should generally be used for this type of protein. Therefore, assuming that dog hair contains 16 % N (Mundt and Stafforst, 1987), the amino acids in hair of this animal make up 92 % of the weight of the hair.

The present study provides original data on the amino acid composition of cat hair. Together with the data from Hendriks *et al.* (1996a), estimating hair growth in adult domestic cats throughout the year, the data in the present study can be used to determine the amino acid requirements for hair growth throughout the year in the adult domestic cat of both sexes. The estimate of the amino acid requirements for hair growth can be used to determine the amino acid requirement of adult domestic cats by the factorial method.

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CHAPTER 4

CATABOLISM OF BODY AMINO ACIDS

Body protein is constantly replaced by newly synthesised protein in mammals. This turnover of body protein results in a continual catabolism of endogenous amino acids. The nitrogen of these catabolised body amino acids is excreted in the urine mainly as urea and ammonia. The aim of this chapter was to provide data on the amount of body amino acids catabolised by the adult domestic cat. Part I of this chapter provides quantitative estimates on the basal endogenous urinary nitrogen excretion of adult cats determined by the protein-free and regression approach. Part II of this chapter provides comprehensive data on the amino acid composition of adult male and female cats which can be used as a pattern for the catabolism of endogenous amino acids.

CHAPTER 4 PART I

ENDOGENOUS URINARY METABOLITE EXCRETION IN THE ADULT DOMESTIC CAT

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4.I.1 ABSTRACT

The study aimed to determine endogenous urinary excretions of total, urea, ammonia and creatinine nitrogen in the adult domestic cat. Endogenous urinary nitrogen metabolite excretions were determined by feeding adult cats a protein-free diet for 10 d or by regression to zero protein intake of urinary nitrogen metabolite excretions for adult cats fed four levels of dietary protein. The mean (± SEM) endogenous total, urea and ammonia nitrogen excretions for the cats fed the protein-free diet were 360 (± 11.3), 243 (± 8.8) and 27.6 (± 0.86) $mg \cdot kg^{-0.75}$body $weight \cdot d^{-1}$, respectively. Lower mean (± SEM) estimates of 316 (± 53.9) and 232 (± 43.4) $mg \cdot kg^{-0.75}$body $weight \cdot d^{-1}$ were obtained for the endogenous excretions of total and urea nitrogen, respectively using the regression technique while a higher endogenous urinary ammonia nitrogen excretion of 33.7 (± 5.68) $mg \cdot kg^{-0.75}$body $weight \cdot d^{-1}$ was found. The differences between the two techniques were not statistically significant. Daily excretions of creatinine nitrogen were not significantly (P = 0.64) different (mean ± SEM, 16.2 ± 0.46 and 17.5 ± 1.19 $mg \cdot kg^{-0.67}$body weight, respectively) for the two methods. The endogenous urinary total and urea nitrogen excretion of the adult domestic cat is higher than values for other mammals such as the human, dog, rat and pig.

4.I.2 INTRODUCTION

Endogenous urinary nitrogen (EUN) excretion is an important component of basal protein metabolism. Moreover, it is used in the calculation of the biological value of dietary proteins and in the factorial determination of crude protein requirements of adult animals.

Estimates of EUN excretion have been obtained by measuring the excretion of nitrogen in the urine of animals given a protein-free diet (Calloway and Margen 1971, Kendall et al. 1982, Moughan et al. 1987). This method, however, has been criticized because it reflects the nitrogen metabolism of animals adapted to protein restriction instead of nitrogen metabolism under normal physiological conditions (Berdanier et al. 1967, Holmes 1965). Alternatively, EUN excretion in animals can be determined under physiologically more normal conditions, by regression to zero nitrogen intake of the urinary nitrogen excretion data of animals fed graded levels of dietary protein (Greaves and Scott 1960, Glem-Hansen and Jørgensen 1973, Moughan et al. 1987).

To the authors' knowledge there are no published estimates of EUN excretion for the adult domestic cat determined using the protein-free method and only one estimate (Greaves and Scott 1960) in the adult cat using the regression technique.

The objective of the present study was to determine and compare endogenous urinary total N, urea N and ammonia N excretion in the adult domestic cat using both the protein-free and regression methods.

4.I.3 MATERIALS AND METHODS

The two studies reported here were approved by the Massey University Animal Ethics Committee.

4.I.3.1 Animals and housing

Twenty-two (11 male, 11 female) domestic short-haired cats, 2 to 6 y of age and with an initial body weight range of 2421 to 4130 g (mean ± SEM, 3296 ± 114.5 g) from the BestFriend Feline Nutrition Research Unit, Massey University (Palmerston North, New Zealand) were used. The cats had been maintained on a casein-based diet for three months prior to the start of the study. The casein-based diet had a calculated

metabolisable energy (ME) content of 21 $kJ \cdot g^{-1}$ dry matter and was similar in composition to a protein-free diet (**Table 1**) with the exception that casein replaced an equal amount (250 $g \cdot kg^{-1}$ diet, as is basis) of starch. The cats were housed individually in a semi-enclosed outdoor area in plastic metabolism cages which allowed total collection of urine. Body weights of the cats were recorded at the start and end of both studies, and at the start of the protein-free feeding period. Water was available at all times and minimum and maximum air temperatures were recorded daily during the experimental periods.

TABLE 1
Ingredient composition of the experimental diets[1]

Ingredient	Dietary crude protein ($g \cdot kg^{-1}$ dry matter)				
	0	40	70	100	130
	($g \cdot kg^{-1}$ as is)				
Fish offal[2]	-	143.8	251.7	359.5	467.4
Water	-	243.8	162.5	81.3	-
Beef tallow[3]	330.0	200.8	194.4	187.9	181.4
Corn starch[4]	337.2	201.9	182.0	162.1	142.4
Sucrose	200.0	129.6	129.6	129.6	129.6
Soybean oil	60.0	38.9	38.9	38.9	38.9
Vitamin-mineral mix[5]	53.0	34.3	34.3	34.3	34.3
Cellulose[6]	19.8	6.9	6.6	6.4	6.0

[1]All diets contained a calculated metabolisable energy content of 20.9 $MJ \cdot kg^{-1}$ dry matter.
[2]Processed at 121° C for 80 min. and contained 24.7 % dry matter.
[3]AFFCO rendering, Horotiu, New Zealand.
[4]Avon Pregel Starch (New Zealand Starch Products Ltd., Onehunga, Auckland, New Zealand).
[5]Composition detailed by Hendriks et al. (1996).
[6]Ahaki Chemical Industry Co. Ltd., Osaki, Japan.

4.I.3.2 Study 1

Total urine from each of six (3 male and 3 female) cats was collected daily over a 2 d preliminary period during which the 25 % casein-based diet was fed and over a subsequent 10 d period during which the protein-free diet (Table 1) was fed. The diets were fed to appetite with the food intake of each cat recorded daily.

4.I.3.3 Study 2

Sixteen (8 male, 8 female) adult cats were equally and randomly allocated to each of four protein-containing diets (Table 1) with the proviso that the groups were balanced for gender. The four diets were formulated to contain 4, 7, 10 or 13 % crude protein on a dry matter basis by varying the amount of fish offal and were kept

isocaloric at a calculated metabolisable energy content of 21 $kJ \cdot g^{-1}$ dry matter. The dry matter content of the protein-containing diets was standardized to 65 % by the addition of water to the 4, 7 and 10 % crude protein diets. The proximate analysis and amino acid composition of the fish offal are given in **Table 2**. The cats received an amount of diet equivalent to 12 g of dry matter per kg body weight daily for 9 d with urine collected quantitatively from each cat during d 7, 8 and 9. The urine collected during the three days were bulked before chemical analysis.

TABLE 2
Nutrient composition of the fish offal used in the experimental diets[1]

Ingredient	Amount ($g \cdot kg^{-1}$ dry matter)
Crude protein	730
Fat	247
Ash	2.8
Cysteine	4.7
Methionine	15.2
Taurine	18.8
Aspartic acid	45.8
Threonine	29.2
Serine	33.2
Glutamic acid	63.6
Proline	27.7
Glycine	45.5
Alanine	36.6
Valine	29.5
Isoleucine	20.9
Leucine	40.2
Tyrosine	17.1
Phenylalanine	20.5
Histidine	13.4
Lysine	39.3
Arginine	73.9

[1]Amino acids were determined as described by Hendriks et al. (1996).

4.I.3.4 Urine collection

The urine collection apparatus allowed collection of uncontaminated urine and consisted of two plastic trays (45 x 30 x 15 cm) which fitted inside each other. There was a 2 cm space between the bottom of the two trays when fitted, where urine could collect. The top tray had a 1.0 mm stainless steel wire mesh floor while the bottom tray was solid plastic. The metabolism cage (0.8 x 1.1 x 0.8 m) was made of a solid

white plastic box with a partially covered galvanized barred door fitted at the front. The urine collection apparatus could be securely positioned in the rear left hand corner on the floor of the metabolism cage under a 5° slope and a 3° tilt such that urine collected in one corner of the apparatus. Feces were retained on the wire mesh of the top tray while urine passed through the mesh and collected in the corner of the bottom tray. The cats had previously been trained to use the apparatus and during the study did so habitually. At the start of a urine collection day, 4 mL of 10% (v/v) H_2SO_4 was added to the bottom tray of the urine collection apparatus which was then fixed in the metabolism cage. The next day the acidified urine was quantitatively transferred into a container, the bottom tray was washed using demineralized water and the washing was added to the container. Daily urine voided was stored at -20° C until chemical analysis. During the urine collection periods of Studies 1 and 2, feces were removed frequently (six times daily between 0700 to 1800 h) and the floor of each metabolism cage and the area of the floor opposite each cage were routinely inspected (six times daily between 0700 to 1800 h) for the presence of urine.

4.I.3.5 Chemical analysis

Volumes and weights of the daily urine collected for each cat in Study 1 and the bulked 3 d urine collected for each cat in Study 2 were measured, filtered through a sintered glass crucible to remove hair and an aliquot was taken for analysis of total N, urea, creatinine and ammonia. Total N was determined in the urine samples (as collected) by the Kjeldahl method (Association of Official Analytical Chemists 1995) while the urine samples were diluted (1:50) before determination of urea, ammonia and creatinine. Urea was determined using a commercially available kit (Blood Urea Nitrogen kit no. 535-B, Sigma, St. Louis, MO) and involved the reaction of urea with diacetyl monoxime and colorimetric measurement of the formed complex at 535 nm. Ammonia was determined using a commercially available kit (Ammonia kit no. 171-UV, Sigma, St. Louis, MO) and was performed on a Cobas Fara II autoanalyzer (Hoffman-La Roche, Basel, Switzerland). The procedure involved reaction of ammonia with 2-oxoglutarate in the presence of glutamate dehydrogenase and reduced nicotinamide adenine dinucleotide phosphate, and measurement of the absorbance at 340 nm. Creatinine was determined by a modified Jaffe reaction on a Cobas Fara II autoanalyzer (Hoffman-La Roche, Basel, Switzerland) according to the procedure of Larsen (1972). All analyses were

performed in duplicate.

4.I.3.6 Data analysis

Daily urinary N metabolite excretions were calculated on the basis that urea contains 46.7 % N, creatinine 37.2 % N and ammonia 82.3 % N and were expressed on a body weight basis or a body weight basis less than unity. The daily food intake data were expressed per unit of body weight.

Daily urinary N metabolite excretion and food intake data for the cats in Study 1 were subjected to a repeated measures ANOVA with sex as the variable and time (days after start of the study) as the repeated factor (Cody and Smith 1987). No effect of sex was found on the daily excretion of any of the urinary N metabolites or the food intake data of the cats in Study 1 and the data were thus pooled across sexes. Daily urinary N metabolite excretion data for the cats in Study 2 were subjected to analysis of variance using the General Linear Models procedure in SAS with diet and sex as variables. There was no effect of sex on the daily excretion of any of the urinary N metabolites of the cats in Study 2. The combined data for the daily urinary excretion of total N, urea N and ammonia N for the male and female cats were then subjected to a least squares linear regression analysis with daily N intake as the independent variate and either the daily urinary total N, urea N and ammonia N excretion as the dependent variate. Student's *t* test was used to determine the level of significance between the daily endogenous urinary creatinine N excretions of the cats over the 12 d period in Study 1 and the cats over the 3 d period in Study 2. All statistical analyses were performed using the SAS statistical package (SAS version 6.04, SAS Institute, Gary, NC) and effects were considered significant at $P < 0.05$.

4.I.4 RESULTS

The cats remained healthy throughout both studies. There was no effect of sex on the amount of food eaten per unit body weight for the cats in Study 1. There was, however, an effect of time on the amount of food intake per unit body weight, as analyzed by repeated measures analysis. The mean (± SEM) daily food intake of the six cats in Study 1 over the two day period when the casein-based diet was fed was 14.6 (± 0.62) $g \cdot kg^{-1}$ body weight and decreased to 10.6 (± 1.61) and 8.7 (± 1.14) $g \cdot kg^{-1}$ body weight during the first and second day when the protein-free diet was offered.

The mean (± SEM) food intake of the cats in Study 1 remained relatively constant from d 5 to 12 of the study, at approximately 6.3 (± 0.36) $g \cdot kg^{-1}$body $weight \cdot d^{-1}$ (**Fig. 1**). The cats in Study 2 readily consumed all of the food offered although one female cat on the 4 % crude protein diet failed to consume all of the daily amount of food offered for three of the first six days of the study and was, therefore, excluded from the study. A male cat on the 10 % crude protein diet urinated outside the urine collection apparatus during the 3 d collection period and was also removed from the study. The cats were monitored closely during both studies and coprophagy was not observed.

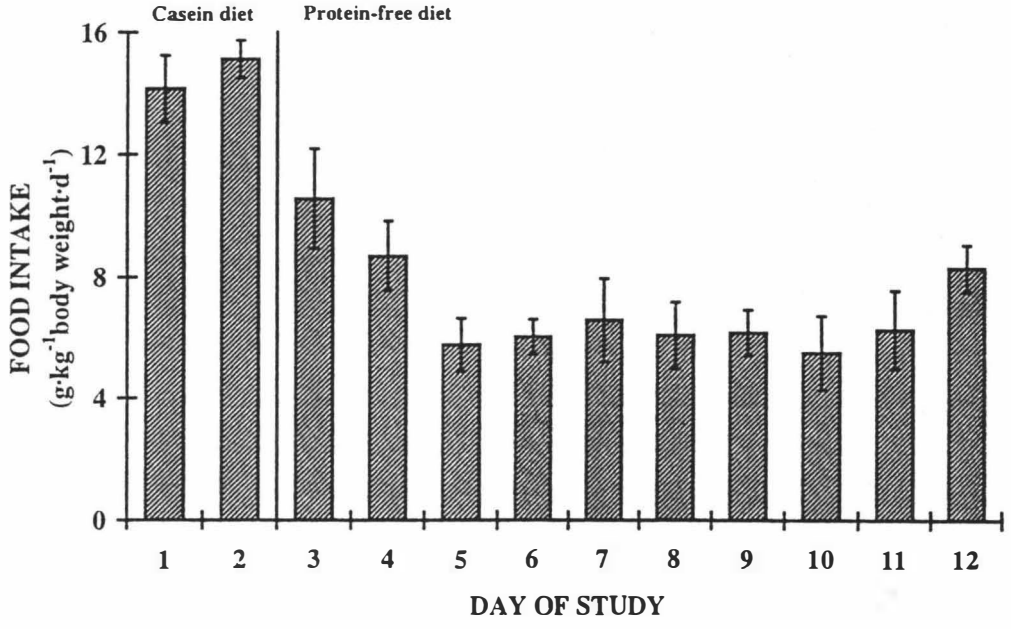

Figure 1 Daily food intakes for adult domestic cats (n = 6) given a casein-based diet for 2 d followed by a protein-free diet for 10 d. Each value represents a mean (± SEM). The changes of food intake over time were significant ($P < 0.001$) for all these measurements as determined by repeated measures ANOVA.

The mean (± SEM) daily weight loss for the cats in Study 1 over the 10 d period when the protein-free diet was fed was 29 (± 2.4) g. The mean (± SEM) daily weight losses for the cats in Study 2 were 30 (± 3.5), 25 (± 3.9), 22 (± 3.7) and 23 (± 1.1) g for the cats receiving the 4, 7, 10 and 13 % crude protein diets, respectively. The average daily minimum and maximum air temperatures over the two studies were 12 and 20°C, respectively.

The overall mean differences between duplicate analyses within samples (expressed as a proportion of the mean) for urea, creatinine, total N and ammonia,

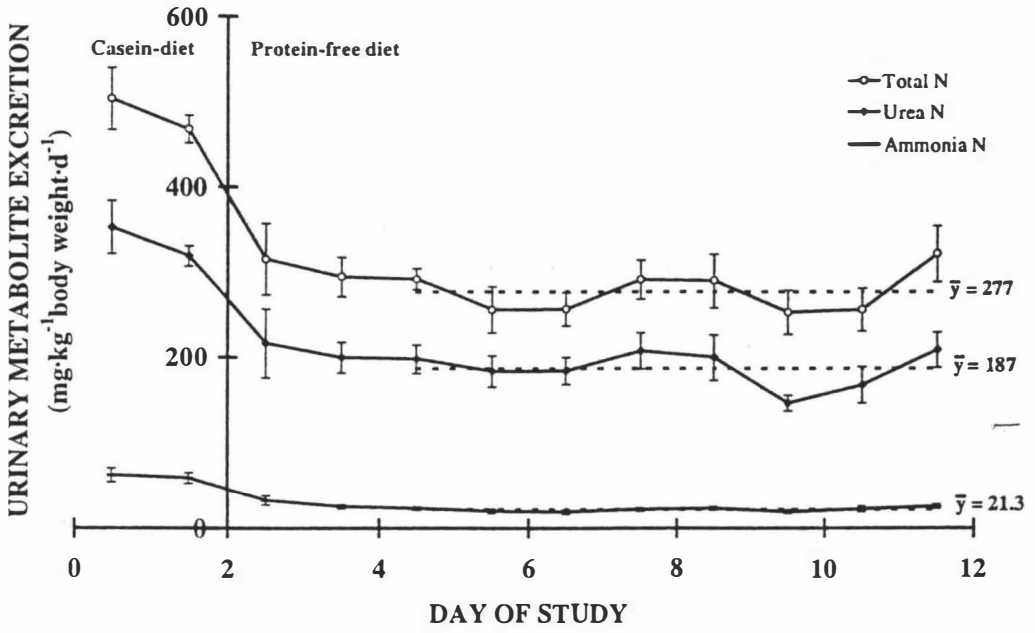

Figure 2 Daily urinary total nitrogen, urea nitrogen and ammonia nitrogen excretions for adult cats (n = 6) given a casein-based diet for 2 d followed by a protein-free diet for 10 d. Each value represents a mean (± SEM). The changes in the excretion of all the N metabolites over time were significant ($P < 0.0001$) for all these measurements as determined by repeated measures ANOVA.

were 4.8 (±0.43), 2.0 (± 0.26), 1.0 (± 0.09) and 0.6 (± 0.05) %, respectively.

The daily urinary excretions of total N, urea N and ammonia N for the cats in Study 1 were high when the casein-based diet was fed and decreased abruptly during the first day when the protein-free diet was introduced, to attain a relatively constant level until the end of the study. The daily excretion of creatinine N in the urine of the cats remained relatively constant throughout Study 1. The mean fraction of undetermined N (total N-urea N-creatinine N-ammonia N) in the urine of the cats in Study 1 was slightly higher when the casein-based diet was fed (mean ± SEM, 75.3 ± 4.81 mg·kg^{-1}body weight·d^{-1}) than when the protein-free diet was offered (mean ± SEM, 56.9 ± 2.97 mg·kg^{-1}body weight·d^{-1}). There was no statistically significant effect of sex on the daily urinary excretion of total N, urea N, ammonia N or creatinine N. There was a significant effect of time on the daily urinary excretion of total N, urea N and ammonia N, but no effect of time on the daily urinary excretion of creatinine N or the fraction of undetermined N in the urine of the cats in Study 1. The overall mean (± SEM) daily urinary excretions of total N, urea N and ammonia

N for the cats in Study 1 are presented in **Figure 2.** The mean (± SEM) daily urinary creatinine N excretion for the cats in Study 1 was 11.5 (± 0.34) mg·kg^{-1}body weight. The mean (± SEM) daily urinary excretions of total N, urea N, ammonia N and the daily amount of undetermined N for the six adult cats over d 5 to 12 in Study 1 were 277 (± 9.0), 187 (± 6.9), 21.3 (± 0.86) and 57.6 (± 3.50) mg·kg^{-1}body weight and 360 (± 11.3), 243 (± 8.8), 27.6 (± 1.06) and 74.3 (± 4.50) mg·kg$^{-0.75}$body weight, respectively.

