The Rat as a Model Animal for Digestion in the Dog

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Kugappiriyai Sritharan

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ABSTRACT

The suitability of the laboratory rat as a model animal for studying protein digestion in the dog was investigated. The work was conducted in two experiments.

In the first study ileal and faecal endogenous excretion of amino acids and nitrogen was measured in adult rats and dogs. Two groups of five adult dogs (two females and three males) and two groups of six adult rats (three males and three females) were fed either a protein-free (PF) or enzyme hydrolysed casein (EHC)-based diet, containing Cr_2O_3 as an indigestible marker. After an 8-10 d equilibration period, 4½ h after the start of hourly feeding, the animals were euthanased and the ileal content was collected from the terminal 20 cm of the ileum and freeze-dried. Faecal digesta samples of the rats and dogs fed the PF diet were obtained one day before digesta sampling from the terminal ileum. The freeze-dried digesta collected from the EHC fed animals were ultrafiltrated before analysis. The amount of endogenous amino acids and nitrogen excreted per gram of dry matter intake at the end of the ileum for the PF and EHC fed animals and over the entire digestive tract for the PF fed animals were determined. Data were analysed using ANOVA with species, diet and the interaction between species and diet as variables. There was no interaction between species and diet on the endogenous ileal excretions of any of the amino acids or nitrogen. Significant ($P < 0.05$) higher endogenous amino acid and nitrogen excretions were found in the dogs compare to the rats when fed the PF and EHC-based diet. Faecal endogenous excretions were higher than ileal endogenous excretion in both species for all amino acid. The pattern of endogenous amino acid excretions was similar in both species with the endogenous excretions of amino acids measured by the ultrafiltration method significantly ($P < 0.05$) higher than the PF method in both species.

In the second experiment the digestibility of a commercial dry dog food was compared between the rat and the dog. A group of five adult dogs (three females and two males) and six adult rats (three females and three males) were fed a commercial dry dog food, containing Cr_2O_3 as an indigestible marker for 10 and 8 days, respectively. On the final day, 4½ h after the start of hourly feeding, the animals were euthanased and the ileal content was collected and freeze-dried. A faecal sample was collected from each animal

one day before ileal digesta sampling. The diet and digesta samples were analysed for amino acids, nitrogen, organic matter and the apparent digestibility of dry matter, organic matter, nitrogen and amino acids were determined at a faecal and ileal level. The true ileal digestibility of nitrogen and amino acids were calculated and all the data were analysed using ANOVA. In the dog, the apparent faecal digestibility of aspartic acid, threonine, serine, proline, glycine and total nitrogen was significantly ($P < 0.05$) higher than the apparent ileal digestibility values whereas for methionine the apparent ileal digestibility value was significantly ($P < 0.05$) higher than the apparent faecal digestibility value. Apparent and true ileal digestibility for most amino acids were significantly ($P < 0.05$) higher in the dog when compared to the rat. Regression analysis showed that there was a significant ($P < 0.001$) linear relationship between the apparent and true ileal digestibility of amino acids between the rat and the dog. Ileal digestibility of amino acids in the dog (Y) could be predicted from respective rat values (X). The following equations were obtained for apparent digestibility: $Y = 0.32 + 0.65\ X$ and true digestibility: $Y = 0.45 + 0.53\ X$.

The present study showed that the rat may be a useful model for studying protein digestion in the dog. However, to make a more general conclusion regarding the use of the rat as a model animal to study protein digestion in the dog, a wider range of dog foods need to be investigated to determine the "strengths" of the regression equation shown above.

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GENERAL INTRODUCTION

The domestic dog (*Canis familiaris*) belongs to the order Carnivora and is one of the most common companion animals kept by man. Modern carnivores are divided into two groups, the aquatic Pinnipeds and terrestrial Fissipeds. Divergence within the Fissipeds occurred in the late Eocene to early Oligocene period, or about 35 million years ago, and resulted in the emergence of three super-families. In these three super-families, the Canoidea contains the domestic dog.

Although the dog is commonly referred to as a companion animal, it serves as much more than mere a companion in our society. Owning a pet has been recognised to have health and psychological benefits such as decreased loneliness, increased self-esteem, increased interaction with others and development of assertiveness. As a result pets are often used in therapy for functionally disturbed children and elderly people in nursing homes. Dogs are actively used to aid the blind and deaf people as well as those confined to wheelchairs. They play a vital role in search and rescue work and are frequently used to detect drugs and bombs at international airports. Furthermore, dogs are indispensable in the farming of sheep in many countries and also have been a valuable animal for studying biochemistry, physiology and basic biology of mammals.

To the owner of a pet the most rewarding service that may be performed is to keep their companion healthy and fit, so that a long and happy life can be enjoyed. The single most important aspect of achieving those ends lies with the diet that is fed to the animal. In many ways the nutritional characteristics of a pet food exceed those of humans or farm animals. Unlike humans, the owner restricts the access of the pet to food. Farm animals on the other hand are fed only for efficient production and rarely are expected to live out their natural lives whereas pets are expected to live long, happy and healthy lives much like humans. When preparing a suitable diet for pets, the life style and life stage of the animal must be considered. As described earlier dogs are used for various tasks, which will result in different requirement for nutrients depending on which task is performed by the animal. Individual animals may be at different life stages and each life stage will require its own particular demands on a diet. Further, dogs are unique among mammals in displaying such a wide range of body weights within a single species (1 kg Chihuahua to 115 kg St Bernard). This wide variation in body weight also has an influence on requirement for nutrients. The nutritional requirement of dogs of only two physiological

states (maintenance and growth) is currently listed by the National Research Council. Additional to the nutrient requirement, data is required on the nutrient digestibility of feed ingredients to accurately formulate diets for dogs. Furthermore, to assess the adequacy of diets to meet the nutrient requirement of dogs, the digestibility of individual dietary nutrients is required.

The digestibility of nutrients in diets for dogs is normally measured in faeces. This method has been criticised due to the microbial action in large intestine. Measuring nutrient digestibilities at the terminal ileum is considered now to be a better method than the traditional faecal method. Moreover, other than the dietary protein, the digesta collected at the terminal ileum also consist of non-dietary endogenous protein. To estimate true digestibility, the gut endogenous protein losses have to be estimated. The two latter procedures are invasive techniques. Practical and ethical reasons hamper the use of invasive techniques in routine estimates of digestibility of nutrients in companion animal diets. Model animals, therefore, may be a better alternative. This thesis aims to develop the rat as a model animal for the digestion of protein and amino acids in the dog.