In general the daily urinary metabolite excretions for the cats in Study 2 increased with increasing dietary N intake although the average daily urinary excretions of total N and urea N for the cats receiving the 10 % crude protein diet were slightly higher than the corresponding values for the cats receiving the 13 % crude protein diet. The daily urinary excretion of creatinine N remained relatively constant with increasing dietary N intake. The daily amount of undetermined N in the urine of the cats on the four protein-containing diets showed no obvious increase or decrease with increasing dietary N intake. There was no significant effect of sex on the daily urinary excretions of total N, urea N, ammonia N, creatinine N or the amount of undetermined N. There was a significant effect of dietary treatment on the daily urinary excretions of total N, urea N and ammonia N, and no significant effect of diet on the daily excretion of creatinine N or the daily amount of undetermined N in the urine. **Figures 3, 4** and **5** show the linear regression lines relating the daily urinary excretion of total N, urea N and ammonia N to daily dietary N intake, respectively. The slope of each line was significant at the 5 % probability level while the intercept of each regression line was significant at the 1 % probability level. The mean (± SEM) daily urinary creatinine N excretion and the daily amount of undetermined N in the urine of the cats in Study 2 were 11.8 (± 0.70) and 38.0 (± 7.82) mg·kg^{-1}body weight, respectively. The estimates (± SE) of endogenous urinary total N, urea N and ammonia N excretion as determined by the extrapolation of the regression relationship to zero protein intake were 227 (± 41.2), 166 (± 34.8) and 24.1 (± 3.96) mg·kg^{-1}body weight·d^{-1} and 316 (± 53.9), 232 (± 43.4) and 33.7 (± 5.68) mg·kg$^{-0.75}$body weight·d^{-1}, respectively.

The mean daily creatinine N excretion of the cats in Study 1, measured over the 12 d period, was not significantly (P = 0.64) different from the mean daily creatinine N excretion for the cats in Study 2, measured over the 3 d period.

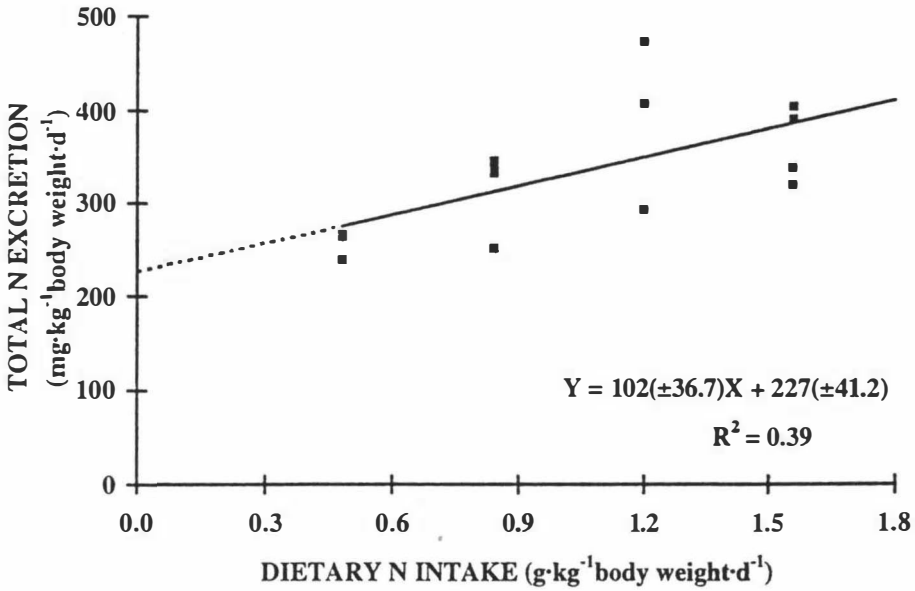

Figure 3 Daily urinary excretions of total nitrogen for adult cats (n = 14) fed four different levels of dietary nitrogen and the linear regression line relating urinary total nitrogen excretion to dietary nitrogen intake. Points represent individual animals. There was a significant effect ($P < 0.05$) of diet on the daily urinary excretion of total nitrogen. The slope and intercept of the linear regression line were significant at ($P < 0.05$) and ($P < 0.01$), respectively.

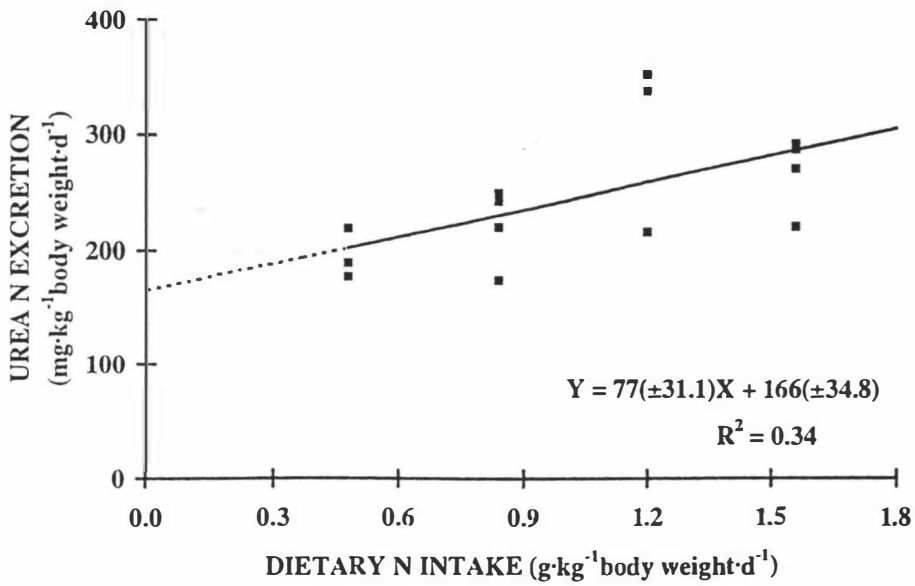

Figure 4 Daily urinary excretions of urea nitrogen for adult cats (n = 14) fed four different levels of dietary nitrogen and the linear regression line relating urinary urea nitrogen excretion to dietary nitrogen intake. Points represent individual animals. There was a significant effect ($P < 0.05$) of diet on the daily urinary excretion of total nitrogen. The slope and intercept of the linear regression line were significant at ($P < 0.05$) and ($P < 0.01$), respectively.

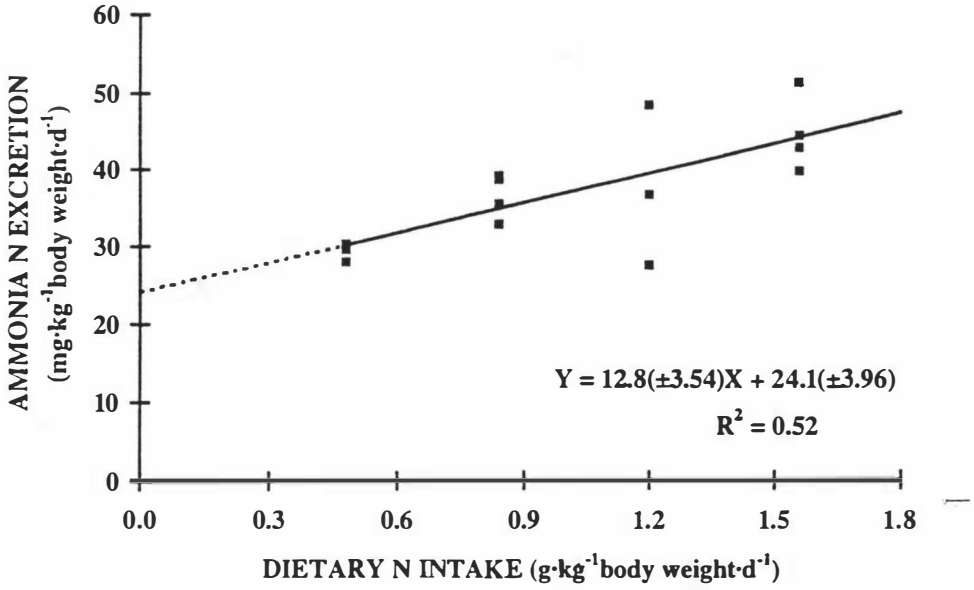

Figure 5 Daily urinary excretions of ammonia nitrogen for adult cats (n = 14) fed four different levels of dietary nitrogen and the linear regression line relating urinary ammonia nitrogen excretion to dietary nitrogen intake. Points represent individual animals. There was a significant effect ($P < 0.05$) of diet on the daily urinary excretion of ammonia nitrogen. The slope and intercept of the linear regression line were significant at ($P < 0.05$) and ($P < 0.01$), respectively.

4.I.5 DISCUSSION

The cats in Study 1 reduced their food intake by approximately half when changed from the casein-based diet to the protein-free diet, with a rapid decline in food intake during the first two days when the protein-free diet was offered (Fig 1). The average daily energy intake of the cats during d 5 to 12 of Study 1 (d 3 to 10 on the protein-free diet) was 132 kJ ME·kg^{-1}body weight. This is similar to the daily energy intake of adult cats given a protein-free diet as recorded by Hendriks et al. (1996) of 117 kJ ME·kg^{-1}body weight and is approximately 53 % of the daily energy requirement for the adult cat housed in a metabolism cage and fed a balanced diet (Goggin et al. 1993, Kane et al. 1981, Miller and Allison 1958). Based on the present results and the limited success of feeding protein-free diets to adult cats reported by others (Greaves 1965, Greaves and Scott 1960, Hendriks et al. 1996, Miller and Allison 1958), it appears that the adult cat does not eat a protein-free diet as readily as other species such as the dog, marmoset, rat and pig (Flurer et al. 1988, Kendall et al. 1982, Moughan et al. 1987, Yokogoshi et al. 1977).

The rate of weight loss (± SE) of the cats in Study 1 as measured over the 10 d

period when the protein-free diet was fed was 0.99 (± 0.024) $\%\cdot d^{-1}$ which is similar to the rate of weight loss for adult cats fed a protein-free diet for 10 d observed by Hendriks et al. (1996) of 1.04 $\%\cdot d^{-1}$. The rate of weight loss of the cats in Study 2 over the 9 d period decreased with increasing protein content of the diet although the cats on the 13 % protein diet lost weight at a similar rate to the cats on the 7 % protein diet. The mean (± SEM) weight loss of the cats on the 4, 7, 10, and 13 % protein diets were 0.86 (± 0.033), 0.73 (± 0.148), 0.68 (± 0.132) and 0.73 (± 0.08) $\%\cdot d^{-1}$.

The cats in the present study were previously trained to use the urine collection apparatus and during the two studies did so habitually. Any urine voided outside the urine collection apparatus would have been easily detected on the white surface of the metabolism cage. Urine, however, could have been sprayed through the partially covered barred door of the metabolism cage. During the daily inspections (six times, between 0700 to 1800 h), no urine was ever found on the floor in front of the metabolism cages.

In mammals such as the marmoset, human, dog and pig there is a rapid decline in urinary total N excretion during the first three days when a protein-free diet is ingested, followed by a slower decline of approximately three days until a relatively steady excretion of urinary total N is achieved over d 6 to 10 (Deuel et al. 1928, Flurer et al. 1988, Kendall et al. 1982, Moughan et al. 1987). The daily urinary excretions of total N, urea N and ammonia N for the cats in Study 1 were high when the casein-based diet was fed and rapidly declined during the first day when the protein-free diet was offered. The daily urinary excretions of total N, urea N and ammonia N reached a relatively steady level during d 4 to 12 (d 2 to 10 after the protein-free diet was offered) of the study. This pattern of decline in the excretion of urinary N metabolites for the adult cats in the present study indicates that the cat metabolises dietary protein rapidly and that the catabolism of body protein is set at a relatively constant level or only slowly adjusts to dietary changes. These data are in accordance with the view that the cat has a limited ability to adapt its hepatic enzymes involved in the catabolism of amino acids (Rogers et al. 1977). The mean (± SEM) excretions of total N, urea N and ammonia N over the period where the food intake of the cats on the protein-free diet in Study 1 was relatively constant (d 5 to 12 of Study 1) which was assumed to represent the endogenous urinary excretion of these metabolites by the adult cat, were 360 (± 11.3), 243 (± 8.8) and 27.6 (± 1.06)

$mg \cdot kg^{-0.75}$body weight$\cdot d^{-1}$, respectively.

The value for the EUN excretion of the adult cat seems to be much higher than the values found for other animals using the same technique. Studies using the protein-free technique have reported EUN excretions of 62, 110, 128, 210 and 163 $mg \cdot kg^{-0.75}$body weight$\cdot d^{-1}$ in marmosets (Flurer et al. 1988), humans (Calloway and Margen 1971), rats (Yokogoshi et al. 1977), dogs (Kendall et al. 1982) and the domestic pig (Moughan et al. 1987), respectively. The main cause of the higher daily EUN excretion of the adult cat in comparison to these other animals was the higher excretion of urea N. Endogenous urinary urea N excretions using the protein-free technique of 60, 70 and 116 $mg \cdot kg^{-0.75}$body weight$\cdot d^{-1}$ have been reported for rats (Yokogoshi et al. 1977), pigs (Moughan et al. 1987) and dogs (Kendall et al. 1982), respectively. The latter values are much lower than the endogenous urinary urea N excretion of 243 $mg \cdot kg^{-0.75}$body weight$\cdot d^{-1}$ found for adult cats in the present study. The higher endogenous urinary urea N excretion of the cat in comparison to other mammals is not unexpected, as this obligatory carnivore has been shown to have a limited ability to conserve nitrogen because of non-adaptive hepatic enzymes involved in the catabolism of amino acids (Rogers et al. 1977). These enzymes are set to handle a high protein diet and the cat, therefore, loses a large amount of amino acid nitrogen even when fed a low protein or protein-free diet (Rogers and Morris 1980).

The protein-free technique for the determination of EUN excretion in animals has been criticized as it reflects nitrogen metabolism in animals deprived of protein rather than nitrogen metabolism in animals under normal physiological conditions (Holmes 1965, Berdanier et al. 1967). Another criticism of this method is that EUN excretion is not constant but declines with time and that a lower EUN excretion may be obtained by increasing the period of protein-free feeding (Dawson and Allen 1961, Flurer et al. 1988, Holmes 1965). Although this has been shown to occur in other mammals such as dogs, humans and marmosets (Deuel et al. 1928, Flurer et al. 1988, Kendall et al. 1982), the present study indicates that this may not occur to such an extent in the adult cat. The regression technique, however, allows determination of EUN excretion under a physiologically more normal situation and involves the regression to zero protein intake of the urinary N metabolite excretion data of animals fed graded levels of dietary nitrogen. When the regression method is used for the determination of endogenous urinary total N, urea N and ammonia N excretion in

adult animals, it is important to supply dietary protein below the maintenance protein requirement of the animal and to minimise the catabolism of amino acids to supply energy. In Study 2, the four protein-containing diets were formulated to contain levels of protein below the minimum protein requirement for adult cats (National Research Council 1986) and the daily energy intake of the cats in Study 2 was set at 251 kJ ME·kg^{-1}body weight·d^{-1}, the dietary energy intake level required by adult cats housed in a metabolism cage (Goggin et al. 1993, Kane et al. 1981, Miller and Allison 1958). Cats develop severe hyperammonaemia when an arginine-free diet is fed because this essential amino acid is required for the conversion of ammonia, generated by the catabolism of amino acids, to urea in the liver (Morris and Rogers 1978). To obtain accurate estimates for endogenous ammonia N excretion in adult cats by the regression technique it is, therefore, important to supply sufficient dietary arginine to allow maximal conversion of ammonia to urea. In cats fed a protein-free diet there is no need to supply a dietary source of arginine as the obligatory body protein degradation of animals fed a protein-free diet will supply the necessary arginine to prevent hyperammonaemia (Morris and Rogers 1978). Growing kittens require approximately 42 g of arginine per kg of dietary crude protein (National Research Council 1986). In the present study, the fish offal protein (Table 2) contained approximately 100 g of arginine per kg which should have been sufficient to allow maximal conversion of ammonia to urea in adult cats.

The estimates for the endogenous urinary total N and urea N made using the regression technique (mean ± SEM, 227 ± 41.2 and 166 ± 34.8 mg·kg^{-1}body weight·d^{-1}, respectively) were lower than the corresponding estimates made using the protein-free technique (277 ± 9.0 and 187 ± 6.9 mg·kg^{-1}body weight·d^{-1}, respectively) while the endogenous urinary ammonia N excretion was slightly higher than the estimate obtained by the protein-free technique (24.1 ± 3.96 vs. 21.3 ± 0.86 mg·kg^{-1} body weight·d^{-1}). The estimates of endogenous total N, urea N and ammonia N obtained by the regression technique, however, had large standard errors and the corresponding estimates for the N metabolite excretions obtained by the protein-free technique all lay within the 95 % confidence limit of the regression estimates, making the differences in the estimates between the two techniques statistically non-significant. The lower values for the endogenous urinary total N and urea N excretion obtained by regression as compared to the protein-free method are in accordance with observations made in other animals. Berdanier et al. (1967) found that the

regression technique gave lower estimates for EUN excretion in rats than the protein-free technique while Moughan et al. (1987) obtained estimates of endogenous urinary total N and urea N by the regression method in pigs which were 11 and 17 % lower than the corresponding estimates obtained by the protein-free method, respectively. Endogenous urinary urea N, ammonia N and undetermined N in the urine of the cats in the present study made up similar proportions of the endogenous urinary total N with both methods (68, 8 and 21%, respectively in Study 1 and 73, 11 and 16%, respectively in Study 2). The higher mean EUN excretion of the cats in Study 1, therefore, was not due to a higher excretion of any one of the N fractions in the urine. The daily urinary excretion of creatinine N of the cats was not significantly different between the two studies and the mean (± SEM) daily excretion of creatinine N as measured over all the cats was 11.7 (± 0.51) $mg \cdot kg^{-1}$body weight and 17.1 (± 0.86) $mg \cdot kg^{-0.67}$body weight. This value is similar to the urinary creatinine N excretion of 14.8 ± 0.61 $mg \cdot kg^{-1}$body $weight \cdot d^{-1}$ (20.0 ± 0.83 $mg \cdot kg^{-0.67}$body $weight \cdot d^{-1}$) for a male cat found by Hammett (1915).

Greaves and Scott (1960) determined EUN excretion for the adult cat by the regression technique and re-analysis of the data of Greaves and Scott (1960) resulted in an estimate of 25 (± 48.6) $mg \cdot kg^{-1}$body $weight \cdot d^{-1}$. This low value is likely to be the result of the dietary protein intake which was in excess of the requirement for body maintenance in the majority of the animals in this study, as evident from the positive N-balance. Fitting a linear regression line through urinary N excretion data of adult animals ingesting protein in excess of requirement will result in a steeper regression line than when a linear regression line is fitted through urinary N excretion data of adult animals fed protein below the requirement for body maintenance.

The present study provides hitherto unavailable data on the endogenous excretion of nitrogen metabolites in the urine of the adult domestic cat. These data can be used for the factorial determination of the protein and amino acid requirements of the adult domestic cat.

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CHAPTER 4 PART II

BODY COMPOSITION OF THE ADULT DOMESTIC CAT (*FELIS CATUS*)

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4.II.1 ABSTRACT

The aim of this study was to determine the chemical body composition of male and female adult cats. The dehaired empty bodies of twenty adult cats were subjected to analysis for dry matter, lipid, ash, crude protein, amino acids, and several minerals (Ca, P, K, Mg, Fe, Mn and Zn). There were no significant ($P > 0.05$) differences in the water (mean ± SEM, 62.3 ± 0.68 %), lipid (11.2 ± 1.18 %) and ash (4.5 ± 0.11 %) contents per unit of wet tissue between the male and female cats. Adult male cats were found to have significantly ($P < 0.05$) higher amounts of crude protein per unit of wet tissue (21.7 ± 0.35 % vs. 20.0 ± 0.60 %), lipid-free matter (24.1 ± 0.22 % vs. 23.0 ± 0.22 %) and lipid-free dry matter (80.7 ± 0.23 % vs. 78.3 ± 0.32 %) than female cats. There was no significant ($P > 0.05$) effect of gender on the whole body amino acid composition (lipid-free dry matter or mol % basis) except for cysteine which was significantly ($P < 0.05$) lower in the male cats in comparison to the female cats. The amount of amino acid nitrogen in the lipid-free dry matter was not significantly ($P > 0.05$) different between the two sexes indicating that the higher amount of crude protein per unit of lipid-free dry matter in the male cats was the result of a higher amount of either non-amino acid nitrogen, amino acids not measured in the present study, or both. The overall mean whole body essential amino acid pattern (relative to 100 mol of lysine) of the cat was: arginine, 82; histidine, 41; isoleucine, 54; leucine, 113; methionine, 32; phenylalanine, 47; threonine 71 and valine, 78. There was no significant ($P > 0.05$) effect of gender on the concentration of any of the minerals per unit of body ash. The mean ± SEM concentrations of Ca, P, K and Mg in the ash fraction were 32.6 ± 0.91, 18.6 ± 0.45, 4.7 ± 0.12, 0.8 ± 0.02 g/100 g, respectively and the mean ± SEM concentrations of Fe, Mn and Zn were 97.5 ± 4.01, 63.7 ± 1.79 and 1.3 ± 0.06 mg/100 g. The Ca:P ratio was not significantly ($P > 0.05$) different between the male and female cats and was on average 1.75 ± 0.011. The body composition of the adult cat is similar to the body composition reported for other adult mammals.

4.II.2 INTRODUCTION

The chemical composition of the body of mammals is of fundamental biological interest. Moreover, there are limited data in the literature on body composition in the cat especially on body amino acid composition. Dunn et al. (1949) determined the amino acid composition of body protein in two adult cats of unknown sex while Stratmann (1988) determined body amino acid composition for one male and two female adult cats. One practical application of information on chemical body composition is in the determination of nutrient requirements using the factorial approach. This study is part of a series of studies at our Centre (Hendriks et al. 1996a, Hendriks et al. 1996b) to determine the amino acid requirements of adult domestic cats following this method.

The present work was undertaken to provide information on the chemical composition of the cat body and specifically to elucidate the body amino acid composition of the adult domestic cat of both sexes. Chemical analysis was made on dehaired empty bodies. Observations on the chemical composition of cat hair are reported separately (Hendriks et al. 1996c).

4.II.3 MATERIAL AND METHODS

The study reported here was approved by the Massey University Animal Ethics Committee. Twenty (14 entire male and 6 non-pregnant female) short-haired cats, 1 to 6 y of age and with a body weight range of 2472 to 5025 g (mean ± SEM, 3819 ± 135 g) were used. The animals had been fed a balanced cat food for one month before being euthanased using carbon dioxide gas. The body weight and empty body weight of each cat was recorded by re-weighing the animal after the contents of the digestive tract, bladder and gall-bladder had been removed. The skin of each cat was removed and dehaired using a depilatory solution (Hendriks et al. 1996a) before the carcass and hairless skin were sealed in a plastic bag and stored at -20° C.

4.II.3.1 Body processing

Upon removal from deep-freeze, each carcass was immediately cut into transverse sections using an electrically driven hand saw. The skin of each animal was manually cut into small pieces and together with the carcass sections ground through a 10 mm

aperture plate with the pieces of skin equally distributed over the number of available carcass sections. The minced contents were thoroughly mixed and minced twice more before the entire content was ground a further three times through a 6 mm aperture plate with thorough mixing of the contents between each mincing. Mixing was conducted as quickly as possible under conditions which minimised evaporative water loss. A representative 150 g sample of the minced material was mixed for 60 sec in a high speed blender and was immediately analysed for dry matter and ash while the remaining material was freeze-dried and frozen (-20° C) until further chemical analysis. This body processing procedure has been shown previously to give satisfactory samples for whole-body chemical analysis (Hendriks and Moughan 1993).

4.II.3.2. Chemical analysis

Dry matter was determined in triplicate by drying samples at 70° C to constant weight while ash was determined by heating the samples at 550° C for 16 h. Lipid was determined by petroleum ether extraction of duplicate freeze-dried samples (AOAC 1980). The lipid-extracted material was ground (1 mm sieve) and samples were taken for the determination of nitrogen, amino acids and minerals. Total nitrogen was determined in duplicate using the Kjeldahl method and crude protein was calculated by multiplying total nitrogen by 6.25. Minerals (Ca, P, K) and trace elements (Mg, Fe, Mn and Zn) were analysed in triplicate using inductively coupled argon plasma atomic emission spectrometry (Lee 1983).

Amino acids were determined in triplicate on 5-mg samples by hydrolysing with 1 mL of 6 mol/L glass distilled HCl (containing 1.0 g phenol/L) for 24 h at 110 ± 2° C in glass tubes, sealed under vacuum. The tubes were opened and 50 μL of 40 mM norleucine was added to each tube as an internal standard whereafter the tubes were dried under vacuum (Savant Speedvac Concentrator AS 290, Savant Instruments Inc., Farmingdale, NY). Amino acids were loaded onto a Waters ion exchange HPLC system (Millipore, Milford, MA) employing postcolumn derivatisation with ninhydrin and detection at 570 nm. Proline was detected at 440 nm. The chromatograms were integrated using dedicated software (Maxima 820, Waters, Millipore, Milford, MA) with amino acids identified and quantified by retention time against a standard amino acid mixture (Pierce, Rockford, Illinois). Cysteine and methionine were analysed as cysteic acid and methionine sulphone by

oxidation with 2 mL of performic acid (1 part 30 % H_2O_2 to 9 parts 88 % formic acid) for 16 h at 0° C and neutralisation with 0.3 mL 48 % (wt/wt) HBr prior to hydrolysis. Tryptophan was not determined. No corrections were made for loss of amino acids during acid hydrolysis. Amino acid concentrations were corrected for recoveries of norleucine and converted to a weight basis using free amino acid molecular weights.

4.II.3.3 Data analysis

The various chemical body components in the hair-free empty body of the cats were expressed either per unit of wet tissue (WT), lipid-free matter (LFM), dry matter (DM), lipid-free dry matter (LFDM) or ash. The data were tested for homogeneity of variance using Bartlett's test (Snedecor and Cochran 1980) and if found to be non-homogeneous were transformed ($\log_{10}$). Student's *t* test was used to determine levels of significance for the difference in concentration of the various body components between the male and female cats. Where there was no significant effect of gender, the data were pooled across sexes with the exception of the amino acid data. Effects were considered significant at $P < 0.05$.

4.II.4 RESULTS AND DISCUSSION

The average Coefficient of Variation for the triplicate amino acid analyses, as determined over all the samples and amino acids was 3.6 %. The mean (± SEM) recovery of norleucine as determined over all the samples was 97 ± 0.3 %. The variations of the triplicate mineral and trace element analyses were found to be satisfactory with an average Coefficient of Variation as determined over all the samples of 7.1, 7.3, 6.3, 7.5, 8.4, 6.5 and 7.3 % for Ca, P, K, Mg, Fe, Zn and Mn, respectively.

The mean body weight, empty body weight, hair weight and concentrations of the chemical body components for the adult cats are presented in Table 1. The mean body weight and empty body weight of the male cats in the present study were significantly higher than for the female cats. This is in accordance with data in the literature (Latimer 1936, Hendriks et al. 1996a) which also show adult male cats to be significantly heavier than adult female cats. The mean ± SEM empty body weight of the cats was 97 ± 0.4 % of the body weight. There was no significant difference in

the amount of hair on the body per unit of empty body weight between the male and female cats.

TABLE 1 **Mean, standard error of the mean and range in body weight, empty body weight, hair weight and concentrations of chemical body components for adult male and female domestic cats.**

	Mean	SEM	Range	
			Min.	Max.
Body weight (kg)				
male	4.00	0.131	3.11	5.02
female	3.39[1]	0.271	2.47	4.45
Empty body weight (kg)				
male	3.88	0.127	3.08	4.85
female	3.25[1]	0.267	2.46	4.35
Hair weight (g/kg EBW[2])	20.6	1.05	13.3	30.3
		(g/100 g wet tissue[3])		
Water	62.3	0.68	53.7	66.8
Lipid	11.2	1.18	6.22	23.6
Crude protein				
male	21.7	0.35	19.1	23.3
female	20.0[1]	0.60	18.0	22.2
Ash	4.5	0.11	3.4	5.5
Undetermined[4]	0.9	0.07	0.2	1.5
		(g/100 g lipid-free matter[3])		
Water	70.2	0.20	68.7	72.5
Crude protein				
male	24.1	0.22	22.9	25.3
female	23.0[1]	0.22	22.5	23.7
Ash	5.1	0.11	3.8	6.1
Undetermined[5]	0.9	0.08	0.3	1.6
		(g/100 g dry matter[3])		
Lipid	29.2	1.98	17.9	50.9
Crude protein	56.7	1.69	38.9	66.0
Ash	12.1	0.35	8.6	14.5
Undetermined[6]	2.0	0.24	0.5	3.7
		(g/100 g lipid-free dry matter[3])		
Crude protein				
male	80.7	0.23	79.4	82.0
female	78.3[1]	0.32	77.5	79.3
Ash	17.1	0.34	13.8	19.6
Undetermined[7]	2.9	0.33	0.9	5.2

The number of observations for the male cats = 14, and female cats = 6. Where there was no significant ($P > 0.05$) effect of gender, the data were pooled across sexes.

[1]Significantly different from females ($P < 0.05$).
[2]Empty body weight.
[3]In the hair-free empty body.
[4]100-(water+lipid+crude protein+ash).
[5]100-(water+crude protein+ash).
[6]100-(lipid+crude protein+ash).
[7]100-(crude protein+ash).

There was no significant difference in the water content per unit of WT and LFM between the two sexes. The ash content and the undetermined component of the body (either on a WT, DM, LFM or LFDM basis) were not significantly different between the male and female cats. The crude protein content was not significantly different between the male and female cats when the data were expressed per unit of DM but was significantly higher in the male cats when the data were expressed per unit of WT, LFM and LFDM. On average the lipid and crude protein contents in the body DM of the cats made up 29 ± 2.0 % and 57 ± 1.7 %, respectively and showed a large degree of inter-animal variation with a range of 18 to 51 % and 39 to 66 %, respectively. Similar proportions of lipid (32 ± 1.7 %) and crude protein (52 ± 1.4 %) per unit of DM in comparison to those found in the present study were reported by Stratmann (1988) analysing a total of 22 adult male and female cats. Statistical analysis of the data presented by Stratmann (1988) also shows a significant difference in the amount of crude protein in the LFDM fraction of the body between male and female cats with the LFDM of the male cats containing 78 ± 1.0 % crude protein which is close to the value found in the present study. The value found for the female cats by Stratmann (1988) of 73 ± 1.0 %, however, is 5.3 % units lower than the corresponding value found for the female cats in the present study. Stanton et al. (1992) also analysed a total of 22 adult male and female cats but found an average amount of 42 and 48 % of lipid and crude protein per unit body DM, respectively.

The chemical body composition of the adult cat is similar to that found in other adult mammals such as the rat, rabbit, dog, pig and human (Pace and Rathbun 1945, Spray and Widdowson 1950, Widdowson and Dickerson 1964). The percentage of water and crude protein per unit of LFM in adult mammals ranges from approximately 70 to 78 % and from 20 to 24 %, respectively. The water content per unit of LFM for the adult cat (70 %) found in the present study is comparable to that in other mammals. The crude protein content of the LFM for adult male and female cats (23 and 24 g/100 g, respectively) is also similar to values quoted for other mammals.

The mean amino acid composition of the body for the adult male and female cats is presented in Table 2. There was no significant difference in the concentration of amino acids in the LFDM and the pattern (mol %) of amino acids in the body of

TABLE 2 **Body amino acid composition and pattern in the adult domestic cat.**

Amino acid	Gender		Gender		Pattern[1]
	Male	Female	Male	Female	
	(g/100 g lipid-free dry matter)		(mol %)[2]		
Essential					
Lysine	5.52 ± 0.046	5.54 ± 0.149	6.10 ± 0.041	6.16 ± 0.124	100
Arginine	5.37 ± 0.056	5.34 ± 0.054	4.97 ± 0.034	4.99 ± 0.037	82
Histidine	2.47 ± 0.050	2.27 ± 0.135	2.57 ± 0.046	2.38 ± 0.122	41
Isoleucine	2.65 ± 0.023	2.70 ± 0.079	3.26 ± 0.025	3.35 ± 0.076	54
Leucine	5.57 ± 0.047	5.70 ± 0.165	6.86 ± 0.045	7.06 ± 0.149	113
Methionine	1.78 ± 0.029	1.83 ± 0.039	1.93 ± 0.034	1.99 ± 0.041	32
Phenylalanine	2.90 ± 0.025	2.92 ± 0.069	2.83 ± 0.023	4.83 ± 0.074	47
Threonine	3.17 ± 0.023	3.25 ± 0.079	4.29 ± 0.035	4.43 ± 0.074	71
Valine	3.44 ± 0.025	3.48 ± 0.078	4.74 ± 0.023	483 ± 0.074	78
Semi-essential					
Cysteine	0.90 ± 0.016[3]	0.97 ± 0.029	1.19 ± 0.023[3]	1.31 ± 0.037	20 (M), 21 (F)
Taurine	0.17 ± 0.009	0.20 ± 0.016	0.22 ± 0.012	0.26 ± 0.020	4
Tyrosine	2.20 ± 0.017	2.27 ± 0.073	1.97 ± 0.017	2.04 ± 0.051	32
Non-essential					
Alanine	5.31 ± 0.070	5.23 ± 0.030	9.63 ± 0.061	9.54 ± 0.050	157
Aspartic acid	6.52 ± 0.059	6.58 ± 0.138	7.91 ± 0.038	8.04 ± 0.096	130
Glutamic acid	10.59 ± 0.085	10.68 ± 0.202	11.62 ± 0.063	11.79 ± 0.126	191
Glycine	7.96 ± 0.148	7.49 ± 0.268	17.12 ± 0.238	16.25 ± 0.690	276
Hydroxylysine	0.28 ± 0.009	0.27 ± 0.017	0.28 ± 0.008	0.27 ± 0.019	5
Proline	5.29 ± 0.074	5.12 ± 0.120	7.41 ± 0.066	7.24 ± 0.197	121
Serine	3.32 ± 0.022	3.36 ± 0.057	5.10 ± 0.031	5.20 ± 0.048	84

Values are means ± SEM. The number of observations for the male cats = 14, and female cats = 6. M = male, F = female.

[1]Body amino acid pattern in mol per 100 mol of lysine.
[2]Mol of each amino acid as a percent of the total mol of amino acids.
[3]Significantly ($P < 0.05$) different from corresponding value for females.

the male and female cats with the exception of cysteine which was significantly higher in the LFDM and on a molar percent basis in the female cats as compared to the male cats. The higher ratio of cysteine in the body protein of the male cat as compared to the female cat may be due to small differences in the relative weights of various organs which have different cysteine contents (Tallan et al. 1954) and resulting in an overall difference of cysteine in the whole-body between male and female cats. Male cats, besides having different reproductive organs, have a heavier pair of kidneys, integument and spleen per unit of body weight than female cats while the brain, pancreas and gastrointestinal tract are heavier per unit of body weight in female cats (Altman and Dittmer 1962). In this respect Kyriazakis et al.

(1993) found that the concentration in the body protein, of lysine and histidine increased whereas the cysteine concentration decreased during the growth of pigs. These authors explained this result by the change in the proportion of body hair protein, which is relatively high cysteine and low in lysine and histidine, to body protein (excluding hair) during growth.

Dunn et al. (1949) determined the amino acid composition of body protein in two adult cats using microbiological assays while Stratmann (1988) determined the amino acid content of the body of three adult cats using modern amino acid analysis techniques. The body amino acid compositions for the 20 cats investigated in the present study agree well with the values reported by Dunn et al. (1949) and Stratmann (1988) although the combined data of the latter two studies show a high degree of variation. In the present study taurine was found to be present in the body of adult male and female cats which differs from observations made by Stratmann (1988) who could not detect taurine in the adult cat body. The amount of nitrogen originating from amino acids in the LFDM fraction was not significantly different between the male and female cats. The nitrogen content in the LFDM fraction of the male cats, however, was significantly higher than in the female cats. From these results it appears that the body of the male cat in comparison to the female cat contains either more non-amino acid nitrogen, amino acids not measured in the present study, or both.

The amino acid composition of the adult cat body is similar to that found in other mammals such as the adult dog, mink, guinea pig, rat and rabbit (Block and Bolling 1947, Dunn et al. 1949, Williams et al. 1954, Glem-Hansen 1992).

The concentrations of minerals in the ash fraction of the body are presented in Table 3. Because the depilatory solution which was used for the dehairing of the skin of the cats in the present study contained NaOH and Na_2S, the concentrations of sodium and sulphur were not determined. There were no significant differences in the concentrations of any of the minerals or trace elements in the ash fraction between the male and female cats. This is in accordance with the data of Stratmann (1988) which, after statistical analysis, show no significant difference in the concentrations of any of the minerals between male and female cats, except for the concentration of zinc which was significantly higher per unit of ash in male cats as compared to female cats. The data of Stratmann (1988), however, were highly variable, in particular for iron, magnesium and zinc. The concentrations of calcium,

potassium, phosphorus, magnesium and zinc in the ash fraction of the cats in the present study are in good agreement with the values of Stratmann (1988). The latter author, however, reported a mean (± SEM) concentration of iron and manganese per unit of ash of (mean ± SEM) 570 ± 459.2 and 3.7 ± 2.39 mg/100 g, which is approximately 6 and 3 times higher, respectively, than values found in the present study. The seven elements investigated in the present study made up 57 ± 1.4 % of the body ash fraction of the cats. The Ca:P ratio in the body was not significantly different between the male and female cats and was on average 1.75 ± 0.011. This ratio is slightly higher than the 1.66 found by Spray and Widdowson (1950) who analysed 3 adult cats and the ratio of 1.68 ± 0.02 found by Stratmann (1988) for 22 adult cats.

TABLE 3 **Mean, standard error of the mean and range for the body concentrations of several minerals in the ash fraction of the adult domestic cat.**

Element:	Mean	SEM	Range	
			Min.	Max.
		(g/100g ash)		
Calcium	32.6	0.91	25.9	40.2
Phosphorus	18.6	0.45	14.6	22.0
Potassium	4.7	0.12	3.9	5.7
Magnesium	0.8	0.02	0.6	1.0
		(mg/100g ash)		
Iron	97.5	4.01	62.3	134.8
Zinc	63.7	1.79	50.4	80.3
Manganese	1.3	0.06	0.9	1.9

The number of observations for the male cats = 14, and female cats = 6. Data were transformed (log_{10}) before statistical analysis. There was no significant ($P > 0.05$) difference in the concentration of any of the elements in the ash fraction between the male and female cats.

The present study provides comprehensive data on the chemical body composition of adult male and female domestic cats and information on the amino acid composition of the adult domestic cat. These data can be used in the determination of amino acid requirements for the adult domestic cat by the factorial method.

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CHAPTER 5

EXCRETION OF FELININE IN THE ADULT DOMESTIC CAT

Felinine is a urinary sulphur-containing amino acid excreted by several members of the Felidae family including the domestic cat (*Felis catus*). Excretion levels by adult cats have been reported to be extremely high and, therefore, may have a significant effect on the sulphur amino acid requirements of this animal. This chapter first presents an overview of the current knowledge on felinine. Based on the current literature it became apparent that accurate quantitative data on excretion levels of felinine in the urine of adult cats are lacking. In order to obtain a felinine standard for quantification experiments using High Performance Liquid Chromatography, the published procedures for the synthesis of felinine were evaluated for their yield. A new synthesis procedure was developed and is presented in Part II of this chapter. Finally, this chapter provides quantitative information on normal excretion levels of felinine in adult domestic male and female cats which can be used in a factorial model which aims to determine the amino acid requirements of adult cats.

CHAPTER 5 PART I

FELININE: A URINARY AMINO ACID OF FELIDAE

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5.I.1 ABSTRACT

Felinine (2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid) has been identified in the urine of several members of the Felidae family including the cat (*Felis catus*). Rates of excretion of 95 mg/day have been recorded for entire male cats with entire female cats excreting around 19 mg/day. These high excretion rates in entire male cats may have a significant effect on the daily sulphur amino acid requirement. The isoamyl moiety of felinine seems to originate from the same isoprenoid pool as used for the synthesis of cholesterol in the cat. The sulphur in the felinine molecule appears to originate from cysteine, although some contradictory evidence exists. The site of synthesis and the method of transportation in the blood remain largely unknown. The biological significance of felinine to the animal is still a matter for speculation, but its function as a precursor to a pheromone seems likely. Recently, an accurate chemical assay for felinine has been developed that will allow investigation of felinine in different tissues and excretions and from a wider range of mammals.

5.I.2 INTRODUCTION

Felinine (2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid) has been recognised for some time as a urinary amino acid excreted in large quantities by the domestic cat and several other Felidae (Datta and Harris, 1951, 1953; Westall, 1953). Another sulphur containing amino acid, isovalthine (2-amino-5-carboxy-6-methyl-4-thiaheptanoic acid), which is structurally similar to felinine, has also been detected in the urine of the domestic cat (Oomori and Mizuhara, 1962) and lion (Greaves, 1965). The biological functions of isovalthine and felinine are unclear, but there is some evidence that felinine has a role in territorial marking involving urine spraying.

There have been several studies carried out 30-40 years ago (Greaves and Scott, 1960; Avizonis and Wriston, 1959; Fukutome, 1961; Roberts, 1963) on these unusual branched chain sulphur amino acids, but little further work has been published until recently when Hendriks *et al.* (1995b), in an attempt to develop an assay for the detection of felinine, showed that several of the published felinine synthesis procedures do not yield felinine but rather a closely related isomer. A new synthesis procedure was developed and described by the latter workers, enabling felinine to be produced in relatively high yields for use as a standard in the quantification of felinine. High-performance liquid chromatography (HPLC) analysis of felinine facilitates new investigations into the metabolism and biological role of this amino acid.