CHAPTER 1

REVIEW OF LITERATURE

1.1 INTRODUCTION

Dietary protein is a particularly important component of the diet as it not only provides essential amino acids required for protein synthesis in the body, but also energy, amino nitrogen and sulphur. Before absorption across the intestinal mucosa, dietary protein has to be degraded into simple absorbable forms. This process of degradation is referred to as digestion and involves a combination of mechanical, chemical and microbial activities. Comprehensive reviews on the anatomy of the gastrointestinal tract, physiology of digestion and the nervous and hormonal control of secretion and motility of the gastrointestinal tract in monogastric animals, including the dog, have been published (Code, 1968; Nickel *et al.*, 1973; Ellenport, 1975; Evans, 1979; Adrian and Bloom, 1981; Johnson, 1981a; Walsh, 1981; Davenport, 1982; Sanford, 1982; Holst, 1986; Jones and Liska, 1986; Greenwood and Davison, 1987; Fioramonti and Bueno, 1988; Strombeck and Guilford, 1990; Dukes, 1993; Johnson, 1994; Guilford *et al.*, 1996). Digestion and absorption of proteins in mammals has also been extensively reviewed (Gitler, 1964; Cuthbertson and Tilstone, 1972; Erbersdobler, 1973; Snook, 1973; Rerat *et al.*, 1976; Kidder and Manners, 1978; Rerat, 1981; Matthews, 1983; Hunt and Groff, 1990; Scharrer and Wolffram, 1990; Rerat and Corring, 1991; Dukes, 1993; Rerat, 1993).

Ingested protein is mixed with endogenous protein secreted from the gut and both are hydrolysed into di- and tri-peptides and free amino acids, predominantly in the stomach and the proximal intestine. Free amino acids and di- and tri-peptides are absorbed along the wall of the entire small intestine, which is accomplished by several transport mechanisms located in the enterocytes. Most of the di- and tri-peptides absorbed by the enterocytes are hydrolysed in the cell and, together with the absorbed free amino acids, can either be metabolised in the enterocytes or released into the hepatic portal vein. Undigested protein leaving the small intestine enters the large intestine where it can be metabolised by the microbial population. Some protein, peptides and free amino acids may escape breakdown in the hind gut and together with the bacteria are excreted in

the faeces. Several factors, including inaccessibility of the protein to proteolytic enzymes, resistance to enzymatic hydrolysis, inhibition to proteolytic enzymes or inhibition to amino acid absorption, may alter the extent of protein digestion and absorption (Donkoh, 1993).

The term digestibility of a nutrient is defined as the difference between the amount of a nutrient ingested in the diet and the amount excreted in the faeces or digesta, expressed as a proportion of the amount ingested. It is a measure of the digestion and subsequent absorption of the nutrient. Measurement of the digestibility of nutrients in diets for animals will help to ensure that diets can be adequately and economically formulated to meet the needs of the animal.

This review first considers various approaches available to determine the digestibility of protein and amino acids in mammals. Secondly, this review will evaluate the suitability of the laboratory rat as a model animal for studying the digestion of protein and amino acids in the dog while finally gut endogenous protein and amino acid losses in the dog will be reviewed.

1.2 DETERMINATION OF THE DIGESTIBILITY OF DIETARY PROTEIN AND AMINO ACIDS

The digestibility of amino acids in food/feeds is highly variable. Knowledge on the amino acids absorbed from a food/feedstuff is an integral part for optimal formulation of diets both in terms of maximising nutrient utilisation as well as optimising health and longevity of the animal. There has been a considerable effort over the last 40 years in the development of techniques to measure the digestibility of dietary nutrients.

1.2.1 Faecal digestibility

One of the earliest procedures to determine the digestibility of dietary nutrients *in vivo* was developed by Kuiken and Lyman (1948). The latter authors calculated the digestibility of a dietary nutrient by comparing the amount of a nutrient ingested with that excreted in the faeces using the following equation (units in mg/g dry matter):

$$\text{Faecal digestibility of a nutrient} = \frac{\text{Nutrient in the diet} - \text{Nutrient in the faeces}}{\text{Nutrient in the diet}}$$

The digestibility of a nutrient can be determined either directly, when the total amount of the nutrient ingested and the amount excreted is measured, or indirectly, in which the ratio of the nutrient to an indigestible marker is measured both in the diet and the faeces.

The measurement of the digestibility of protein and amino acids by the faecal method is a relatively straightforward approach. However, this method has been criticised for the measurement of the digestibility of dietary protein and amino acids as microorganisms in the large intestine may metabolise dietary and endogenous proteins and amino acids. Considerable bacterial deamination, decarboxylation and transformation occurs in the large intestine, as well as synthesis of bacterial protein from amino acids arriving from the small intestine and non-protein nitrogenous compound diffusing from the blood (Gitler, 1964; Mason, 1980; Wrong *et al.*, 1981; Zebrowska, 1982; Just, 1983). In general this leads to a net disappearance of amino acids and nitrogen between the ileum and the rectum, although sometimes a net appearance of amino acids may occur. Especially for methionine and sometimes for lysine there may be a net gain, due to the microbial action (Just, 1980; Low, 1980; Sauer and Ozimek, 1986; Moughan, 1991). An indication of the significance of hind gut microbial flora metabolism is that around 62-76% of human faecal nitrogen is present in microbial bodies (Mason, 1984). The extent of microbial activity depends on the type and numbers of microorganism present, the type of feedstuff and the time of residence of material in the hind gut. It is thus a function of both species of animal and diet (Moughan and Donkoh, 1991).