This review highlights the metabolism and biological properties of felinine and emphasises its apparent uniqueness to some members of the Felidae family.

5.I.3 CHARACTERISATION AND SYNTHESIS OF FELININE

During a survey of the urinary and plasma amino acid patterns of various animal species, Datta and Harris (1951) found a previously unknown amino acid in the urine of domestic cats (*Felis catus*) and an ocelot (*Felis pardalis*). The amino acid migrated to a position similar to leucine in the two dimensional paper chromatograms, when phenol and collidine-lutidine were used as the solvents. The amino acid was tentatively named "cat spot." Two years later, Westall (1953) isolated cat spot as an amorphous solid from cat urine using large ion-exchange columns and renamed the amino acid felinine. The simplest empirical formula that

could be derived from degradation experiments was $C_8H_{17}O_3NS$, and the structure of this sulphur amino acid was provisionally determined as 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid (Fig 1).

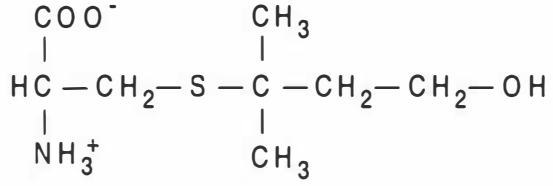


Fig. 1. Molecular structure of felinine, 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid (Westall, 1953).

Chemical synthesis of felinine was first achieved by Trippett (1957) by the reductive S-debenzylation of 3-methyl-3-thiobenzylbutan-1-ol in the presence of sodium in liquid ammonia and β-chloroalanine. The synthetic material behaved exactly as a sample of "natural" felinine obtained from Westall (1953). Trippett (1957) further demonstrated that natural felinine has the L-configuration. A subsequent chemical synthesis of felinine was published by Eggerer (1962) in which the main route of synthesis used a phosphate derivative of isopentenol in a reaction with cysteine. Two other routes, which were also claimed to yield felinine, used isopentenol and cysteine in the presence of pyridine (Eggerer, 1962). Further synthesis procedures reporting yields of felinine of up to 80 % where published by Schöberl *et al.* (1966, 1968).

More recently and to obtain a standard for HPLC, Hendriks *et al.* (1995b) evaluated the synthesis procedures of Trippett (1957), Schöberl *et al.* (1966, 1968) and two of the three procedures reported by Eggerer (1962), using nuclear magnetic resonance, mass spectrometry and HPLC procedures. Most of the published procedures were shown to produce an isomer of felinine, whereas the only evaluated procedure leading to felinine (Trippett, 1957) was found to give low yields. Hendriks *et al.* (1995b) also published a new procedure for the synthesis of felinine leading to high yields of this amino acid.

5.I.4 OCCURRENCE OF FELININE

5.I.4.1 Effect of species

The urine of many mammals has been investigated for the presence of felinine (Table 1). All animals found to excrete felinine belong to the Felidae family, although it appears that not all members of this family excrete the amino acid. The method for detection of felinine in these studies (Datta and Harris, 1951, 1953; Roberts, 1963), however, was two-dimensional paper chromatography, a rather insensitive technique which does not give accurate quantitative measurements. A failure to detect felinine in the urine of certain animals does not necessarily mean that the animal does not excrete felinine. Because dietary factors seem to influence the excretion of felinine (Datta and Harris, 1953), detection of this amino acid could have been difficult, especially in combination with the low sensitivity of paper chromatography.

Table 1 Reported occurrence of felinine in the urine of different mammalian species.

	Reference*		
Species	1	2	3
Cat (*Felis catus*)	Yes	Yes	Yes
Ocelot (*Felis pardalis*)	Yes	Yes	-
Leopard (*Panthera pardus*)	-	Yes	No
Indian leopard cat (*Felis bengalensis*)	-	Yes	-
Bobcat (*Felis rufus*)	-	-	Yes
Cheetah (*Acinonyx jubatus*)	-	-	No
Puma (*Felis concolor*)	No	No	No
Tiger (*Panthera tigris*)	No	No	No
Lion (*Panthera leo*)	No	No	No
Serval (*Felis serval*)	-	No	-
Fishing cat (*Felis viverrina*)	-	No	-
Dog (*Canis familiaris*)	No	No	-
Binturong (*Arctictis binturong*)	No	No	-
Genet (*Genetta tigrina erlangeri*)	No	No	-
Pole cat (*Putorius putorius*)	-	No	-
Bear (*Ursus* ?)	-	-	No
Rat (*Rattus norvegicus*)	No	No	-
Mouse (*Mus musculus*)	No	No	-
Guinea-pig (*Cavia porcellus*)	No	No	-
Golden hamster (*Mesocricetus auratus*)	-	No	-
Rabbit (*Oryctolagus cuniculus*)	No	No	-
Rhesus monkey (*Macacus rhesus*)	-	No	-
Horse (*Equus caballus*)	No	No	-
Cow (*Bos taurus*)	No	No	-
Goat (*Capra hircus*)	-	No	-

*Reference: 1, Datta and Harris (1951); 2, Datta and Harris (1953); 3, Roberts (1963).
-, not determined

This may be the reason for the discrepancy between the two studies measuring felinine in the urine of the leopard (*Panthera pardus*). Datta and Harris (1953) detected felinine in leopards' urine whereas Roberts (1963) failed to show the presence of felinine in the urine of this species. With the abovementioned difficulties in detecting felinine using traditional methods, a re-evaluation of the excretion of felinine by members of the Felidae family and other species of the order Carnivora seems to be warranted.

5.I.4.2 Distribution in fluids and tissues of the cat

Published quantitative data on levels of felinine in fluids have been obtained using paper chromatography (Westall, 1953; Roberts, 1963) and ion exchange chromatographic methods (Tallan *et al.*, 1954; Avizonis and Wriston, 1959; Shapiro, 1962). Ion exchange chromatography requires a felinine standard of known purity, and in several of the studies using ion exchange chromatography the origin of the felinine standard was not given or when reported contained contaminants. Because paper chromatography does not allow an accurate quantification of felinine, the published quantitative data of felinine need to be interpreted with some caution. Westall (1953) determined felinine in the urine of the cat (1.0-1.2 g/l) but was unable to detect any felinine in ultrafiltered blood plasma. Datta and Harris (1953) subsequently confirmed the absence of felinine in blood plasma. High urinary levels of felinine were also reported by Tallan *et al.* (1954) using the ion exchange chromatographic method of Moore and Stein (1954) and presumably using the felinine isolated by Westall (1953) as a standard. A felinine concentration of 1.85 g/l was recorded in the urine of a male cat, whereas only trace amounts were detected in the plasma and in a bladder tissue extract. No felinine was found in tissue extracts of the kidney, liver, brain or gastrocnemius muscle. These observations led the authors to suggest that felinine may be formed in the kidney and excreted directly or that it may be present in a bound form in the plasma. Roberts (1963), however, detected felinine in tissue extracts of the liver, kidney and skin of the cat and suggested that felinine is carried in the blood at low concentrations from these tissues and that the low blood levels are maintained by a rapid excretion by the kidney. Rates of excretion of more than 8 g of felinine per litre urine have been reported by Avizonis and Wriston (1959) in a cat fed a cystine enriched diet for 3 days. Using ion exchange chromatography and Westall's (1953) "standard", the lowest urinary

concentration reported by these authors was 1.5 g/l. Shapiro (1962) reported urinary excretion rates of 1.7 and 1.0 g/l for a male and female cat, respectively, but did not report the synthesis procedure that gave the impure felinine standard used.

5.I.4.3 Effect of gender

Measuring felinine levels in the urine of 31 cats, Roberts (1963) noted that the amount of felinine excreted in the urine of the domestic cat is sex dependent. This study was based on measurements using paper chromatography and reported that adult male cats had higher urinary concentrations of felinine than adult female cats. However, Roberts (1963) failed to report whether the male and female cats investigated were entire or castrated and did not measure the daily urine volumes. Roberts (1963) was able to raise the level of urinary felinine in a female cat to that found in entire males, with 8 days of testosterone treatment. Oestrogen treatment did not affect felinine excretion in an entire male cat. Moreover, felinine could not be detected in the urine of kittens of either sex. A female kitten, the urine of which was analysed monthly, failed to show any felinine excretion until the attainment of 8 months of age. By 11 months of age, the cat was excreting felinine at the same rate as an adult female cat. The age of attaining sexual maturity is variable in female kittens but can be expected to occur around 9 months (Beaver, 1992). The work of Roberts (1963) was extended by Hendriks *et al.* (1995a), who assayed 24 hr urinary felinine excretions in a total of 28 entire and castrated cats of both sexes. Using HPLC analysis and a synthetic felinine standard of known purity, obtained according to the synthesis procedure described by Hendriks *et al.* (1995b), the average felinine concentration in the urine of entire male cats was found to be 2.0 g/l (range 0.4-3.6 g/l) with entire females excreting on average 0.3 g/l. The higher concentration of felinine in the urine of entire male cats as compared to entire female cats was found to result in higher amounts of felinine excreted per day. The average amounts of felinine excreted (mg/24 hour) were 95 for entire male cats, 29 for castrated males, 19 for entire females and 13 for spayed females.

5.I.5 BIOSYNTHESIS

In studies to elucidate the biosynthesis of felinine, Avizonis and Wriston (1959) showed that cystine, leucine and mevalonic acid can contribute to the formation of

felinine in the cat. The inclusion of additional cystine and leucine in the diet of cats and intraperitoneal injections of leucine and mevalonic acid all resulted in an increased felinine concentration in the urine. The participation of leucine and mevalonic acid in felinine biosynthesis was confirmed with isotope studies (Avizonis and Wriston, 1959) in which 2 % of DL-2-[^{14}C]leucine and 0.5 % of DL-2-[^{14}C]mevalonic acid radioactivity was incorporated into felinine. Furthermore, Avizonis and Wriston (1959) reported that other workers from the National Institutes of Health (Bethesda, MD) had incorporated [^{14}C]acetate and DL-2-[^{14}C]mevalonic acid into felinine. Shapiro (1962) injected cats with 2-[^{14}C]acetate and found radioactivity in both felinine and cholesterol, indicating a relationship between the latter two metabolites. Further evidence for such a relationship was obtained in a study on the rate of incorporation of DL-2-[^{14}C]mevalonic acid into felinine and cholesterol (Shapiro, 1962). Similar rates of incorporation of the radioactivity into felinine and free cholesterol were observed after injection. Wang (1964) also found similar rates of incorporation of radioactivity into felinine and cholesterol when 2-[^{14}C]acetate was used. Shapiro (1962) suggested that mevalonic acid is diverted to the metabolic pathways for cholesterol and felinine and that these pathways use the same isoprenoid pool. Radioactivity from injected DL-2-[^{14}C]leucine was also incorporated into felinine (Shapiro, 1962). This supports the observations made by Avizonis and Wriston (1959) that leucine is a precursor to felinine. However, the latter authors found leucine to be a better precursor to felinine than mevalonic acid.

Although Shapiro (1962) found that 4.0 % of injected L-[^{35}S]cystine was incorporated into felinine, Roberts (1963) failed to detect any radioactivity in felinine after cats were injected intravenously with [^{35}S]cystine. However, Roberts was also unable to incorporate any 2-[^{14}C]mevalonic acid into felinine. Even after supplementing the diet of a male cat with [^{35}S]-yeast, in which methionine as well as cystine where radioactively labelled, no radioactive felinine could be detected. Roberts (1963) concluded that cystine and methionine are not immediate precursors to felinine. Support for this conclusion was provided by Wang (1964). Wang isolated radioactive felinine from cat urine after administering 2-[^{14}C]acetate, 2-[^{14}C]mevalonic acid and 2-[^{14}C]leucine. The stepwise chemical degradation of these radioactively labelled felinine samples showed that the radioactivity for the first two precursors was found in the isoamyl alcohol moiety of felinine. The distribution of the radioactivity in the isoamyl alcohol moiety was found to be consistent with the

postulated view that this portion of the felinine molecule originates from an isoprenoid unit or isopentenylpyrophosphate. The radioactivity of the labelled leucine, however, was found in the alanine moiety of felinine. A metabolic pathway from leucine to the formation of serine and the subsequent incorporation of the radioactivity from serine into cysteine was given as a possible explanation of this latter finding (Wang, 1964). Figure 2 illustrates the proposed metabolic pathway (Eggerer, 1962; Shapiro, 1962; Wang, 1964; Borchers *et al.*, 1967) according to which felinine is believed to be synthesised in the cat (*F. catus*).

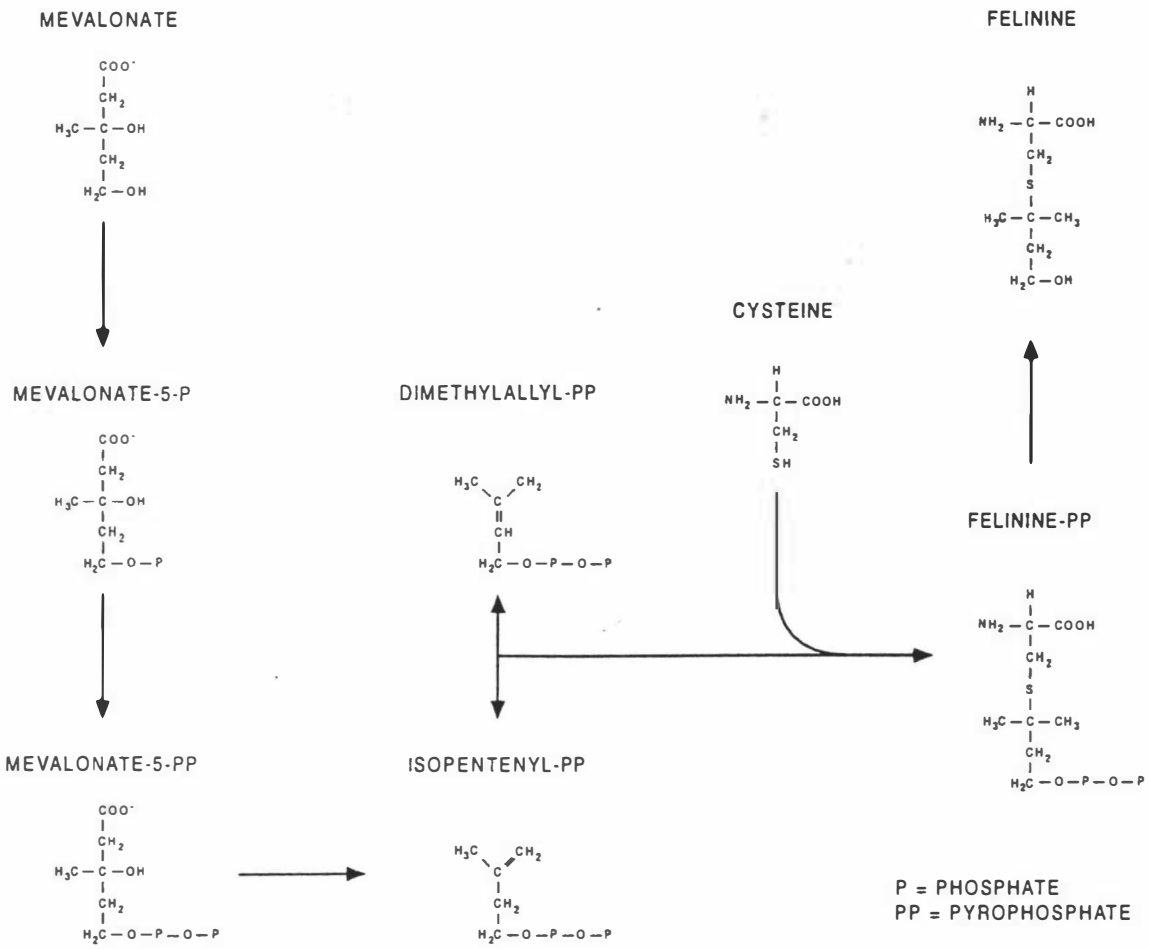


Fig. 2. Proposed metabolic pathway for the biosynthesis of felinine in the domestic cat (Eggerer, 1962; Shapiro, 1962; Wang, 1964; Borchers *et al.*, 1967).

Felinine is believed to be synthesised from the same isoprenoid units as cholesterol in the cat. The major sites of synthesis of cholesterol in the rat, squirrel monkey, guinea-pig, rabbit and hamster are the liver, skin, gastrointestinal tract and

carcass muscles (Spady and Dietschy, 1983). Similar studies in the cat have not been reported, but it may be assumed that cholesterol synthesis occurs in the same tissues. Roberts (1963) found felinine in tissue extracts of the liver, kidney and skin, and this would suggest that felinine is synthesised in similar tissues to cholesterol. Although Tallan *et al.* (1954) found traces of felinine in the plasma, other researchers have failed to demonstrate the presence of felinine in cat blood (Datta and Harris, 1953; Westall, 1953). These latter findings have been confirmed using modern HPLC analysis of ultrafiltered (<10.000 Dalton) tom cat plasma (W. Hendriks, unpublished observations). This raises the question as to how felinine is transported from the site of synthesis to the kidney.

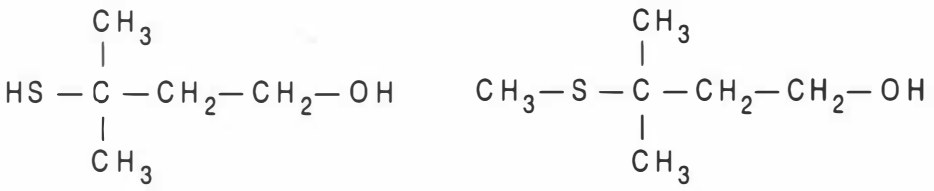
A possible solution to the question concerning the transportation of felinine in the blood was provided by Kuwaki *et al.* (1963). Using guinea pig liver homogenates, they showed that isovalthine could be formed *in vitro* as part of a glutathione-isovaleric acid conjugate (GSIV). The reason for the use of guinea pig liver instead of cat liver was not given by the authors. Hydrolysis of the GSIV in 2 M HCl for 20 hr was reported to yield, besides glycine and glutamic acid, isovalthine.

In vitro synthesis of felinine in liver homogenates of the cat has largely been unsuccessful. In the many experiments conducted, no felinine was ever found to be produced (Roberts, 1963; Wang, 1964). Wang (1964) also attempted to demonstrate the presence of a glutathione conjugating system in the liver and glutathionase activity in the kidney of the cat. However, no conjugates of glutathione could be detected and no glutathionase activity could be demonstrated in the kidney. The author indicated that the failure to detect any conjugates of glutathione could have been due to the use of isopentenylphosphate instead of isopentenylpyrophosphate in the experiments. Furthermore, Wang (1964) calculated, based on the experiment of Kuwaki *et al.* (1963), that if a glutathione isopentenylphosphate conjugate would have been formed, the amounts would have been too small to detect by paper chromatography. If felinine is transported in the blood in a glutathione conjugate, it would be expected that hydrolysis of the blood would yield felinine. However, it has been observed (W. Hendriks, unpublished observations) that synthetic felinine is an acid-labile amino acid that is destroyed under the conditions of 2 and 6 M HCl hydrolysis at 110° C for 24 hr.

5.I.6 BIOLOGICAL SIGNIFICANCE OF FELININE

The biological role of felinine in the animal is still a matter of speculation, although several theories have been proposed. Shapiro (1962) and Roberts (1963) hypothesised that the excretion of felinine and isovalthine may be a mechanism for the regulation of steroid metabolism in the cat. Wang (1964) hypothesised that felinine could play a regulatory role in cholesterol biosynthesis in the cat. Although felinine and cholesterol seem to be synthesised from the same isoprenoid pool, no direct evidence for a regulatory effect of felinine on cholesterol or steroid metabolism has been found.

A more likely role for felinine, as a urinary component for territorial marking, was suggested by MacDonald *et al.* (1984). The observed differences in urinary felinine excretion rates between male and female cats (Roberts, 1963; Hendriks *et al.*, 1995a) are in accordance with this role. Further evidence to support this hypothesis was published by Joulain and Laurent (1989). They observed that "fresh" cat (*F. catus*) urine was mostly odourless but upon "ageing" an odour developed reaching a peak at ~12-24 hours. The extent of the odour depended upon the diet and the period within the sexual cycle of the cats. These authors suggested that the compound responsible for the tom cat urine odour originates from decomposition of felinine due to microbial activity and/or oxidation in air into 3-mercapto-3-methyl-1-butanol (I) (Fig 3). This latter compound was detected in cat urine (Joulain and Laurent, 1989) and subsequently found in bobcat (*Felis rufus*) urine by Mattina *et al.* (1991). Furthermore, compound I was characterised as having a "catty" odour at concentrations of 10-1000 ppb (Joulain and Laurant, 1989). Another volatile compound (3-methyl-3-methylthio-1-butanol) (II), which may also be formed on degradation of felinine, was found in cat (Joulain and Laurent, 1989) and bobcat urine (Mattina *et al.*, 1991) (Fig 3). These authors also found other volatile components (disulfides and trisulfides) in the urine of these animals which are derivatives of compounds I and II. It was noted by Hendriks *et al.* (1995b) that odourless synthetic felinine stored as a lyophilisate at -20° C and room temperature developed an odour that was similar to the odour of tom cat urine. It seems likely, therefore, that felinine degrades to another compound or other compounds to give rise to the distinct tom cat urine smell.