Although there may be some absorption of amino acid across either the caecal or colinic mucosa in new-born mammals (Batt and Schachter 1969; James and Smith, 1976; Heine *et al.*, 1987), it appears that in growing and adult animals, amino acid absorption across the large intestinal mucosa is nutritionally not significant (Hoover and Heitmann, 1975; Schmitz *et al.*, 1991; Darragh *et al.*, 1994). Olszewski and Buraczewski (1978) and Niiyama *et al.* (1979) showed that amino acids may be absorbed across the caecal-colonic mucosa of the pig. However, these authors did not measure any metabolic variables that would identify whether the amounts of amino acids absorbed were of nutritional significance. Robinson *et al.* (1973) demonstrated absorption of amino acids across the

large intestine *in vitro* in dogs, but there was no evidence that such capacity would also occur *in vivo*. Zebrowska (1973) and Just *et al.* (1981) showed almost complete digestion and absorption of nitrogen from infused protein in the caecum of pigs, but most of this nitrogen was not utilised by the animal and was excreted in the urine. In general it appears that amino nitrogen is absorbed from the hind gut mainly in the form of ammonia, which under normal circumstances is of no nutritional value to the animal.

Overall it appears that only a very low proportion of the faecal amino acids excreted directly related to the flow of the undigested dietary amino acids entering the large intestine (Moughan and Donkoh, 1991). The traditional faecal measurement of amino acid digestibility is, therefore, an inaccurate method to assess the amounts of amino acids absorbed.

1.2.2 Ileal digestibility

It is now well recognised that a more accurate approach to estimate the digestibility of protein and amino acids by an animal is the ileal digestibility method (Rerat, 1981; Tanksley and Knabe, 1984; Sauer and Ozimek, 1986; van Weerden, 1989). In this method total digesta is collected at the terminal ileum and the concentration of the nutrient in the diet is compared with that in the digesta using following equation (units in mg/g of dry matter):

$$\text{Ileal digestibility of a nutrient} = \frac{\text{Nutrient in the diet} - \text{Nutrient in the ileal digesta}}{\text{Nutrient in the diet}}$$

Total collection of digesta, however, is time consuming, requires major surgery on the animal, and is not possible in all situations. An indigestible marker can also be added to the diet and used to determine the digestibility of nutrients using following equation (units in mg/g of dry matter):

$$\text{Ileal digestibility of a nutrient (N)} = \frac{(\text{N in the diet} / \text{Marker in the diet}) - (\text{N in the ileal digesta} / \text{Marker in the ileal digesta})}{(\text{N in the diet} / \text{Marker in the diet})}$$

Selection of an appropriate marker, however, poses some difficulties. To obtain reliable digestibility estimates, the marker must be indigestible, pass chemically unaltered through the gut, be non toxic, conveniently analysed for and move through the gut uniformly with the digesta. Although a wide range of markers has been used with their unique advantages and disadvantages, currently there is no ideal marker, although chromic oxide seems to be the marker most commonly used in digestibility studies (Kotb and Luckey, 1972).

Whatever method is used for estimating ileal digestibility, collection of digesta from the terminal ileum is not a straightforward approach. Numerous methods have been developed. The main method used involves the surgical implantation of cannula. The ileo-ileo and ileo-caecal re-entrant cannulation involves total transaction of the ileum, causing some physiological changes. In addition these cannulas may block up with digesta because of dietary particle size, dietary crude fibre content, feed intake, and factors that increase the viscosity of the digesta (Sauer and de Lange, 1992). Furthermore, leakage around the cannula has been reported (Sauer and Ozimek, 1986).

Another approach allowing total collection of digesta is ileo-rectal anastomosis or ileo-rectal shunt (IRS), which is a modification of the latter method. Ileo-rectal anastomosis and IRS preserve the functional role of the ileocecal valve and do not have the limitation of the ileo-ileo and ileo-caecal re-entrant cannulation, so a variety of feedstuffs including high fibre diets can be tested at normal feed intake levels. Moreover, animals prepared with an IRS require much less time and effort to maintain than animals fitted with re-entrant cannulas (Sauer and de Lange, 1992). However, certain precaution have to be taken with IRS fitted animals. More water should be provided to the animals, diets should include more vitamin B and minerals and as there is leakage, the area around the anus should be cleaned daily (Sauer and de Lange, 1992). However, questions concerning the physiological normality of anastomoised animals exists (Moughan, 1991).

Several methods have been developed to overcome the problems previously discussed with the ileo-rectal anastomosis method. Some of these are post-valvular T-caecum cannula, post-valvular ileo-colic fistulation, the ileo-colic post-valve procedure and the simple T-cannulation method. The latter methods have the distinct advantage that they avoid the transection of the small intestine so functional integrity of the small intestine and of the ileocaecal valve are maintained and, as a result, the animal is in a more normal physiological state. However, there are a number of concerns with the latter

approaches, including the ability to obtain representative samples and the possible shortcoming of digestible markers (Sauer and de Lange, 1992). Depending on whether the first or the last fractions of a meal arrives at the ileocecal junction are being considered, the time for the food to reach the terminal ileum may vary from 4-16 hours. To obtain representative samples, therefore, attention should be paid to factors such as the frequency and duration of sampling in relation to time and frequency of feeding (Sauer and de Lange, 1992). It should be mentioned that with high fibre diets any cannula is susceptible to blockage (Donkoh *et al.*, 1994a). Moreover, any form of cannulation including simple T-cannulation is likely to disturb normal physiological function (nutrition flow and absorption) of animals (Wenham and Wyburn, 1980).

An alternative to collection of digesta using a cannula is to sample digesta from the terminal ileum while the animal is under anaesthesia (Moughan and Smith, 1987). This method has the distinct advantage of minimal disruption of normal digestive functions and allows samples of digesta to be taken from several parts of the digestive tract. Furthermore, there is no limitation as to the type or form of diet given to the test animals (Donkoh *et al.*, 1994a). However, and as previously discussed, this technique may also have difficulties in obtaining representative samples of digesta. However, Moughan and Donkoh (1991) suggested that when a frequent feeding regime is adapted with this technique, digestibility data may not be more variable than the previous discussed methods.

1.2.2.1 Limitation of ileal amino acid digestibility

Although the ileal digestibility assay is widely accepted as being superior to the faecal method to determine amino acid digestibilities, it has been criticised due to various reasons.

There may be interference from a population of microorganisms present in some segments of the stomach and terminal ileum where a slowing down of digesta transit occurs (Cranwell, 1968; Bergner *et al.*, 1986; Dierick *et al.*, 1986).

A main technical criticism, as described earlier, is the difficulty in obtaining a representative sample of digesta. In cannulated animals, when a regular feeding regime is employed, sampling of large amounts of digesta throughout 12-hour periods for several successive days will provide a representative sample of digesta (Donkoh, 1993). With the

slaughter method, collection of digesta at a predetermined optimal time, may be used to generate reliable digestibility estimates (Moughan and Donkoh, 1991).