(I) (II)

Fig. 3. Molecular structure of 3-mercapto-3-methyl-1-butanol (I) and 3-methyl-3-methylthio-1-butanol (II).

Bobcat urine has been shown to have a repellent effect on the snowshoe hare (*Lepus americanus*) (Sullivan *et al.*, 1985a), the black-tailed deer (*Odocoileus hemionus columbianus*) (Sullivan *et al.*, 1985b) and the white-tailed deer (*Odocoileus virginianus*) (Swihart, 1991). Although felinine and the volatile compounds I and II have been found in the urine of bobcats, there is no direct evidence that felinine degrades to compounds I and/or II, or whether any of these compounds or derivatives of these compounds actually have a repellent effect. It is notable in this respect that Baines *et al.* (1988) have patented a synthetic deer repellent based on an acid fraction obtained after Soxhlet extraction of lion faeces (*Panthera leo*) and felinine. These authors refer to felinine as "another animal repellent" although no proof is given to substantiate this statement. However, the synthesis procedure of felinine in this patent has recently been shown to produce an isomer of felinine (Hendriks *et al.*, 1995b). Boag and Mlotkiewicz (1994) subsequently demonstrated a repellent effect of this patented mixture with wild rabbits.

Related to the possible repellent effect and territorial marking roles of felinine, is its possible role as a pheromone to attract the opposite sex. Although no direct evidence is known to the authors to substantiate this hypothesis, Joulain and Laurent (1989) noted that the sexual cycle has some effect on the occurrence of the odour of tom cat urine. Further research into the function of felinine as a pheromone is warranted.

5.I.7 NUTRITIONAL ASPECTS

Westall (1953) observed that dietary factors may have some influence on the urinary excretion of felinine because cats were found to decrease felinine and simultaneously

increase taurine excretion when transferred from a mixed diet to a fish-based diet. Datta and Harris (1953) also studied the influence of the diet on felinine excretion. Before dietary manipulation, they detected taurine and only trace amounts of felinine in the urine of a female cat. After including 2-3 % cod liver oil in the diet, taurine was present in only trace amounts and the greatest component of the ninhydrin-reacting substances was identified as felinine. Felinine excretion persisted for 14 days after removal of the cod liver oil from the diet. Datta and Harris (1953) suggested that the component of cod liver oil which stimulated the production and excretion of felinine might have been stored in the body and pointed to the high amounts of vitamin A and D present in cod liver oil. These investigators further noted that felinine and taurine were never found together in urine in significant concentrations and they hypothesised that cysteine was a precursor for taurine but when felinine synthesis was induced, cysteine was converted to felinine instead of taurine.

Greaves and Scott (1960) studied the excretion of felinine and taurine in the urine of cats fed diets varying in protein content (13-46 %). Taurine was present in the urine of the male cats on all but the two lowest protein (13 and 19 %) diets and in the case of the female cats on all but the 13 % protein diet. Felinine was always found to be present in the urine. The cats were found to be in negative nitrogen balance on the 19 and 13 % diets (Greaves and Scott, 1960). Dietary supplementation with leucine and cysteine has been shown to increase felinine concentrations in the urine of cats (Avizonis and Wriston, 1959; Shapiro, 1963). Shapiro (1963) also observed that increasing dietary fat levels increased felinine concentrations in the urine of a cat. In light of the previously discussed precursors of felinine, it would seem likely that an increasing dietary fat level would provide more acetate, a felinine precursor, to become available for felinine synthesis.

MacDonald *et al.* (1984) calculated that the total quantity of dietary sulphur amino acids needed to provide for the synthesis of felinine would not exceed 0.05 % of the diet for an adult male cat. The National Research Council (1986) lists the sulphur amino acid requirement of an adult domestic cat for maintenance as being 3 g/kg diet, which would mean that, according to the MacDonald *et al.* (1984) estimates, a maximum of 17 % of the total maintenance requirement for sulphur amino acids would be required to excrete felinine. Using more recent data from Hendriks *et al.* (1995a), and assuming that these are normal excretion levels of

felinine, it can be shown that entire male cats would need 0.12 mmol of sulphur/kg body weight (BW)/day to produce felinine. If the assumption is made that the sulphur in the felinine molecule originates from cysteine, although some contradictory evidence exists on the incorporation of cysteine into felinine, 15 mg of this amino acid would be required. The total sulphur amino acid maintenance requirement for an active adult male cat can be calculated to be ~48 mg/kg BW/day (National Research Council, 1986; Burger and Smith, 1987). To sustain normal levels of felinine excretion in tom cats fed at maintenance, ~30 % of the total daily sulphur amino acids may be required. If methionine is also used for felinine synthesis, this percentage would be even higher. Although felinine levels can be elevated by the inclusion of felinine precursors in the diet, it is unknown at present whether felinine levels of cats fed to requirement would fall below the levels reported by Hendriks *et al.* (1995a). In this respect Greaves and Scott (1960) found felinine to be present in the urine of cats in negative nitrogen balance. From the above calculations, it can be seen that the excretion of felinine may have a significant effect on the sulphur amino acid requirements of the entire male cat. This effect is lower for castrated male, entire female and spayed female cats because of the lower felinine excretion rates. Kittens apparently do not excrete felinine in any significant quantity, and there is thus no requirement for sulphur to provide for felinine synthesis.

5.I.8 CONCLUSION

There is contemporary interest in the amino acid felinine. Felinine has been positively identified in the urine of only a few members of the Felidae family, namely the cat (*F. catus*), ocelot (*F. pardalis*), leopard (*P. pardus*), indian leopard cat (*Felis bengalensis*) and bobcat (*F. rufus*). Various investigators have failed to find felinine in other members of this family (cheetah [*Acinonyx jubatus*], puma [*Felis concolor*], tiger [*Panthera tigris*], lion [*P. leo*], serval [*Felis serval*] or fishing cat [*Felis viverrina*]) but sensitivity of the earlier analytical methods is suspect and further studies are warranted. Recently an accurate chemical assay for felinine has been developed which will allow investigation of felinine in different tissues and excretions and from a wider range of mammals.

Felinine seems to be synthesised from the same isoprenoid pool as cholesterol in the cat. Although radioactively labelled sulphur in cysteine has been incorporated

into felinine, contradictory evidence still exists on the use of cysteine as an immediate precursor of felinine. Furthermore, the site or sites of felinine synthesis remain unknown, as does the method of transportation of felinine from extra-renal tissues.

The biological significance of felinine to the animal is still a matter for speculation but its function as a precursor to a pheromone seems likely but remains unproven. Furthermore, it can be calculated that the biosynthesis of felinine may have a substantial effect on the sulphur amino acid requirements of the entire cat.

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CHAPTER 5 PART II

SYNTHESIS OF FELININE, 2-AMINO-7-HYDROXY-5,5-DIMETHYL-4-THIAHEPTANOIC ACID

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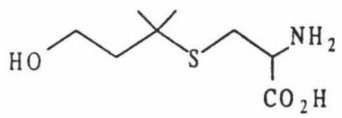
5.II.1 ABSTRACT

Several published synthesis procedures reported to lead to (±)-felinine, 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid, a sulfur-containing urinary amino acid of cats (*Felis domesticus*), were evaluated for their yield. Most of the procedures were found to produce an amino acid isomeric with felinine and the structural assignment of this isomer has been determined as 2-amino-7-hydroxy-7-methyl-4-thiaoctanoic acid. The yield for the only evaluated synthesis procedure shown to produce felinine was found to be low. A new high yielding method for the synthesis of (±)-felinine is presented.

5.II.2 INTRODUCTION

During a recent study at our institute on the amino acid metabolism of adult domestic cats it became necessary to quantify the amount of an important sulfur containing amino acid, felinine, known to be present in large amounts in cat urine (*1-2*). This amino acid was first isolated from cat urine by Westall (*2*) in 1953 and tentatively assigned structure **1**, 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid, on the basis of a Raney nickel-induced desulfurization of the felinine and subsequent isolation of alanine and isopentenol (Structure **1**).

Confirmation of the structural assignment, by synthesis, was achieved in 1957 by Trippett (*3*) and subsequent attempts by various research groups (*4—6*) to substantiate this assignment have all purported to lead to this compound. In these procedures characterization was accomplished, for the most part, using two dimensional paper chromatography in addition to melting-point determinations on products obtained by solvent-induced precipitation from water. No spectroscopic or high resolution chromatographic validation of any of these products has hitherto been reported.



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STRUCTURE 1

Recently, bobcat (*Lynx Rufus*) urine has been shown to have a repellent effect on the behavior of several herbivorous animals (*7—9*). This effect is believed to be caused by several volatile sulfur-containing compounds which could originate from the degradation of felinine (*10*), although direct evidence is lacking. Since felinine has been found in bobcat urine and the levels of this amino acid have been reported to be higher in the urine of entire male than entire female cats (*11*), felinine may have a role as a pheromone or precursor to a pheromone in Felidae.

To obtain a standard for high performance liquid chromatography (HPLC), the synthesis procedures of Trippett (*3*), Schöberl *et al.* (*5*), and Schöberl *et al.* (*6*) and the two procedures published by Eggerer (*4*) which used isopentenol were followed. This contribution reports the results of the evaluation of the latter-mentioned synthesis procedures and gives the structural reassignment of the product obtained in

three of these procedures. Nuclear magnetic resonance (NMR), mass spectrometry (MS) and HPLC data for natural and synthetic felinine are also presented to corroborate these findings and a new procedure for the synthesis of felinine is reported.

5.II.3 EXPERIMENTAL

5.II.3.1 Chemicals

L-Cysteine, L-cysteine hydrochloride, L-cysteine methyl ester hydrochloride, (±)-β-chloroalanine, 3-methylbut-2-en-1-ol and dimethylformamide were purchased either from Aldrich Chemical Co. (St. Louis, MO) or Sigma Chemical Co. (St. Louis, MO). The 3-methylbutan-1,3-diol was synthesized by performing a Reformatsky reaction between ethylbromoacetate and acetone and subjecting the resulting β-hydroxy ester to reduction by lithium aluminum hydride. The diol was purified by distillation under vacuum. The 3-methyl-3-thiobenzylbutan-1-ol and 3-mercapto-3-methylbutan-1-ol were prepared by Michael addition of benzylmercaptan and hydrogen sulphide, respectively, to methyl-3,3-dimethylacrylate followed by reductions by lithium aluminum hydride of each of the adducts. The structures of these synthesized compounds were authenticated by NMR spectroscopy.

All other reagents were of AR grade and solvents were distilled prior to use.

5.II.3.2 Apparatus and Methods

^{1}H and ^{13}C NMR spectra were recorded as solutions in either D_2O or deuterochloroform ($CDCl_3$) on a Bruker AC300 instrument operating at 20° C and all chemical shift data obtained in D_2O solutions are cited with respect to the HOD signal corrected to δ 4.90.

Fast atom bombardment-mass spectrometry (FAB-MS) was performed on a VG70-250S double focusing magnetic sector mass spectrometer (VG Analytical, Manchester) equipped with standard LSIMS ion source and associated ion gun. Chemical ionization (CI) mass spectrometry data were obtained using a dedicated instrument identical to the FAB-MS instrument.

Amino acid analyses were performed on a Waters (Millipore, Milford, MA) ion exchange HPLC system employing postcolumn derivatization with *O*-phthalaldehyde and detection by fluorescence spectrometry. A Waters ion-exchange

amino acid analysis column was used with HPLC conditions as described by the manufacturer. Chromatograms were integrated using dedicated software (Waters Maxima 820) and the retention times of the synthesized amino acids were compared with those of a standard amino acid mixture. All published synthesis procedures were carried out precisely as described in the original publications.

5.II.3.3 Isolation of "Natural Felinine"

Urine, from male cats housed in metabolic cages, was collected, pooled and immediately frozen prior to processing. The urine (200 ml) was filtered, deproteinized, treated with activated charcoal (*2*), and acidified to pH 1.5 with hydrochloric acid. The liquor was applied to a cation exchange column (Dowex-W50 in the acid form) and eluted with a sodium citrate buffer (67 mM, pH 2.1). Fractions were collected and analyzed by HPLC for the presence of the peak believed to be felinine. Those fractions containing what was believed to be relatively pure felinine were pooled and concentrated to dryness under reduced pressure. The resulting solid (0.5 g) was redissolved in a small volume of water and desalted by adsorption on a Dowex-W50 (H^+ form) column and eluted with an aqueous ammonia solution (0.2 M). The eluate was reconcentrated and the desalting procedure repeated. In this manner, an homogeneous white lyophilisate (20 mg) was obtained. FAB-MS: (-) *m/z* 205, 206; (+) *m/z* 207, 208 ($C_8H_{17}NO_3S$ requires *m/z* 207 amu). ^{1}H NMR (D_2O): δ 1.46 (s, 3H), 1.49 (s, 3H), 2.01 (2x overlapping t, J = ca. 7.4 Hz, 2H), 3.10 (m, 2H (H_β)), 3.92 (2x overlapping t, J = ca. 7.4 Hz, 2H), 4.32 (t, J = 5.4 Hz, 1 H (H_α)). ^{13}C NMR: 164.1 (C_Q), 61.56/61.48 (CH_2), 58.9 (CH), 47.08/46.77 (C_Q), 45.9 (CH_2), 34.0 (CH_2), 31.06/31.02 (CH_3).

5.II.3.4 Synthesis of "Schöberl Felinine," 2-Amino-7-hydroxy-7-methyl-4-thiaoctanoic Acid (**7a**)

The use of either 3-methylbutan-1,3-diol or 3-methylbut-2-en-1-ol (isopentenol) in the procedures of Schöberl *et al.* (*5, 6*) gave rise to the same crude product that was shown by NMR spectroscopy and HPLC analysis to mainly comprise a single amino acid. However, attempts to purify the product either by crystallization or ion exchange chromatography were unsuccessful. Spectral data recorded on the lyophilized crude product were clearly resolved so as to permit a structure assignment. FAB-MS: (-) *m/z* 206; (+) *m/z* 208 ($C_8H_{17}NO_3S$ requires *m/z* 207 amu).

^{1}H NMR (D_2O): δ 1.34 (s, 6H), 1.90 (m, 2H), 2.74 (t, J = 8.2 Hz, 2H), 3.20 (qd, J_{AB} = 14.6 Hz, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 2H (H_β)), 4.01 (dd, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 1H (H_α)). ^{13}C NMR 175.4 (C_Q), 74.0 (C_Q), 55.0 (CH), 45.2 (CH_2), 34.0 (CH_2), 30.5 (CH_3), 30.5 (CH_3), 29.6 (CH_2).

The Eggerer (*4*) synthesis procedures followed were essentially the same reactions as that of Schöberl *et al.* (*6*) except that the reaction of L-cysteine hydrochloride with 3-methylbut-2-en-1-ol (isopentenol) was carried out in the presence of pyridine.

5.II.3.5 Synthesis of Methyl 2-Amino-7-hydroxy-7-methyl-4-thiaoctanoate (**7b**)

The use of L-cysteine methyl ester in place of the free amino acid in each of the procedures described by Schöberl *et al.* (*5, 6*) gave rise to identical (NMR) crude products. These were isolated by extraction of the aqueous acid phase with dichloromethane (2 x 20 ml), followed by neutralization with saturated aqueous sodium bicarbonate solution and extraction again with dichloromethane (2 x 20 ml). The organic phase was dried and concentrated to a mobile liquid and subjected to flask chromatography over silica (50 % ethylacetate/hexane) to give a colorless mobile liquid (ca. 40 % yield, based upon cysteine methyl ester). CI(+): MH^+ *m/z* 222.1160 (Calcd for $C_9H_{20}NO_3S$ *m/z* 222.1164); $[MH-H_2O]^+$ *m/z* 204.1058 (Calcd for $C_9H_{18}NO_2S$ *m/z* 204.1058). ^{1}H NMR ($CDCl_3$): δ 1.62 (s, 6H), 2.02 (m, 2H), 2.73 (m, 2H), 2.88 (qd, J_{AB} = 13.0 Hz, $J_{A\alpha}$ = 7.1 Hz, $J_{B\alpha}$= 4.9 Hz, 2H (H_β)), 3.68 (dd, $J_{A\alpha}$ = 7.1 Hz, $J_{B\alpha}$ = 4.9 Hz, 1H (H_α)), 3.77 (s, 3H). ^{13}C NMR 174.4 (C_Q), 69.7 (C_Q), 54.2 (CH), 52.1 (CH_3), 45.9 (CH_2), 37.3 (CH_2), 32.4 (CH_3), 32.3 (CH_3), 28.0 (CH_2).

5.II.3.6 Synthesis of "Trippett Felinine," 2-Amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic Acid (**1**)

Using the method of Trippett (*3*) of reductive *S*-debenzylation of 3-methyl-3-thiobenzylbutan-1-ol in the presence of (±)-β-chloroalanine, a crude product comprising two species was isolated. NMR spectra revealed the presence of the mono (or di-)-sodium salt of 3-mercapto-3-methylbutan-1-ol as the major (≥80 %) component and a compound displaying ^{1}H and ^{13}C signals characteristic of felinine **1**. The crude material was dissolved in a small volume of water, acidified to pH 4, and extracted with dichloromethane (4 x 15 ml) to remove the free mercaptoalcohol and the aqueous phase filtered through celite and freeze-dried, to give an off-white

lyophilisate. This product was not subjected to further purification. HPLC analysis confirmed the presence of a compound coeluting with natural felinine. FAB-MS: (-) *m/z* 205; (+) *m/z* 207 ($C_8H_{17}NO_3S$ requires *m/z* 207 amu). ^{1}H NMR (D_2O): δ 1.56 (s, 6H), 2.06 (t, J = 7.4 Hz, 2H), 3.30 (qd, J_{AB} = 16.3 Hz, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 2H (H_β)), 3.95 (t, J = 7.4 Hz, 2H), 4.16 (dd, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 1H (H_α)). ^{13}C NMR 175.3 (C_Q), 61.3 (CH_2), 56.9 (CH), 47.7 (C_Q), 45.8 (CH_2), 30.9 (CH_2), 30.9 (CH_3), 30.8 (CH_3).

5.II.3.7 Synthesis of (±)-Felinine, 2-Amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic Acid (**1**), an Adapted Procedure

To a stirred solution of 3-mercapto-3-methylbutan-1-ol (2.0 g, 16.7 mmol) and (±)-β-chloroalanine (1.9 g, 15.2 mmol) in dry dimethylformamide (40 ml), sodium hydride (0.67 g, 60 % dispersion, 16.7 mmol) was added in a single portion. The solution was stirred at ambient temperature for 0.5 h, after which time a second equivalent was added. A third equivalent of sodium hydride was added after an additional 1 h and the solution stirred for 4 h before being quenched with dilute hydrochloric acid (pH 2, 30 ml) and extracted with ether (3x 20 ml). The aqueous acid phase was filtered through celite and concentrated under reduced pressure to give a light yellow semisolid (2.5 g) that could be freed of dimethylformamide by pumping at 0.2 mm Hg. The homogeneity of the product was subsequently confirmed by HPLC analysis which showed a single amino acid coeluting with natural felinine. A sample of this product (0.2 g) was dissolved in dilute hydrochloric acid and applied to a Dowex-W50 column in the H^+ form and washed to neutrality with 3 vol water before being eluted with dilute ammonia solution (ca. 0.2 M) and prepared as a white lyophilisate (0.1 g). A sample of this material was crystallized (with <30 % recoveries) by dissolving in a tetrahydrofuran/methanol mixture to give (±)-felinine as white rosettes, mp 185-187° C (dec). Elemental analysis: C, 44.27; H, 8.32; N, 6.35 ($C_8H_{17}NO_3S.0.5H_2O$ requires C, 44.44; H, 8.33; N, 6.54). FAB-MS: (-) *m/z* 206; (+) *m/z* 208 ($C_8H_{17}NO_3S$ requires *m/z* 207 amu). ^{1}H NMR (D_2O): δ 1.56 (s, 6H), 2.06 (t, J = 7.4 Hz, 2H), 3.30 (qd, J_{AB} = 16.3 Hz, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 2H (H_β)), 3.95 (t, J = 7.4 Hz, 2H), 4.16 (dd, $J_{A\alpha}$ = 7.3 Hz, $J_{B\alpha}$ = 4.4 Hz, 1H (H_α)). ^{13}C NMR 175.3 (C_Q), 61.3 (CH_2), 56.9 (CH), 47.7 (C_Q), 45.8 (CH_2), 30.9 (CH_2), 30.9 (CH_3), 30.8 (CH_3). No change in the chemical shifts of these signals was observed after treating the sample with dilute ammonia (to pH 10.5) and lyophilizing the resulting solution.