Reliability of digestibility measurements determined by the slaughter or simple T-piece cannulation methods, further, is influenced by the validity of the marker being used. As mentioned earlier, currently there is no ideal marker. It is generally accepted that comparisons between the digestibility of different feedstuffs can be made within a trial using the same marker. But it must be stressed that the behaviour of a marker may be influenced by the physico-chemical property of the material with which it moves (Donkoh, 1993).

In heat processed feedstuffs the ileal amino acid digestibility values do not always accurately represent the availability of amino acids (Fuller *et al.,* 1981; Moughan, 1991; Moughan *et al.,* 1991; Batterham *et al.,* 1990; Batterham, 1992). There are some amino acids (mainly lysine but also methionine, cystine, and tryptophan) in feedstuffs which have undergone heat processing or prolonged storage, that may reacted with other compounds present in the feedstuff (e.g. Maillard reaction) and become nutritionally unavailable (Hurrell and Carpenter, 1978; Hurrell and Carpenter, 1981; Hurrell and Finot, 1985). During the acid hydrolysis step of conventional amino acid analysis, which is used to break down the protein into its constituent amino acids, a proportion of the formed derivatives may revert back to the amino acid. As a consequence, conventional amino acid analysis may lead to an overestimation of the actual availability of the amino acid in the digesta. Lysine is a prime example as this amino acid reacts with other compounds in the diet to become unavailable. Upon hydrolysis the bound lysine is liberated and measured as being available to the animal. However, by determining the ileal digestibility of reactive lysine (unbound lysine) an estimate of the lysine which is available to the animal can be made (Moughan and Rutherfurd, 1996).

Nevertheless, besides these drawbacks of the ileal digestibility assay, studies by Low and Partridge (1984) and Moughan and Smith (1985) indicate close correlation between the apparent ileal digestibility assay and animal performance like carcass retention, daily weight gain and feed conversion. Tanksley and Knabe (1984) concluded that ileal digestibility values offer great potential for increasing the precision of diet formulation for growing pigs. Moreover, apparent ileal digestibility coefficients have been shown or described to be sensitive in detecting small differences in protein digestibility (Rudolph *et al.,* 1983; Vandergrift *et al.,* 1983; Sauer and Ozimek, 1986).

1.2.2.2 Factors influencing ileal digestibility values

There are various factors influencing the ileal digestibility of amino acids. Factors such as digesta collection method, food intake, dietary protein concentration, dietary fibre content, processing, anti nutritional factors, antibiotics and environmental temperature have been shown or are suspected to influence the ileal digestibility coefficients.

Different methods have been used for sampling digesta and comparisons of these different methods have been performed to investigate their effect on the estimates of digestibility (Moughan and Smith, 1987; Kohler *et al.*, 1990; Leterme *et al.*, 1990a; Kohler *et al.*, 1991; Van Barneveld *et al.*, 1991). Results indicate that the method of sampling does not have a large effect on digestibility estimates. However, the variability in the digestibility values realised to be greater when using the slaughter method and as a result a larger number of replications may be required if the slaughter technique is used.

There are remnants of plant cells that are resistant to hydrolysis by the alimentary enzymes of mammals. Dietary fibre includes non-starch polysaccharides, resistant starch and lignin. These different types of dietary fibre may influence the digestibility in different ways. Bueno *et al.* (1981) have shown that addition of cellulose affects gastrointestinal transit time in the dog, which again influences the digestibility of nutrients. Further, fibre may interfere with digestion by adsorbing proteolytic enzymes (trypsin and chymotrypsin) and lowering the access of the proteolytic enzymes to the substrate (Schneeman, 1978). In contrast, some studies have shown that pectin (5 % of diet) increases pancreatic amylase activity and lipase output (Isaksson *et al.*, 1983). Fibre is capable of adsorbing amino acids and peptides and withholding them from being absorbed (Bergner *et al.*, 1981). Another effect of dietary fibre is, to influence endogenous nitrogen excretion by increasing mucus production and increasing sloughing of mucosal cells (Schneeman *et al.*, 1982; Low, 1989).

Krawielitzki *et al.* (1977) suggested that the digestibility of nutrients may vary with food intake, however, studies by Haydon *et al.* (1984), Van Leeuwen *et al.* (1987) and Sauer *et al.* (1989) showed that dry matter intake has no effect on the ileal digestibility of amino acids in pigs.

Quantity and type of fat has been shown to influence the digestibility of protein in pigs (Nielsen *et al.*, 1985; Ozimek *et al.*, 1985). Sauer *et al.* (1980) showed that when the fat concentration increased from 4.5 to 26.8 % of diet dry matter, digestibility of crude protein and amino acids increased by 3 % units.

During processing, foodstuffs may undergo varying degrees of pressure and temperature and this will affect the digestibility and availability of protein and other nutrients. Schutte *et al.* (1987) demonstrated that the ileal digestibility of nutrients decreases with increasing heat treatment. One effect of heat is that during processing, some amines and amino acids may react with carbohydrates in the foodstuff and one example is non-enzymatic browning or Maillard reaction (Hurrell and Carpenter, 1978; Hurrell and Carpenter, 1981). Most of the digestive enzymes do not cleave peptide bonds of an amino acid, which are adjacent to a carbohydrate moiety. Trypsin is the enzyme specific for peptide bonds containing a carboxyl group (lysine, arginine) and –amino group. The carbohydrate that is bound to the free epsilon group of lysine interferes with the ability of trypsin to break the peptide bound. Another effect of processing is that during processing particular size of the food or feed ingredients is often reduced. The ileal digestibility of amino acids, however, has been shown to increase with decreasing particular size (Sauer *et al.*, 1977; Owsley *et al.*, 1981). Overall, one effect of processing reduces the digestibility whereas another effect increases the digestibility.

There are some compounds found naturally in food prevent digestion and absorption of nutrients from the gut. These substances are called anti-nutrients. Anti-nutrients affect protein digestion either directly by reacting with food proteins and making them less digestible or indirectly by reacting with gut cells or enzymes and affecting their digestive, absorptive, secretary and protective functions (Jaffe, 1980; Vandergrift *et al.*, 1983; Ozimek and Sauer, 1985; Leterme *et al.*, 1990b).