5.II.4 RESULTS AND DISCUSSION

Felinine isolated from tom cat urine has been reported to coelute with citrulline between glutamic acid and glycine on a cation exchange column (*12, 13*). In the present study when adult tom cat urine was analyzed by HPLC, a large peak was observed (Fig. 1, peak 6) to elute between glutamic acid and glycine with clear resolution on both sides. Variation of the chromatographic conditions and a citrulline standard showed that citrulline was not present in the cat urine. The species believed to be felinine was subsequently isolated by semipreparative HPLC, but remained contaminated with small amounts of impurities (Fig. 1). Nonetheless, the 1D and 2D NMR (^{1}H-^{1}H and ^{1}H-^{13}C COSY) spectral data for this species, as isolated, were consistent with expectations of the structure of felinine (*2*). The two contiguous methylene groups appear as multiplets at δ 2.01 and δ 3.92 and are disconnected to the methine and methylene groups of the cysteinyl moiety which appear as multiplets at δ 4.32 and δ 3.10 (Fig. 2).

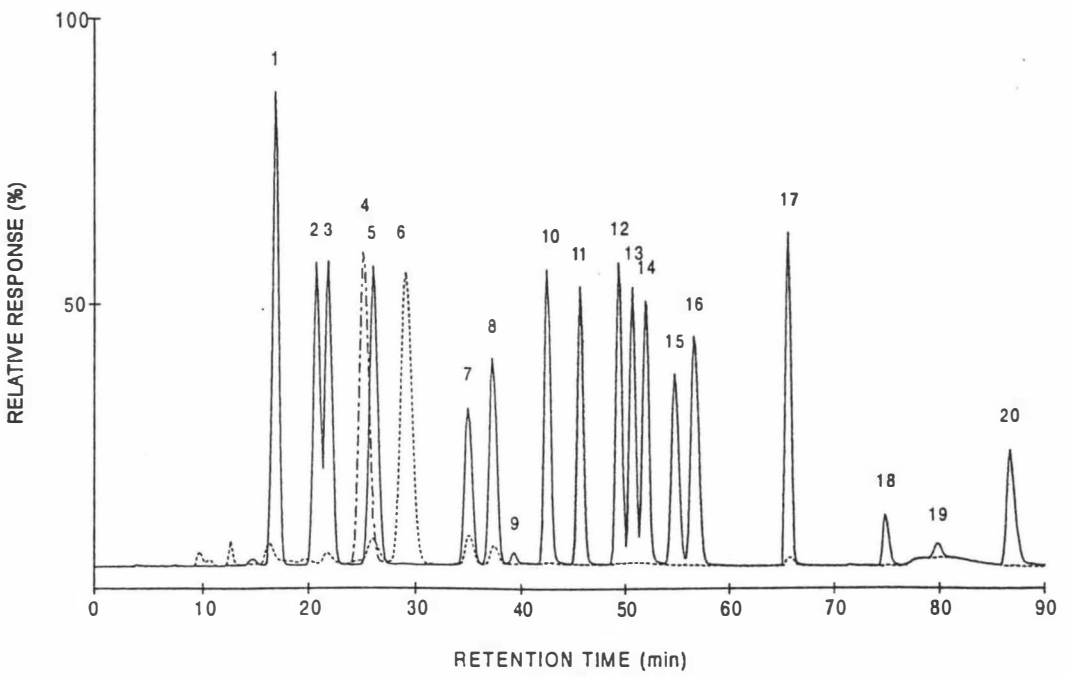


Fig. 1. Superimposed HPLC chromatograms of a standard amino acid mixture (—), the product mixture from the Schöberl *et al.* (*5*) procedure (· · ·) and felinine isolated from tom cat urine (—). Peak: 1, Asp; 2, Thr; 3, Ser; 4, 2-amino-7-hydroxy-7-methyl-4-thiaoctanoic acid; 5, Glu; 6, Felinine; 7, Gly; 8, Ala; 9, Cys; 10, Val; 11, Met; 12, Iso; 13, Leu; 14, Norleucine; 15, Tyr; 16, Phe; 17, His; 18, Lys; 19, NH_3; 20, Arg.

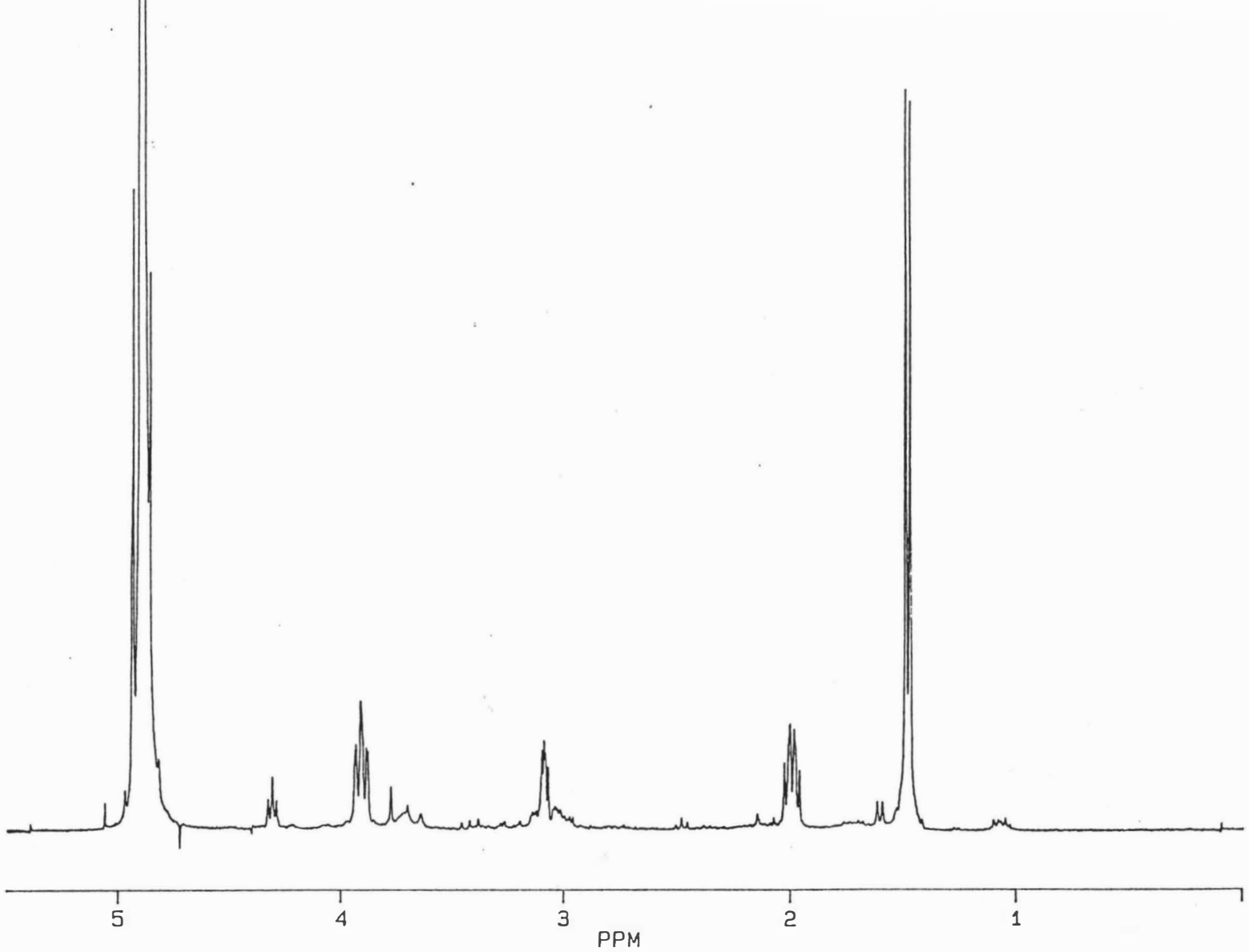
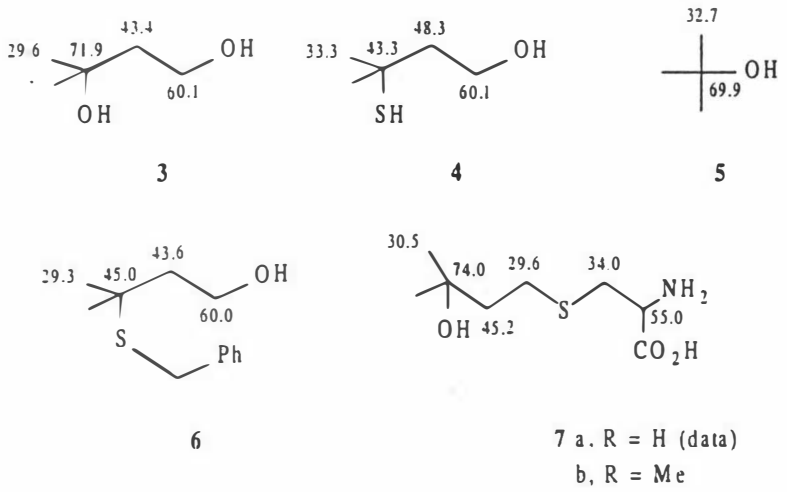


Fig 2. ^{1}H NMR spectrum of felinine isolated from tom cat urine.

structure we have assigned to this isomeric species is 2-amino-7-hydroxy-7-methyl-4-thiaoctanoic acid **7a** (Structures **3-7b**).

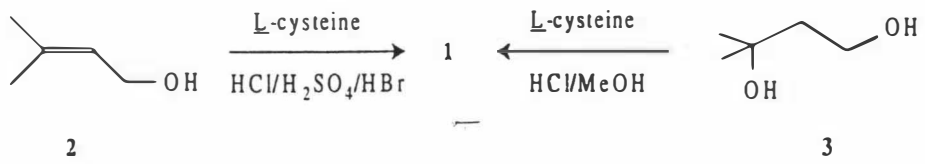


STRUCTURES 3-7

Chemical shift data and the corresponding multiplicities were discernable and showed the signals associated with the cysteinyl moiety with H_α at δ 4.01 (dd $J_{A\alpha}$ = 7.3 and $J_{B\alpha}$ = 4.4 Hz) and H_β at δ 3.20 (qd J_{AB} = 14.6 Hz and $J_{A\alpha}$ = 7.3 and $J_{B\alpha}$ = 4.4 Hz) and those of the contiguous methylene units of the "isopentenol" moiety as triplets (J = 8.2 Hz) at δ 2.74 and δ 1.90. Moreover, in accordance with expectation, discrete ^{1}H-^{1}H and ^{1}H-^{13}C correlations were observed for each 'unconnected' moiety. Mechanistically, compound **7a** appears to result from a nucleophilic displacement of the protonated primary hydroxyl function. Capture of any incipient tertiary electrophilic center at C3 (derived from **2** or **3**), which would give rise to felinine appears to be the less-favored pathway.

As the purification of **7a** was consistently unsuccessful we undertook the same reaction but with L-cysteine methyl ester instead of L-cysteine and isolated, by conventional flask chromatography, a mobile homogeneous liquid in ca. 40 % yield. This compound **7b** was found, from examination of the ^{13}C and ^{1}H NMR (Fig. 3) spectra, to be the methyl ester of **7a**. Connectivity plots and accurate mass measurements were consistent with expectations. In addition, when both the Schöberl *et al.* (*5, 6*) procedures were evaluated by two dimensional paper chromatography according to the procedure of Datta *et al.* (*15*) using phenol and collidine/lutidine as solvents, the isomer **7a** was found to have the same R_f values as

The multiplicities of these resonances are not, however, those expected of a first order analysis, a phenomenon that we believe is attributable to diastereotopic effects due to the chiral α-carbon of the amino acid unit. FAB-MS data clearly confirmed that the isolated compound had a molecular weight which corresponds to that for structure **1**. It was concluded, therefore, that the compound isolated from tom cat urine was the same compound studied by Westall (*2*) and named felinine.



2 3

SCHEME 1

With the purpose of preparing a synthetic standard for quantitation experiments, we elected to utilize the simple procedure described by Schöberl *et al.* (*5*) for the synthesis of felinine. In contrast to the results reported by these authors, the procedure in which cysteine is added as an electrophile to 3-methylbut-2-en-1-ol **2** (isopentenol) (Scheme **1**), gave a multicomponent mixture that contained no amino acid that coeluted (HPLC) with natural felinine. Employing the second method described by Schöberl *et al.* (*6*) that utilizes 3-methylbutan-1,3-diol **3** instead of isopentenol **2** (Scheme **1**), a similar outcome was observed. HPLC analysis again showed no compound coeluting with natural felinine.

The crude products from each of these reactions, when prepared as lyophilisates and subjected to HPLC analysis, did reveal the presence of a single amino acid species (≥85 % product) but this did not coelute with natural felinine. This amino acid was found to be clearly resolved and to be more mobile than natural felinine (Fig. 1, peak 4). ^{1}H and ^{13}C NMR data recorded for this compound were consistent with a species isomeric (FAB-MS (-) *m/z* 206, (+) *m/z* 208) with the expected structure in that the correct number of carbon types was observed in addition to the anticipated ^{13}C-^{1}H and ^{1}H-^{1}H connectivities. Chemical shift data, however, were not consistent with that expected for felinine. Particularly obvious was the absence of a signal attributable to a methylene bearing a hydroxyl function. In addition, the appearance of a single quaternary carbon resonance at 74.0 ppm is consistent with oxygen substitution rather than sulfur substitution at this center (*14*). Based upon chemical shift data recorded here for the model compounds **3-6**, the

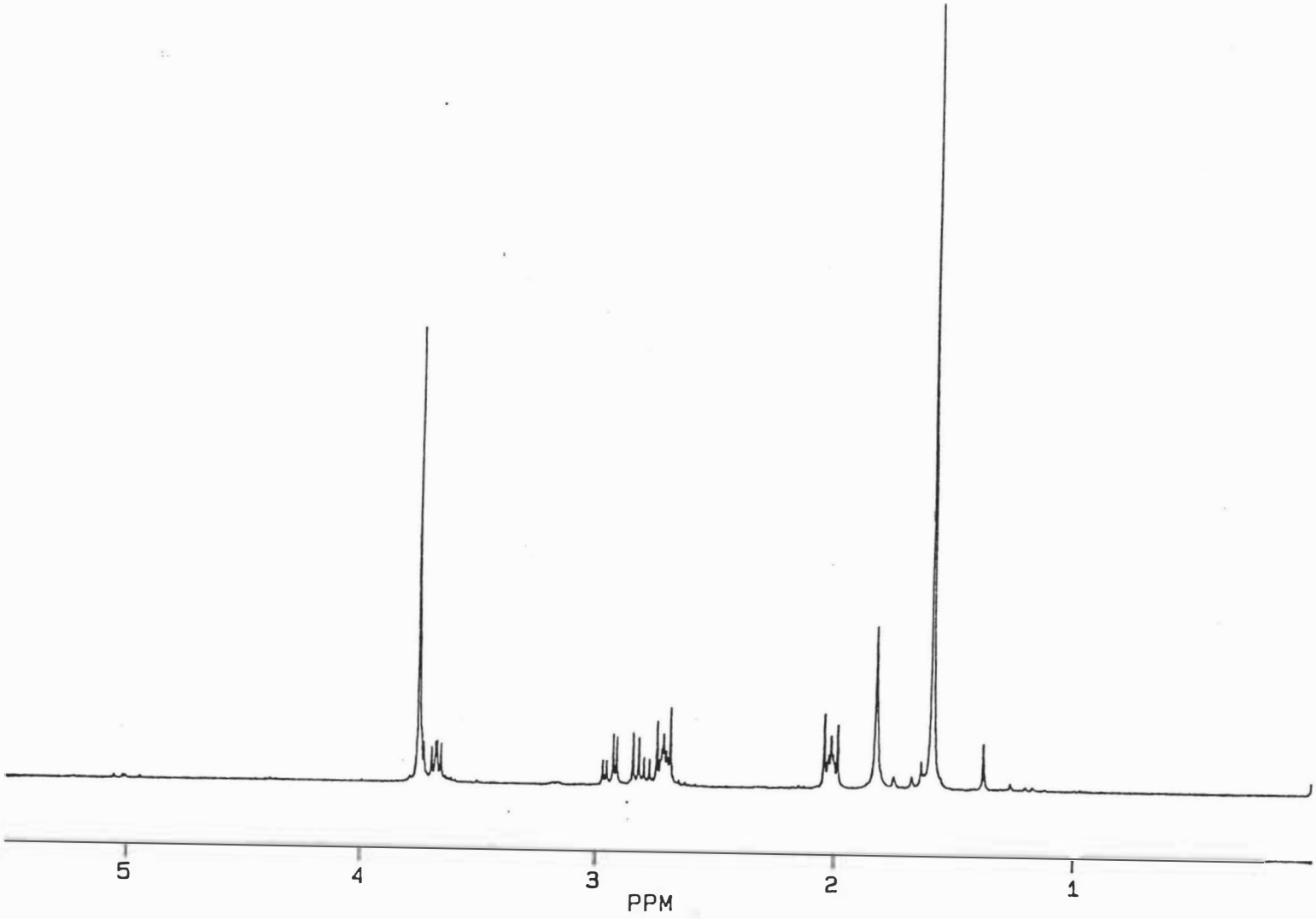
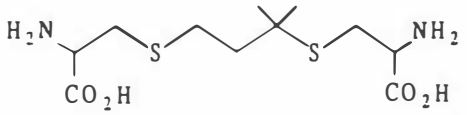


Fig. 3. ^{1}H NMR spectrum of the compound obtained from the use of L-cysteine methyl ester instead of L-cysteine in the Schöberl *et al.* (5) procedure.

natural and "Trippett's" felinine. The color of this amino acid after reaction with ninhydrin, however, was a distinct purple compared to that of natural and Trippett's felinine which gave a yellow-purple color. Since the procedure from (*5*), and therefore also the procedure from (*6*) which was compared with (*5*), relied mainly on paper chromatography for the identification of felinine (*16*), the identical R_f values might be the reason why these procedures were believed to yield felinine.

Reaction of cysteine at the primary center in **3** (to give **7a**) is not without a precedent. A by-product having the stoichiometry of a 2:1 adduct **8** has already been isolated by Schöberl *et al.* (*5*) from the acid-mediated condensation of cysteine with the diol **3** (Structure **8**). Moreover, this compound, for which no analytical or spectroscopic data were presented, is purported to be formed by prolonging the same reaction at 80° C from 1 h to 4 days and also by reacting the (his) putative felinine with 6 M HCl at 80° C for 4 h.



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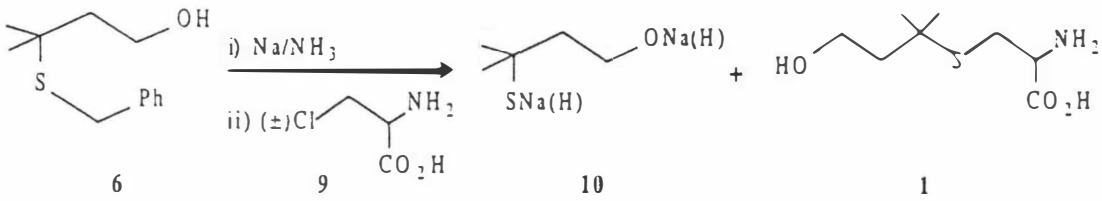
STRUCTURE 8

Under the basic conditions (stoichiometric pyridine) described in two of Eggerer's (*4*) three routes, which utilize isopentenol and which might perhaps be expected to disfavor reaction at the primary hydroxyl center, we observed the formation of an insoluble precipitate (precisely as described, and believed to be cystine) with only small recoveries of product mixtures, which contained no felinine as analysed by HPLC, but only a small amount of the isomer **7a**. The main procedure published by Eggerer (*4*) that utilizes a phosphate-protected derivative of isopentenol in a reaction with L-cysteine hydrochloride has not been evaluated in the present work. We have no reason to doubt that the route is other than as described.

The Trippett's (*3*) synthesis of felinine, involving a reductive *S*-debenzylation of 3-methyl-3-thiobenzylbutan-1-ol **6**, followed by nucleophilic addition to (±)-β-chloroalanine **9** (Scheme **2**), was successful. In addition to the supposed mono- or disodium salt of 3-mercapto-3-methylbutan-1-ol **10**, a small amount (≤10 %) of a product component shown to coelute (HPLC) with natural felinine was observed.