Although several studies (Just *et al.*, 1980; Livingstone *et al.*, 1982; Macgregor and Armstrong, 1984; Parker *et al.*, 1984) showed an increase in apparent digestibility of whole or some amino acids with antibiotics (nebacetin or avoparcin) in various species, Moughan *et al.* (1989) found no significant effect of the inclusion of antibiotics (avoparcin or zinc bacitracin) in the diets on the apparent ileal digestibility of amino acids in milk-fed calves.

Lowering the ambient temperature has been shown to cause a marginal reduction in the digestibility of nutrients at the ileal level (Fuller and Boyne, 1972; Phillips *et al.*, 1982). Christopherson and Kennedy (1983) explained the latter effect that at a lower temperature there is an increased rate of passage of digesta through digestive tract, which results in a reduction in the digestibility of nutrients.

1.2.2.3 Faecal verses ileal digestibility

The differences between ileal and faecal digestibility of amino acids does not appear to be constant, as can be seen from Table 1.1. Depending on the amino acid and on the feedstuff considered the digestibility value obtained with the faecal method may over or underestimates the ileal digestibility. Large differences between ileal and faecal digestibility have typically been found for low digestible protein sources (Zebrowska and Buraczewski, 1977; Jorgensen and Sauer, 1982; Moughan, 1995), as it allows more undigestible material from the ileum to disappear at the large intestine.

Table 1.1 The differences between apparent faecal and ileal digestibility (%) of amino acid for the growing pig

Amino acid	Soya bean meal	Meat and bone meal
Lysine	+3.3	+14.7
Threonine	+7.1	+21.5
Methionine	-2.1	+9.3
Average of all amino acids	+2.9	+16.5

Source: Donkoh (1993)

Table 1.2 illustrates the overestimation of the faecal digestibility method in different species in determining the digestibility of protein. The degree of the difference between the ileal and faecal digestibility depends on the type and numbers of microorganism present, the type of feedstuff and the time of residence of material in the hind gut. It thus varies with species of animal and diet (Moughan and Donkoh, 1991). The amount of amino acid disappearing in the large intestine usually varies from 5 to 35 % of the total amino acids ingested in pigs (Moughan, 1995).

Furthermore, the phenomenon of a distinct depressive effect of overheating of feedstuffs on the ileal but not on the faecal digestibility of amino acids has been observed (van Weerden *et al.*, 1985; Schutte *et al.*, 1987). This indicates that the ileal digestibility method is more sensitive than the faecal digestibility method to detect small differences in digestibility value.

Table 1.2 Faecal and ileal crude protein digestibility measured in different animals

Species	Digestibility *(%)*	
	Faecal	Ileal
Piglet	0.97	0.90
Growing pig	0.81	0.66
Pre -ruminant calf	0.94	0.88
Adult Human	0.89	0.87
Chicken	0.86	0.78
Growing rat	0.78	0.69

Source: Moughan and Donkoh (1991)

1.2.3 True digestibility

Accepting that amino acid digestibilities should be based on measurements made at the terminal ileum of monogastric animals, it needs to be recognised that ileal digesta contains appreciable quantities of non dietary protein such as bacteria, hair and endogenous secretion. To obtain true estimates of the digestibility of nutrients, therefore, corrections should be made for non-dietary components that may be present in the digesta. Digestibility values determined without taking into consideration the endogenous losses of amino acids are termed apparent, while digestibility values that have been corrected for endogenous amino acid excretions are termed true. True digestibility can be calculated by the following equation (units in mg/g dry matter):

$$\text{True amino acid (AA) digestibility} = \frac{\text{AA in diet - (AA in digesta - Endogenous AA loss)}}{\text{AA in diet}}$$

1.2.3.1 Apparent verses true digestibility

Studies by Sauer *et al.* (1980), Furuya and Kaji (1989) and Donkoh (1993) showed that the level of protein present in the diet has a positive effect on the apparent digestibility of amino acids. This is because at low dietary protein levels the proportion of amino acids of endogenous origin will be greater. Apparent digestibility values are, therefore, greatly

influenced by the condition of the assay used in its determination. On the other hand true amino acid digestibility values are unaffected by the dietary conditions under which that ingredient is fed to the animal (Furuya and Kaji, 1989; Donkoh, 1993). True digestibility values, therefore, are more accurate in detecting differences in the digestibility of various protein sources (Furuya and Kaji, 1989; Zuprizal *et al.*, 1991; Donkoh, 1993). Moreover, the true digestibility values are more additive than the apparent digestibility values as these are corrected for endogenous losses (Furuya and Kaji, 1991). Overall, true digestibility is a fundamental property of a food ingredient, while apparent estimates of digestibility are variable and are open to error based on the assay methodology. True, as opposed to apparent estimates of digestibility, therefore, should more clearly describe the amino acids absorbed from a diet.

Although it is accepted that true digestibility more accurately estimates the digestibility of protein in feedstuff, there is some controversy concerning the application in practice of true as opposed to apparent coefficients of amino acid digestibility. When deciding upon which type of digestibility value to use for diet formulation, the methodology used for the estimation of the nutrient requirement of the animal must be considered. Most estimates of nutrient requirements are based on empirical methods or growth models, which have usually taken into consideration the endogenous losses of amino acids by the animal. So if apparent digestibility values were used in conjunction with these estimates of requirement there would be a double penalty against the feed. True digestibility values in these instances will be a fair representation of the protein source (Darragh *et al.*, 1995).

1.2.3.2 Methods developed to measure endogenous losses of amino acids and nitrogen

Various approaches have been taken to measure the endogenous nitrogen and amino acids remaining unabsorbed at the end of the terminal ileum. Traditionally the animal was fed a protein-free diet and the amino acids or nitrogen present in the digesta was measured. Although this is a straightforward approach, this method has been criticised as it may create a physiologically abnormal metabolism in the animal (Low, 1980). Millward *et al.* (1976) suggested that cell replication and cell protein turnover might be reduced in the gastrointestinal tract in the absence of dietary protein. Lower proteolytic enzyme activities in the pancreas and intestine have been reported for animals fed a protein-free

diet (Puigserver *et al.*, 1986). Fauconneau and Michel (1970) suggested that protein-free feeding reduce the secretion of mucus protein and the turnover rate of epithelial cells. Furthermore, the breakdown and re-utilisation of secreted enzymes may be greater in the protein-free fed state. Therefore, the protein-free feeding method may not provide accurate estimate of endogenous excretions, as for this reason various methods have been developed to estimate the endogenous gut secretion under more normal feeding conditions.

Skilton *et al.* (1988) and Darragh *et al.* (1990) measured endogenous amino acid excretions in pigs after feeding a diet containing synthetic amino acids as the sole nitrogen source but devoid of specific non-essential amino acids. In another approach, De Lange *et al.* (1989) fed a protein-free diet to pigs with simultaneous intravenous infusion of amino acids and measured endogenous gut excretions. In the above two approaches, although the animals were in positive nitrogen balance, estimates of endogenous amino acid excretions were similar to those estimates by the protein-free feeding method. It can be concluded, therefore, that negative nitrogen balance has no effect on endogenous amino acid excretion in the gut.

An alternative to determining endogenous amino acid and nitrogen losses at the terminal ileum of animals is to use the regression approach (Moughan *et al.*, 1987; Furuya and Kaji, 1989). In this approach animals are fed a series of diets of different protein content, the amino acid flows are measured, and the endogenous excretion of each amino acid is extrapolated to zero protein intake assuming that there is a linear relationship between level of feed intake and the excretion of the endogenous amino acid. The latter approach has advantages over the protein-free method as the animal is fed a more physiological normal diet with the energy intake of the animal being close to its energy requirement, which is often not the case in the protein-free method (Hendriks *et al.*, 1996). However, the regression method has been criticised for various reasons. First of all, there may not be a linear relationship between feed intake and endogenous amino acid excretion. Further, the increase in amino acid flow with increase in dietary protein intake is attributed entirely to increase amounts of food protein, the assumption being that there is no change in the amount of endogenous amino acid excretion. There is evidence; however, that the rate of protein secretion into the intestine varies with the amount of protein consumed (Snook and Meyer, 1964). Consequently some of the increased dietary protein intake is possibly the result of enhanced secretion of endogenous proteins. In

addition, any increase in protein level in the diet is always associated with other changes in dietary composition, which complicates the interpretation of the results (Donkoh *et al.*, 1995).

In labelling methods, whether using labelled dietary proteins (Patridge *et al.*, 1985) or labelling the animal and its secretion (De Lange *et al.*, 1990), the contribution of endogenous constituents to ileal nitrogen flow can be elucidated and the amount of endogenous constituents estimated. This method can be considered as superior to other methods as it allows the detection of the effects of fibre, fat, antinutritional factors and the level or type of protein in the diet on endogenous gut secretions. However, with labelling methods the endogenous amino acid composition is determined using the endogenous nitrogen loss measured and an estimated endogenous amino acid composition usually based on protein-free feeding. Moreover, several of the assumptions and techniques required in the application of tracer methodology are questionable. The cost of tracer labelled substances and the use of specialised equipment further detracts the routine use of this methodology in determination of endogenous excretions.

Hagemeister and Erbersdobler (1985) proposed a procedure for determining the endogenous nitrogen and amino acid losses, in which the lysine in dietary protein has been chemically transformed to homoarginine. Further, endogenous lysine flow can be indirectly calculated by feeding a partially guanidinated diet assuming that homoargine is absorbed to a similar extent as lysine. As homoarginine is not used for protein synthesis, it does not appear in endogenous protein. An additional advantage is that homoarginine can be transformed to lysine by arginase in the liver (Carpenter, 1973; Prior *et al.*, 1975), thus preventing lysine deficiency. However, it must be assumed that the homoarginine does not cause altered protein metabolism in the animal and that there is no significant arginase activity within the gastrointestinal tract. Moughan and Rutherfurd (1990) showed that the assumption regarding similar absorption rates of homoarginine and lysine is reasonable. These authors also showed that the degree of guanidination of gelatin has no significant effect on the lysine flow determined at the terminal ileum. An advantage of this method is that it allows the influence of the type and quality of protein and fibre or fat on endogenous excretion to be studied. However, this method provides direct information on the endogenous loss of lysine only. In addition, due to a slow rate of conversion of homoarginine to lysine, there is a possibility for accumulation of

homoarginine in the body and homoarginine may interfere with the urea cycle leading to an accumulation of ammonia in the body.

Butts *et al.* (1991) developed a method to determine the endogenous losses of amino acids at the terminal ileum in pigs fed a diet in which the protein (casein) was present in peptides (M.W< 10,000 Dalton) and amino acids. After collection of ileal digesta, a large volume ultrafiltration device was used to separate the low molecular weight fraction from the high molecular weight (M.W>10,000 Da) fraction. The high molecular weight fraction obtained this way provides a measure of endogenous amino acids flow. Any remaining dietary amino acids and small peptides being unabsorbed at the terminal ileum are removed in the low molecular weight fraction. But in addition to the unabsorbed dietary amino acids and peptides, the low molecular weight fraction will also contain non-protein nitrogen compounds, free amino acids and small peptides of endogenous origin. The latter, however, are expected to be low (Butts *et al.*, 1992). Nevertheless, their removal in the low molecular weight fraction may lead to some underestimation of the actual endogenous loss of amino acids. Further, this ultrafiltration method may be influenced in some way by the enzymic hydrolysate of casein itself and thus be an artefact of this particular dietary treatment. The homoarginine and ultrafiltration method, however, provide similar estimates of endogenous losses (Moughan and Rutherfurd, 1990). Another criticism of this method is that Williams *et al.* (1972) showed that in the blood there was binding of free amino acids and peptides with plasma protein. Therefore, it is a possibility that undigested dietary free amino acids and peptides bind to the endogenous protein in the ileal digesta. This could result in an overestimation of endogenous loss. In contrast, the endogenous amino acid flow with a synthetic amino acid diet was not higher than those for the protein-free diet, indicating that the dietary free amino acids were not "trapped" in endogenous protein.

In conclusion, the enzyme hydrolysed casein or ultrafiltration method allows endogenous nitrogen and amino acid excretions to be determined under the condition of protein-feeding which is physiologically more normal than the protein-free method. Additionally endogenous losses of all amino acids can be determined directly without having to resort to assumptions, as is the case in the homoarginine and labelling methods.