SCHEME 2

When reacted with 1 M sulfuric acid and repeatedly (3x) extracted with ether, the original lyophilized product could easily be freed from the mercaptobutanol "salt" **10** to give a product (judged to be ≥80 % pure) showing resonances and ^{13}C-^{1}H and ^{1}H-^{1}H connectivities characteristic of those expected of felinine. In particular, the ^{1}H-^{1}H COSY plot revealed again two discrete sets of vicinal connectivities with the chemical shifts of the resonances consistent with those already described. ^{1}H and ^{13}C signals characteristic of the hydroxyl-bearing methylene carbon were observed at δ 3.95 (t, J=7.3 Hz) and 61.3 ppm, respectively. The ^{13}C signal due to the sulfur-substituted quaternary carbon was observed at 47.7 ppm. Chemical shifts and multiplicities of the contiguous cysteinyl methylene (δ 3.30 qd, J_{AB}=16.3, $J_{A\alpha}$ = 7.3, and $J_{B\alpha}$ = 4.4 Hz) and methine (δ 4.16, dd, J_{AB}=16.3, $J_{A\alpha}$ = 7.3, and $J_{B\alpha}$ = 4.4 Hz) protons were clearly resolved. The stoichiometry of this compound was confirmed by FAB-MS which revealed an identical spectrum to that recorded for the natural felinine with an obvious and prominent molecular ion at *m/z* 207 (40 %).

By modifying this reductive *S*-debenzylation route (*4*) we have achieved a quantitative synthesis of felinine. Thus, treatment of an equimolar mixture of 3-mercapto-3-methylbutan-1-ol **4** and (±)-β-chloroalanine **9** in anhydrous dimethylformamide with stepwise additions of three molar equivalents of sodium hydride gives felinine **1** in yields of ca. 65 %. Purification could be achieved, although with considerable loss, using ion-exchange chromatography to give (±)-felinine as a white lyophilisate. A sample of this product was crystallized by judicious dissolution in a tetrahydrofuran/methanol mixture to give the amino acid as white rosettes, mp. 185-187°C. This purified synthetic compound coeluted with natural felinine as analyzed by HPLC and the FAB-MS(+) spectrum showed an obvious molecular ion at *m/z* 208 (MH^+). The ^{1}H-NMR spectrum of the lyophilized product is shown in Fig. 4.

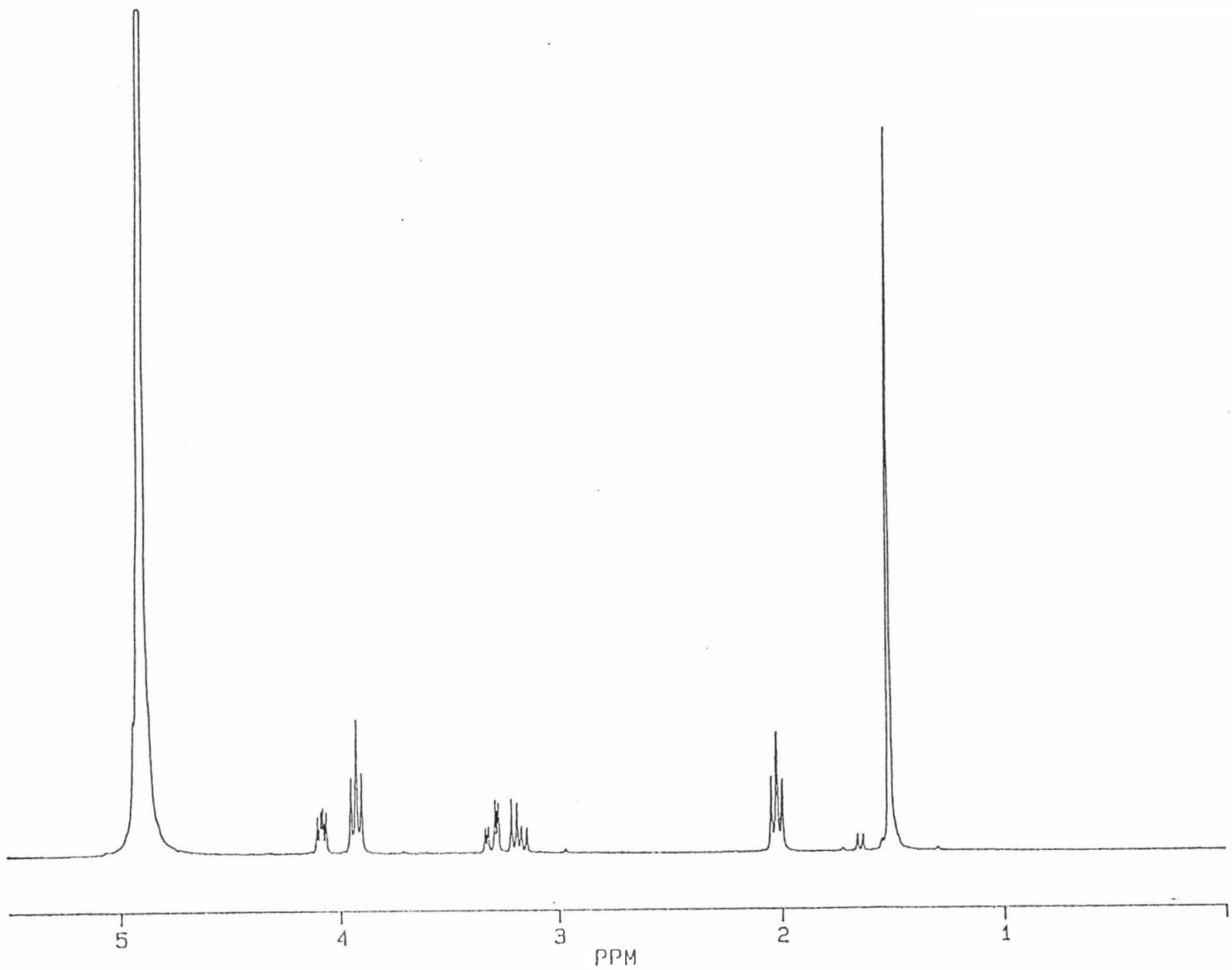


Fig. 4. ^{1}H NMR spectrum of the compound obtained from the new synthesis procedure of felinine.

When the ^{1}H NMR spectra are compared to that recorded for natural felinine (Fig. 2) several contrasting features warrant explanation. First, the appearance of two discrete quaternary methyl singlets and multiplicities due to the three methylene and methine units in the ^{1}H NMR spectrum of natural felinine which are dissimilar to those observed for the racemic synthetic product (Fig. 4). This is unusual, but not unexpected. The effect of chirality at the α-carbon of the amino acid is evidently transmitted through the entire molecule to the point where different components of the molecule experience different chiral environments (diastereotopism), at least in aqueous media. It is perhaps unusual that this phenomenon is not observed in the spectrum recorded for the felinine isomer **7a**, since this was synthesized from L-cysteine, but isolated as a neutral species. However, two discrete methyl carbon resonances were clearly discernable. Second, in all the ^{1}H NMR spectra recorded on various preparations of felinine (which have been shown to coelute by HPLC with the natural amino acid) we observed minor variations in chemical shifts, most notably with the α-cysteinyl proton. These effects are no doubt concentration dependent and they are seen to be pH dependent as samples of each of the synthetic materials, prepared either from **4** or **6** and lyophilized from acid or alkaline pH give rise to α-CH resonances with a range of ca. δ 0.5. The difference is seen to be more significant (δ 0.4) simply as a result of the use of sulfuric acid in place of hydrochloric acid in the quenching process.

It is notable that during the studies into the synthesis of (±)-felinine, synthetic and natural felinine when stored as a lyophilisate at -20° C or room temperature were observed to slowly decompose, with the development of a characteristic 'catty' odor. The process by which this appears to take place and its relevance to the production of (urinary) volatile sulfur-containing compounds is currently being investigated.

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CHAPTER 5 PART III

TWENTY-FOUR HOUR FELININE EXCRETION PATTERNS IN ENTIRE AND CASTRATED CATS

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5.III.1 ABSTRACT

The purpose of this study was to determine the 24 h urinary excretion of a sulphur containing amino acid called felinine in entire and castrated cats of both sexes. Entire male cats excreted (mean ± SEM) 122 ± 23.6 μmol of felinine per kg bodyweight per day with castrated males, entire females and spayed females excreting 41 ± 8.4, 36 ± 7.3 and 20 ± 3.8 μmol, respectively. There was an overall significant difference between groups in the amounts of felinine excreted in 24 hours [$F(3,24) = 11.8$, $p < 0.0001$] with there being significant differences between entire males and castrated males ($p < 0.001$) and castrated males and spayed females ($p < 0.05$). There was no difference in excretion between entire and spayed females. Urine volumes were not significantly different for the 24 h period. The differences in excretion levels were caused by different concentrations of felinine in the urine with entire male cats excreting (mean ± SEM) 2.0 ± 0.55 g of felinine per litre of urine. The data obtained in the present study support the concept that felinine, which has been found in Felidae species only, may be testosterone dependent. Felinine may be involved in territorial marking.

5.III.2 INTRODUCTION

Felinine has been known for some time to be a major component in the urine of the domestic cat and some other members of the Felidae family (3,6,10). This sulphur containing amino acid has not been found in the urine of other mammals such as ungulates and primates (2,3). Urinary concentrations of felinine have been reported to be higher in male then female cats (6) although accurate quantitative data are lacking. This latter report led to the hypothesis that felinine might be a urinary component for territorial marking (5). Further evidence to support this hypothesis was found during studies (4) into the synthesis of felinine where it was noted that after storage of synthetic odourless felinine, either at -20° C or room temperature, a characteristic "catty" odour developed.

At present there is a growing interest in ethically acceptable methods for controlling feral cats and the normal prey of cats such as crop damaging species of birds, rabbits and hares. A pheromone from a carnivorous animal, like the cat, may be valuable in controlling such populations.

Recently, high performance liquid chromatography and a new synthesis procedure for felinine (4) have enabled accurate measurements of this amino acid in biological fluids. The purpose of the present study was to measure the 24 h urinary excretion of felinine in entire and castrated cats of both sexes to obtain data on urinary concentrations and daily excretion rates.

5.III.3 METHOD

5.III.3.1 Animals and housing

This study was carried out in the BestFriend Feline Nutrition Research Unit at Massey University using 28 cats (6 entire males; 10 castrated males; 7 entire females and 5 spayed females) ranging from 2-6 years of age. The cats were kept in groups according to gender (max. 8 per group) in large cages and fed, to appetite, a standard commercially available canned cat food (g/100g DM: protein, 50.6; fat, 28.9; ash, 9.5; cys, 0.40; met, 1.14; tau, 0.15). Water was available at all times.

5.III.3.2 Urine collection

At the start of the 24 h urine collection period, the bladder of each cat was palpated

by an experienced veterinarian. If the bladder was judged empty, the cat was transferred to a metabolic cage specially designed for accurate collection of urine and the time was recorded. Cats with a palpable bladder had gentle manual pressure applied to the bladder for 20 s and the cats were then returned to their cage. Bladder palpation precipitated micturition in most cats within 15 min. The cats were re-examined after 20 min and treated in the above manner until the bladder was found to be empty whereafter they were transferred singly to metabolic cages to start the 24 h urine collection period. Food and water were withheld during the collection period. The following day each cat was reexamined for a palpable bladder and treated as described above. When the bladder was judged empty, the collection period ended, the time was recorded and the cat returned to its colony cage. The urine was collected, the volume measured and a sample was deep frozen (-70°C) pending felinine analysis. All urine volumes were adjusted to a 24 h period. Urine samples were analysed for felinine immediately after the 24 h collection periods had ended.

5.III.3.3 Felinine analysis

The urine samples were thawed, mixed with a small amount of activated charcoal and filtered though a 0.45 µm filter just prior to loading on a High Performance Liquid Chromatographic (HPLC) system. Exposure of the urine to the air and room temperature, during this study, was minimised. Felinine analysis was performed on a Waters (Millipore, Milford, MA) ion exchange HPLC system employing postcolumn derivatisation with *O*-phthalaldehyde and detection by fluorescence spectrometry. A Waters ion exchange amino acid analysis column was used with HPLC conditions as described previously (4). Chromatograms were integrated using dedicated software (Waters Maxima 820) and the retention time of felinine was compared with a felinine standard of known purity (4).

5.III.3.4 Statistical analysis

A linear model with gender as the fixed effect was fitted to the data using the General Linear Model (GLM) procedure in SAS (7). Where a significant *F* value was obtained the a priori tests were three further comparisons using Student's *t* test: entire male versus castrated male, castrated male vs. spayed female and entire female vs. spayed female.

5.III.4 RESULTS

Table 1 shows the concentrations of felinine in the urine and the 24 h urine volumes and felinine excretions for the different groups of animals. The 24 h urine volumes were not significantly different in the four groups.

TABLE 1
BODY WEIGHT (BW), URINARY FELININE CONCENTRATION, 24 h URINE VOLUME AND DAILY URINARY FELININE EXCRETION FOR ENTIRE MALE, CASTRATED MALE, ENTIRE FEMALE AND SPAYED FEMALE CATS

Gender	n	Body Weight (kg)	Felinine Concentration (mM)	24 h Urine Volume (ml/kg BW)	24 h Felinine Excretion (μmol/kg BW)
Entire Male	6	3.8 ± 0.08	9.7 ± 2.68*	17.2 ± 3.74	122 ± 23.6†
Castrated Male	10	3.7 ± 0.19	2.4 ± 0.61	19.5 ± 2.14	41 ± 8.4‡
Entire Female	7	2.7 ± 0.13	1.6 ± 0.32	21.8 ± 2.05	36 ± 7.3
Spayed Female	5	3.1 ± 0.16	1.0 ± 0.22	20.3 ± 1.28	20 ± 3.8
ANOVA F(3,24), p<			0.0003	0.649	0.0001

Values are presented as mean ± SEM.
*†Significantly different from castrated males by Student's *t* test: *$p < 0.05$, †$p < 0.001$.
‡Significantly different ($p < 0.05$) from spayed females by Student's *t* test.

The mean concentration of felinine in the urine was higher in the entire male group (range 0.4-3.6 g/l) than in the other groups and statistical analysis showed significant differences between groups [$F(3,24) = 9.5$, $p < 0.0003$]. There was a statistically significant difference between entire males and castrated males ($p < 0.05$) but no difference ($p > 0.05$) between castrated males and spayed females or entire females and spayed females. The mean urinary felinine excretion for the 24 h period was influenced by gender of the animal (entire male > castrated male > entire female > spayed female) with entire males excreting approximately 3 times more felinine then castrated males (Fig. 1). There was an overall statistically significant difference in the amount of felinine excreted over the 24 h period [$F(3,24) = 11.8$, $p < 0.0001$] with there being significant differences between entire males and castrated males ($p < 0.001$) and castrated males and spayed females ($p < 0.05$). There was no difference in excretion between entire and spayed females.

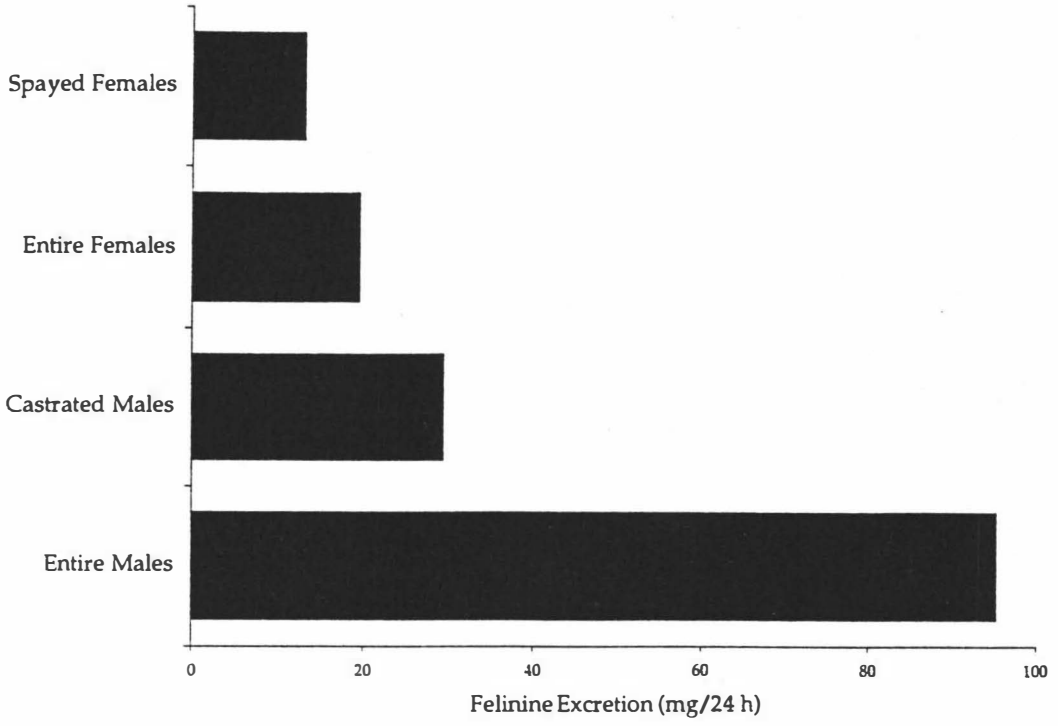

FIG. 1. Mean 24 h excretions of felinine in entire male, castrated male, entire female and spayed female cats.

5.III.5 DISCUSSION

In a preliminary trial, it was found that felinine levels in tom cat urine did not degrade measurably during the time period from when the urine was thawed until felinine analysis (W. Hendriks, unpublished observations). However, some degradation of felinine may have occurred during the 24 h period the urine was being collected from the animal. Thus it is possible that values reported here for urinary felinine are underestimates.

The high felinine concentration in the urine of the entire males (9.7 mM) is in accordance with values obtained by other workers. Earlier studies recorded concentrations of 9.2 (9) and 8.2 mM (8) for adult male cats. One study reported a fourfold higher concentration of felinine in the urine of adult male compared to adult female cats. However, this study used paper chromatography which does not permit accurate quantitative measurements. Furthermore only the concentrations of felinine were determined rather then daily excretions and it is not reported whether the male and female cats investigated were entire or castrated. In the present study a

significant difference between the 24 h felinine excretions of entire male and female cats was found. Castration of male cats significantly reduced the 24 h excretion of felinine to levels observed in female cats. Castration of female cats did not reduce the 24 h excretion of felinine significantly. These observations are in accordance with the view that the male hormone testosterone may be involved in the excretion and/or production of felinine. Treatment of a female cat with testosterone has been shown to elevate the urinary concentrations of felinine to adult male levels (6). However, in this study the amount of urine excreted by the female cat was not determined. Therefore there may have been a difference in the daily urine volumes which may have caused the different felinine concentrations. It does, however, appear likely that felinine is in some way controlled by testosterone in the cat.

It has been suggested that felinine may be a urinary component for territorial marking (5) and the present study supports this hypothesis. The acute and distinctive smell of the urine of the entire male cat is well known. Although synthetic felinine is odourless it has been reported that after storage of synthetic felinine for some time, an odour develops which can be characterised as "catty" (4). It is therefore likely that felinine degrades to another compound or other compounds which produce the distinctive smell. It is interesting to note in this context that felinine is listed as an ingredient in a patented deer repellent (1).

To the knowledge of the authors there has been little work done on the use of pheromones to attract cats. The current interest in the control of feral cats, rabbit and hares means that the use of felinine as a possible attractant to cats or a repellent to its prey should be investigated further.

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CHAPTER 6

GENERAL DISCUSSION

The domestic cat is believed to have evolved on a diet which consisted mainly of animal tissue. The relatively constant composition and the high and balanced nutrient content of such a diet is likely to be the reason for several metabolic adaptations in the cat. Examples of these adaptations are the inability of the cat to regulate hepatic amino acid catabolic enzymes, to synthesise niacin from tryptophan and to synthesise taurine and several urea cycle intermediates in sufficient quantity. The cat, furthermore, excretes several unusual sulphur-containing amino acids.

The data presented in this work reflect some of these metabolic adaptations. Endogenous ileal proline excretion in the adult cat fed a protein-free diet (Chapter 2) was not elevated, while in other mammals such as the human, pig and rat an elevated excretion has been observed. This may be due to a low activity of the enzyme, pyrroline-5-carboxylate synthase (Rogers and Phang 1985) which is involved in the synthesis of two urea-cycle intermediates, ornithine and citrulline. Furthermore, feeding a protein-free diet to cats did not result in an elevated endogenous ileal glycine excretion which is often observed in other animals fed a protein-free diet but instead resulted in a high endogenous ileal taurine excretion. This is likely to have been related to a difference in the conjugation of bile acids, which in the cat predominantly occurs with taurine while other animals such as the rat and pig predominantly use glycine. The inability of the cat to regulate hepatic amino acid catabolising enzymes was evident from the non-decreasing levels of endogenous urinary nitrogen metabolites for the cats fed a protein-free diet for 10 days (Chapter 4 part 1). That these amino acid catabolising enzymes are set to handle a high protein diet could clearly be seen from the high endogenous urinary total nitrogen excretion in the cat in comparison to that in other mammals such as the human, marmoset, dog, rat and pig (Chapter 4 part 1). The excretion of the amino acid felinine (Chapter 5) further emphasises, the uniqueness of the cat's metabolism.