1.2.3.3 Factors affecting endogenous losses

Endogenous excretion of amino acids and nitrogen has been shown to be affected by various factors. Dietary protein affect the endogenous secretion of nitrogen both quantitatively and qualitatively through modifying secretions and increasing the stability of the enzymes (Snook, 1965). Ozimek *et al.* (1983) suggested that the amount as well as quality of fat would influence the endogenous excretion of lipase. However, Corring *et al.* (1989) cited evidence that the level of dietary fat was the primary influence on lipase excretion. In addition, and depending on the type and percentage, fibre present in the diet influences the endogenous amino acid and nitrogen excretions in different ways. Dietary fibre increases the activity and the level of enzymes and mucus secretions (Schneeman *et al.*, 1982) and the sloughing of mucosal cells (Low, 1989). Various antinutritional factors have also been shown to influence endogenous gut secretions of amino acids and nitrogen (Schneeman, 1982).

1.3 THE RAT AS A MODEL ANIMAL

Model animals are often used for ethical or practical reasons because direct measurements on the animal of interest may be difficult or even sometimes impossible to obtain. It is now well accepted that the ileal digestibility assay is a better method for determining the digestibility of amino acids. This method, however, often involves major surgery to place a cannula in the small intestine or caecum of the animal, or the animal has to be euthanased. Ileal digestibility values, therefore, are expensive to obtain and often carry a high ethical cost. Under these circumstances, an animal model such as the rat would be highly valuable to allow rapid determination of ileal digestibility values of newly formulated diets.

1.3.1 Reasons for the rat as a model animal

Several factors make the rat (*Rattus rattus*) a valuable model animal. The rat is a docile, hardy animal that thrives well in small areas and has the ability to learn. Rats breed easily throughout the year, grow rapidly and are sexually mature at approximately seven weeks of age (Waddell and Desai, 1981), making it an excellent animal for studying genetics. With an average litter size of 14 (Waddell and Desai, 1981) and its small size, rats are

relatively inexpensive to obtain and keep. Furthermore, a variety of experimental and surgical techniques have been developed on the rat to allow accurate sampling of digesta, excreta, body fluids and tissue samples. Additionally, for a long period after weaning, the animal continues to gain weight (Dunn *et al.*, 1947) and is thus useful in nutritional studies, which monitor weight gain over extended periods.

There are, however, some drawbacks in using the laboratory rat as a model animal in nutritional research. Many strains of rats are susceptible to chronic respiratory diseases, middle ear disease, pinworm and scabies. Furthermore, the fact that the rat practices coprophagy may result in biased data. However, despite the above-mentioned disadvantages, the rat has been extensively used in nutritional science. The rat has been validated as a model animal for digestion in pigs and humans (Bodwell *et al.*, 1980; Pelletier *et al.*, 1983; Moughan *et al.*, 1984; Picard *et al.*, 1984; Smith *et al.*, 1990; Skilton *et al.*, 1991; Donkoh *et al.*, 1994b). The following section will compare the anatomy and physiology of the gastrointestinal tract of the rat and the dog to determine the suitability of the rat as a model animal in digestibility studies on dogs.

1.3.2 Comparative anatomy and physiology of the digestive tract of the rat and the dog

The rat and the dog are both mammals and, therefore, major similarities are apparent in the anatomy and physiology of their digestive tracts. The gastrointestinal wall of the entire digestive tract consists of four layers in both species. The mucosal layer is made up of epithelial connective tissue, lamina propria and muscularis mucosa. The submucosa layer consists of connective tissue, blood vessels, lymphatics and nerve plexus. The muscular layer consists of three separate layers of visceral muscles and a nerve plexus. The serosal connective tissue covered by mesothelium is the fourth layer covering the entire tract. The physiology of secretion and absorption of nutrients are similar between the rat and dog. The different feeding habits throughout evolution of these two species, however, have resulted in some anatomical and physiological differences in the digestive tract.

1.3.2.1 Mouth cavity.

The mouth is the first part of the digestive tract and is adapted to consume food and reduce particular size of food to make it easy to swallow. The basic anatomy and function of the mouth cavity is similar between the rat and the dog. However, according to the type of diet, there are some adaptations in distribution and type of teeth, surface structure of the tongue, size and movement of the jaw and composition of salivary glands and their secretion.

The origin and the structure of teeth are similar in both species; however, there is a difference in the type of teeth present. Maskell and Johnson (1993) point out that the dental formula of the dog, like that of the rat, is able to handle omnivorous diets. The dog's saliva plays a special role in regulation of body temperature and, therefore, the secretion of saliva per unit of gland weight is higher in the dog in comparison to the rat (Schneyer and Schneyer, 1968). Furthermore, secretion of saliva is continuous in the rat while in the dog this only occurs in the presence of food.

Table 1.3 Comparative enzymology of salivary secretion in the dog and the rat

Species	Enzyme activity				
	Acid phosphatase	Esterase	Pseudocholin esterase	Galactosidase	Amylase
Dog					
Parotid	2	2	2	2.0	0
Submaxillary	2	3	2.5	3.0	trace
Rat					
Mixed[1]	3	3.5	3	3.5	4.5

Source: Ellison (1968)

[1]Mixed secretion of main salivary glands

Comparison of the enzymology of saliva of the dog and the rat (Table 1.3) shows that except for amylase the enzyme activity in the saliva of the rat and dog is similar. Nevertheless, salivary amylase is not significant to overall carbohydrate digestion (Maskell and Johnson, 1993).

1.3.2.2 Oesophagus

In both species the oesophagus has the same basic structure and function. However, there are some differences between the rat and the dog in the anatomy and physiology of the oesophagus.

In the dog and in most other animals a lamina propria intervenes between the muscularis mucosa and the lining epithelium. This connective tissue layer, however, is absent in the rat (Schofield, 1968). Furthermore, tubulo alveolar sub mucosal glands, present throughout the length of the canine oesophagus, cannot be found in the rat (Dellmann, 1971). A further difference between the rat and the dog is that the circular muscle coat of the dog consists of smooth muscles at the region immediately above the cardiac orifice (Dellmann, 1971), which makes the oesophagus distensible in this animal. The dog furthermore has a particularly well developed vomiting centre making vomiting quite common in this species (Maskell and Johnson, 1993), while it is a rare phenomenon in the rat.