Several studies have been carried out aiming to establish the protein requirement of the adult domestic cat. There is, however, a paucity of information on the amino acid requirements of this companion animal. The present work provides data which allows a

factorial estimate to be made of the amino acid requirements of the adult cat. Although a factorial model will not be generated here, a detailed description of how to formulate such a model and a critical overview of the individual components in such a model will be made.

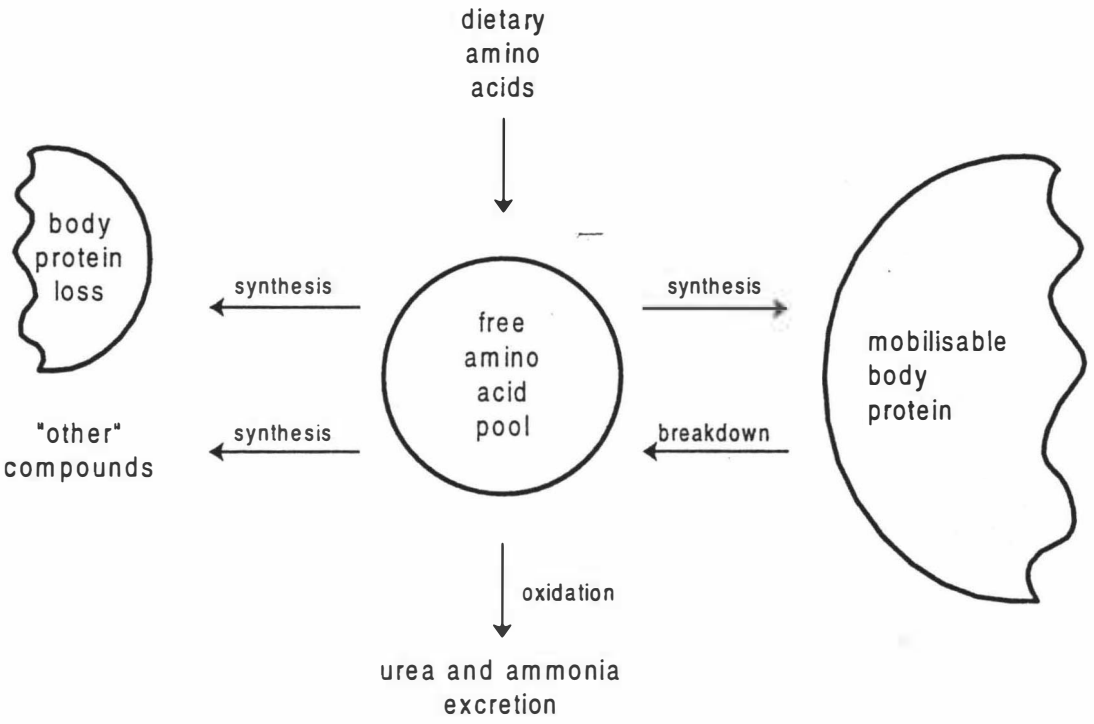

Figure 1 Simple model for whole-body protein metabolism in adult animals.

Whole-body protein metabolism in adult animals can be represented schematically using a simple model (Fig. 1) consisting of a free amino acid pool (metabolic pool) and a mobilisable body protein pool. The latter pool is regarded in this model as a single pool which continually recycles amino acids back to the metabolic pool (protein turnover). Several metabolic processes give rise to the continual loss of amino acids from the body in adult animals. These losses of amino acids need to be replaced and such replacement constitutes the maintenance requirement.

The processes giving rise to body amino acid loss are:

1. Amino acids are drawn from the metabolic pool to synthesise proteins which are lost from the body and therefore, these amino acids cannot be re-utilised for other metabolic processes. This pool of body protein is represented in Fig. 1 as "body protein loss" and consists of protein which is used for the growth of hair, skin and nails, and protein which is lost through the digestive and urinary tracts.

2. Amino acids are lost from the body associated with the continual resynthesis of body protein (protein turnover). An explanation for the loss of amino acids during body protein turnover is that amino acids enter the metabolic pool where they come in to contact with degradative enzymes which catabolise amino acids even at low substrate concentrations. Any amino acid lost through oxidation will cause an imbalance of amino acids for the synthesis of body protein and therefore, will result in the catabolism of amino acids which are in excess of the requirement for body protein synthesis. This catabolism of body amino acids associated with body protein turnover can, therefore, be expected to occur in a pattern similar to the pattern of amino acids in whole-body protein. It has been argued, however, that certain amino acids may be preferentially retained in the cell during protein turnover (Simon 1989) and are not released into the metabolic pool. If this preferential retention of amino acids occurs to a significant extent, then the pattern of amino acid catabolism associated with body protein turnover may differ from the pattern of amino acids in whole body protein.

3. Amino acids are withdrawn from the metabolic pool to synthesise "other" compounds such as non-amino acid nitrogen containing compounds (e.g. histamine, serotonin) or amino acid nitrogen containing compounds (e.g. 3-methylhistidine, hydroxyproline, hydroxylysine, purines, creatine). The use of amino acids for these purposes is usually considered to be small (Reeds 1988, Moughan 1995). In some cases, however, the amounts of amino acids used for the synthesis of "other" compounds can possibly be quantitatively significant. Methionine is used in the synthesis of creatine and the amount of methionine used depends on the supply of other compounds such as choline, betaine and tetrahydrofolate. In the case of the adult cat, felinine is a compound which may have a significant drain on the amino acids required for its synthesis.

4. Amino acids can be catabolised to provide energy in the form of ATP. Also, amino acids can supply precursors for the synthesis of glucose and fatty acids. A significant catabolism of amino acids can be observed when a cat is fed a diet containing balanced protein but low non-protein digestible energy.

Finally, to express the animal's requirement for amino acids at the tissue level to a dietary requirement, a term for the efficiency of utilisation of dietary amino acids needs to be included in the factorial model. Absorbed dietary amino acids, not metabolised in the intestine, are transported to the liver where they come in contact with catabolic enzymes. As a result, a proportion of the absorbed amino acids will be degraded. This

catabolism seems to be an "inevitable" consequence of the operation of mechanisms controlling the degradation of amino acids in the body (Heger and Frydrych 1989). Oxidative losses of dietary amino acids are also believed to occur because they are consumed at a rate which is in excess of the rate at which net protein synthesis can occur and the animal cannot accumulate essential amino acids (Millward and Rivers 1988).

The present work provides estimates for the loss of amino acids in the adult domestic cat from several of the above described routes. Using the latter data, a factorial model can be generated which allows estimation of the essential amino acid requirements of the adult domestic cat. The following section provides a critical overview of the estimates provided in the present study for the losses of amino acids in the adult cat which occur due to the digestion of food, replacement of hair (body protein loss, Fig. 1), catabolism of body amino acids associated with body protein turnover and the synthesis of "other" compounds. Also, inefficiency of utilisation of dietary amino acids for protein synthesis will be discussed.

Amino acids of endogenous origin are constantly being lost from the gastrointestinal tract of animals. These amino acids originate mainly from desquamated cells and mucus although some endogenous amino acids will originate from digestive enzymes. In accordance with other animals, the amounts of endogenous amino acids excreted at the terminal ileum of the domestic cat are underestimated by the protein-free method (Chapter 2). Estimates of endogenous ileal amino acid excretion for adult cats under the condition of peptide alimentation should, therefore, be used in a factorial model. In addition, an estimate of large bowel endogenous amino acid excretion should be included. Potentially, the latter can be obtained by subtracting the ileal endogenous amino acid excretion (determined by feeding the protein-free diet) from the faecal endogenous amino acid excretion when the protein-free diet plus antibiotics was fed to the cats. However, the digestive system of the cats on the protein-free diet with added antibiotic-fungicide mixture was not completely cleared of microorganisms and some catabolism or synthesis of amino acids may have occurred. Furthermore, the effect of the antibiotic-fungicide mixture *per se* on endogenous amino acid excretions is unknown and may have influenced endogenous protein secretion and reabsorption.

The main sources of endogenous amino acids at the terminal ileum is mucus and desquamated cells (Snook 1973) and it can be expected that mucus and sloughed epithelial cells also constitute the main source of the endogenous excretions by the

large intestine. An estimate of the amount of endogenous amino acids excreted by the large intestine can, therefore, be obtained by subtracting the ileal nitrogen excretion of the cats fed the protein-free diet from the faecal nitrogen excretion of the cats fed the protein-free diet. This approach can be expected to give a relatively accurate estimate of endogenous amino acid excretions by the large intestine when the difference in the amount of nitrogen-containing material entering and leaving the large intestine through the intestinal wall, is small in proportion to the total amount of nitrogen excreted by the large intestine. Whether the latter is an accurate assumption for the adult domestic cat remains to be proven. Another approach is to estimate endogenous excretions in the large intestine based on the excretion estimates made in the upper digestive tract. This may be a reasonable approach as mucus and sloughed cells can be expected to constitute the main sources of the endogenous excretions in the small and large intestine. The surface area of the small and large intestine will be an important variable in endogenous excretions between the small and large intestine. As digesta have a longer residence time in the large intestine than in the small intestine, more endogenous material can be expected to be excreted in the large intestine per unit food dry matter intake. Transit times of digesta need to be taken into account in a theoretical estimation along with the surface area of the small and large intestines. It can be calculated from data published by Wood (1944) and Marshell and Johnson (1993) that the surface area of the large intestine in the adult cat is approximately 3.1 % that of the surface area of the small intestine. The transit time of food in the small intestine of adult cats is approximately 2.5 h (Humphreys and Scott 1962), while the transit time in the large intestine is approximately 40 h (Fucci *et al.* 1995). It can be calculated from the above data that per unit of dry matter intake the large intestine would excrete approximately 50 % of the amount of endogenous material excreted by the small intestine in the adult domestic cat. The latter value is similar to the estimate of 70 % which is obtained according to the approach described above based on subtracting the ileal nitrogen excretion of the cats fed the protein-free diet from the faecal nitrogen excretion of the cats fed the protein-free diet. It seems, based on the data presented in Chapter 2 and the theoretical estimate generated here, that the large intestine in adult cats makes a significant contribution to the overall excretion of gut endogenous material. This is unlike the pig where endogenous excretions from the large intestine have been found to contribute only 11 % to the total endogenous excretions of the gut (Krawielitzki *et al.* 1990).

Total gut endogenous amino acid excretions appear to be linearly related to food dry matter intake in animals (Butts *et al.* 1993). A high food dry matter intake will result in a high amount of gut endogenous amino acids being excreted and a factorial model should include the effect of the amount of food dry matter consumed. Animals, including the cat, eat an amount of food according to the first limiting component in the diet and in many cases this component is energy. Kane *et al.* (1981) and Kane *et al.* (1987) concluded that adult cats regulate their daily food intake on the basis of energy density. Earle and Smith (1991) presented an allometric equation describing the digestible energy intake for non-obese adult cats varying in body weight from 2.5 to 6.5 kg required to maintain body weight. Together with the digestible energy content of the diet, the latter equation can be used to determine the daily food dry matter intake of adult cats of various live weights. From the estimates of food dry matter intake the gut endogenous amino acid losses can be predicted.

Hair is constantly being replaced in the adult domestic cat. Hair growth and hair loss show sinusoidal patterns throughout the year with maximum and minimum hair growth occurring approximately 75 days earlier than the maximum and minimum hair loss, respectively. The growth and loss of hair in adult cats, furthermore, seems to be optimally regulated to obtain the densest coat during the coldest period of the year and the sparsest coat during the warmest period of the year. As hair mainly consists of protein, the amino acid requirements of the adult cat are not constant throughout the year but change according to the change in hair growth.

The present work, provides non-linear functions from which quantitative estimates of hair growth and hair loss in adult domestic short-haired cats during specific periods of the year can be obtained. As hair growth reflects the physiological requirement for amino acids more accurately than hair loss, the function describing hair growth rate in adult domestic cats throughout the year (Chapter 3 part 1) should be used in a factorial model. In conjunction with the amino acid composition of cat hair, determined using the compartmental model (Chapter 3 part 3), an estimate can be obtained of the amino acids required for the growth of hair at any period during the year. As no difference was found in this study for the amino acid composition of differently coloured (ginger, grey, black and white) cat hair, the estimates obtained for the amount of amino acids required for the growth of hair apply to the majority of adult short-haired cats. Care should be taken in extrapolating these estimates to cats having other types of coats such as long-haired coats, hairless coats and angora coats and in

extrapolating these data to cats living in other geographical locations.

Small amounts of protein and amino acids are lost in the urine which originate from mucus and sloughed cells from the urinary tract, amino acids not absorbed by the kidney and small amounts of protein. These losses are negligible in comparison to the losses of endogenous nitrogenous material from other routes (Linder 1991). An independent estimate of these losses in the adult domestic cat has not been made in the present work. The latter losses of amino acids, however, are included in the endogenous urinary nitrogen estimate (Chapter 4 part 1).

Attempts to estimate the loss of body amino acids associated with body protein turnover have been made by measuring urinary urea and ammonia excretion of animals fed a protein-free diet. However, it can be argued that the loss of body amino acids associated with the turnover of body protein when a protein-free diet is fed, reflects adaptation of the animal to the protein-free state. In omnivorous animals such as the dog, pig, rat, marmoset and human the degradative enzymes involved in the catabolism of amino acids in the liver adapt to dietary protein intake. At high dietary protein levels, the activity of hepatic amino acid catabolising enzymes is increased to cope with the high flux of amino acids while at dietary protein levels lower than requirements the activity of these enzymes is decreased to minimise loss of amino acids. This adaptation can be observed when the latter species are fed a protein-free diet and the daily excretion of nitrogen is measured in the urine. The excretion of nitrogen per unit metabolic body weight slowly decreases to reach a relatively steady level after approximately 10 days but can be further reduced by prolonging the protein-free feeding period (Deuel *et al.* 1928, Dawson and Allen 1961, Flurer *et al.* 1988). At about a dietary protein intake level required for nitrogen balance, the activities of the hepatic amino acid catabolising enzymes in humans, rats, dogs and pigs, therefore, are higher than under the condition of protein-free feeding and more endogenous amino acids are lost through catabolism. In the cat, however, the hepatic amino acid catabolising enzymes have a limited ability to adapt to dietary changes (Rogers *et al.* 1977) and a relatively steady level of endogenous nitrogen excretion is reached from day 2 onwards when cats are fed a protein-free diet (Chapter 4 part 1). In the adult cat, therefore, the loss of body amino acids associated with the turnover of body protein can be expected to be similar when the cat is fed a low protein diet or is given a protein-containing diet. Feeding a protein-free diet to animals may result in an increased mobilisation of body amino acids to supply the first-limiting amino acid for the

synthesis of proteins which are lost from the body and for the synthesis of "other" compounds. This will result in an imbalance of amino acids for the synthesis of mobilisable body proteins resulting in the catabolism of the amino acids. The protein-free approach is, therefore, expected to over-estimate the loss of amino acids associated with body protein turnover.

A physiologically more normal approach for estimating the loss of amino acids associated with the turnover of body protein may be the regression to zero protein intake of the nitrogen excretion data of animals fed graded levels of dietary protein. The animal is fed protein, supplying amino acids needed for the synthesis of proteins which are lost from the body and for the synthesis of "other" compounds. Little or no additional body protein needs to be mobilised and this method, therefore, should result in a more accurate estimate of the loss of amino acids associated with body protein turnover. It follows that amino acids are lost in a pattern similar to the pattern of amino acids in body protein as the loss of any amino acid to catabolism leads to an imbalance of amino acids for resynthesis which results in a loss of all other amino acids proportional to their concentration in body protein. Chapter 4 part 1 provides estimates of endogenous urea and ammonia nitrogen excretion for adult domestic cats determined by the regression technique, which in conjunction with the pattern of amino acids in whole body protein (Chapter 4 part 2), can be used to estimate the losses of amino acids associated with body protein turnover.

The quantitative contribution of amino acids for the synthesis of "other" compounds is assumed, as in the case for other animals (Reeds 1988), to be small in the adult cat. The cat, unlike many other mammals, however, excretes the amino acid felinine in its urine in significant quantities. The biological significance of felinine to the animal is still a matter for speculation but it is likely that this amino acid has a role as a precursor to a pheromone. As such, felinine may be important in communication between animals. The excretion of felinine constitutes a continuous loss of the amino acids involved in the synthesis of felinine in the adult cat and these amino acids need to be replaced. It is evident from the literature that cysteine and, therefore, also methionine are likely to be the precursors for felinine which provide the cysteine moiety. The isoamyl-alcohol moiety of felinine is believed to originate from mevalonic acid which can be formed from acetate and, therefore, no amino acids should be required to supply this part of the felinine molecule. The levels of felinine excretion reported in the present study for entire male, castrated male, entire female and spayed

female cats (Chapter 5 part 3) can be used to determine the amounts of sulphur amino acids needed for the synthesis of felinine. It must be noted, however, that the levels of felinine excretion presented in Chapter 5 part 3 may overestimate the felinine excretion of cats fed protein at maintenance, as these animals were fed a commercial canned cat food to appetite. The data presented in Chapter 5 part 3, however, are at present the most accurate published data available on the normal excretion levels of felinine in adult cats.

Amino acids can be catabolised to provide energy in the form of ATP or can be used to synthesise glucose. It is believed that gluconeogenesis from amino acids is permanently "switched on" at a high level in the cat resulting in large amounts of amino acids being ultimately catabolised to provide energy. Kettelhut *et al.* (1980) studied glucose homeostasis in the cat and concluded that administration of a low protein carbohydrate-rich diet to the carnivorous animal reverts glucose homeostasis to the usual pattern observed in animals fed balanced diets. The cat does, therefore, seem able to maintain glucose homeostasis on diets low in protein as long as carbohydrate is present in the diet. Further, it seems that the cat is able to effectively conserve essential amino acids and it is believed that the cat uses dietary non-essential amino acids to provide energy (Chapter 1, pp. 24-28). It follows that essential amino acids are not preferentially catabolised by the cat to provide energy and that a factor for the amount of essential amino acids required to provide energy does not need to be included in a factorial model which aims to determine the essential amino acid requirements of the adult cat. If, however, body protein was specifically mobilised to provide amino acids for energy yielding processes by the cats in the regression study (Chapter 4 part 1), then the estimate of the catabolism of body amino acids associated with body protein turnover would be an over-estimate. This then would result in an over-estimation of the amount of essential amino acids required for basal protein turnover.

To express the physiological requirement at the tissue level of the adult cat for amino acids as a dietary requirement, an estimate of the inefficiency of utilisation of dietary amino acids needs to be included. This inefficiency is mainly due to the presence of degradative enzymes in tissues and because dietary amino acids are consumed at a rate which is in excess of the rate at which net protein synthesis can occur. The latter effect can be expected to be small in adult cats as the domestic cat eats many small meals throughout a 24 h period, showing no diurnal cycle (Kane *et al.* 1981, Kane *et al.* 1988). Heger and Frydrych (1989) showed that the efficiency of

utilisation varies with dietary amino acid intake in growing rats with a high efficiency at relatively low amino acid intake levels and a lower efficiency at higher amino acid intake levels. At a maintenance amino acid intake level, the efficiency of utilisation of dietary essential amino acids was high ranging from 0.9-1.0. Based on these observations, it is believed that the inefficiency of utilisation of dietary amino acids by the adult cat is low and that a correction factor of 0.9-1.0 should be used in a factorial model which aims to determine the amino acid requirements of the adult cat. Further research is needed, however, to obtain direct estimates of the inefficiency of utilisation of dietary amino acids in the adult cat.

There are several limitations to the factorial model described above. One limitation is inherent to the modelling process. A model can be tested and evaluated but cannot be validated. It is only possible to show that it is not valid. Other limitations are associated with the accuracy of estimates used in the model. Gut endogenous amino acid excretions have been determined using a purified enzymatically hydrolysed casein-based diet and it is unknown whether similar amounts of endogenous amino acids are excreted using other types of diet or diets containing other ingredients. This will affect the amino acid requirements of the animal making the requirement dependent on the diet fed. Also, assumptions need to be made as to the contribution of the large intestine to total gut endogenous amino acid excretion and the appropriateness of these assumptions remains unproven. The model described here applies to short-haired domestic cats and estimation of hair growth in cats with other types of hair coat will need to be made. Furthermore, geographical location may influence hair replacement patterns and assumptions will have to be made to estimate the amino acids required for hair growth for cats in other parts of the world. The excretion levels of felinine in the present work were determined in adult cats fed a commercially canned cat food to appetite which may have resulted in elevated felinine excretion levels. Furthermore, there is some evidence in the literature to suggest that cysteine and methionine may not be the immediate precursors of felinine and this would alter the requirement for the sulphur amino acids should it be found that sulphate or taurine are used to synthesise felinine. Further investigation into the origin of the sulphur in felinine seems warranted.

Although there are limitations to a factorial model for the determination of the amino acid requirement of adult cats, the estimates obtained by this technique should provide a useful point of origin to further refine the estimates of the minimum dietary

levels of essential amino acids required by the adult domestic short-haired cat. The present study provides the underlying biological information required for the formulation of such a model.

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