1.3.2.3 Stomach

The stomach is the largest dilation of the alimentary canal and is a musculo glandular organ. It aids digestion by promoting mixing and grinding of the diet, while its intrinsic glands intermittently add enzymes, mucus and hydrochloric acid. In both species again the basic structure, type of secretion, physiology of secretion, gastric motility and the function of the stomach are similar. However, some differences exist which are related to the feeding habit and type of diet normally consumed by these two species.

In the dog the stomach is a single chamber lined throughout by a glandular mucous membrane. In the rat, although the stomach is a single chamber, only the distal part leading to the duodenum is glandular; the lining of the remainder is non-glandular (Hebel and Stromberg, 1986). Because the rat is a continuous feeder and used to consuming large quantities of food of relatively low nutritional value, the stomach of this species is capacious and serves to store food. The dog, on the other hand, is a meal feeder; the proper gastric mucosa of this species is, therefore, able to expand for the temporary storage of food (Maskell and Johnson, 1993).

Similar enzymes are secreted by the stomach of the rat and the dog (Davenport, 1982; Maskell and Jonhnson, 1993; Hamosh, 1994; Wright *et al.*, 1994; Strombeck and Guilford, 1996). The rat, however, secretes gastric juice continually whether or not food

is present in the stomach. In the case of the dog, both pepsinogen and electrolytes are secreted at a very low rate or not at all in the basal state (Hirschowitz, 1968). Furthermore, pepsin activity, which is important for initiating digestion of collagen, is higher in the dog than the rat (Maskell and Johnson, 1993). Nevertheless, these authors also point out that pepsin secretion is not essential for digestion and absorption of a meal; pancreatic proteases are important for completion of protein degradation and can cope adequately in the absence of pepsin if necessary.

The effective function of the stomach, besides being determined by the enzymes secreted, is also determined by the retention time of ingesta in the stomach. The gastric retention time is influenced not only via the structural and physiological characteristics of the stomach but also by the physical and nutritional characters of the diet. Warner (1981) showed that the gastric retention time of food in the rat and the dog is similar. Code and Carlson (1968), furthermore, showed that the frequency of peristaltic movement does not differ between these two species.

1.3.2.4 Small intestine

The small intestine is an important part of the digestive tract and it plays a main role in the digestion and absorption of nutrients. The anatomy of the small intestine, the organic and inorganic composition present in the secretions, the physiology of secretion and motility, and its function are similar between the rat and the dog. Furthermore, the mechanism of absorption of nutrients is similar between these two species.

The efficiency of digestion and absorption of nutrients depends, among other factors, on the enzymes secreted into the small intestine, the rate at which digesta moves through the digestive tract and the absorbable surface area of the intestine. The rat, like the dog, excretes the enzymes amylase, sucrase, isomaltase, maltase, lactase, trichalase, enterokinase, lysozyme, neutral endopeptidase, dipeptidyl peptidase, aminopeptidases, carboxypeptidase, nucleotidase, nucleosidase and alkaline phosphatase (Rhodes, 1968; Davenport, 1982; Malagelada, 1981; Strombeck, 1996). As can be expected in typical carnivores, the activity of amylase is low. Amylase activity of animals increases with the increased ingestion of dietary starch. The amplitude of the increase and the time taken for this adaptation varies with type of the diet and animal species. In typical carnivores, such as the cat, the level of adaptation of amylase activity is low and the time taken for this adaptation to occur is high. In the dog, however, the level and time for the adaptation of

amylase activity to occur, resemble more that of omnivorous animals (Maskell and Johnson, 1993).

In mammals, nutrients cross the intestinal mucosa by similar transport mechanisms (Scharrer and Wolffram, 1990). The rate of absorption, however, varies between animals. Carnivores are unable to regulate the rate of small intestinal absorption of monosaccharides in response to the level of carbohydrate in the diet. However, dogs, like rats, are able to do the latter (Maskell and Johnson, 1993). The rate of amino acid transport can be expected to be high in carnivorous animals. Nevertheless, it was shown in the rat that the rate of amino acid transport is modulated if the diet contains a high protein content (Wolffram *et al.*, 1984; Scharrer, 1989). Furthermore, the absorptive capacity of fat can also be expected to be high in carnivores. Although experimental findings are not yet available for dogs, in the rat it has been shown that the capacity of fat absorption adapts to the fat level in the diet (Scharrer and Wolffram, 1990).

The efficiency of digestion and absorption, among other factors, also depends upon the rate at which digesta moves through the digestive tract, which in turn is influenced not only by the digestive tract itself, but also by the physical and chemical properties of the diet. The gastrointestinal transit time (Clemens and Stevens, 1980) and the frequency of rhythmic segmental contractions of the small intestine of the rat and the dog show a high degree of similarity compared to the other animals (Hightower, 1968).

Table 1.4 Mucosal surface area of different segments of the small intestine in the rat and the dog

Species	Small intestinal segment				
	Duodenum	Jejunum	Ileum	Average	Reference
	(cm^2/cm of serosal length)				
Rat	8.2	8.5	4.4	5.4	Permezel & Webling (1971); Wood (1944)
Dog	101	54	39	49	Warren (1939)

The amount of nutrients absorbed is also determined by the available mucosal surface area of the small intestine. The length and width of the small intestine and the length and shape of the villi determine the mucosal surface area of the entire small intestine. The latter parameters are different between the rat and the dog (Evans, 1979;

Nickel *et al.*, 1973; Dukes, 1993; Hebel and Stromberg, 1986). Table 1.4 shows the mucosal surface area of different parts of the small intestine in the rat and the dog. The average mucosal surface area per unit of serosal length in the dog is much larger than in the rat. However, the available mucosal surface area per unit of body weight is similar in the rat and dog (Table 1.5).

Table 1.5 The mucosal surface area of the small intestine (SI) of the rat and the dog

Species	SI mucosal area	Body weight	Mucosal area/bwt[1]	Reference
	(cm^2)	(g)	(cm^2/g)	
Rat	516	325	1.58	Fisher and Parsons (1950)
Dog	16200	10000	1.62	Warren (1939)

[1]body weight

The liver and pancreas are two accessory glands present in all mammalian species. These glands drain their secretions into the small intestine, which plays a main role in digestion of nutrients. The organic and inorganic components present in the secretion and the function of the accessory glands are similar between the dog and the rat (Hofmann, 1968; Rhodes, 1968; Davenport, 1982). However, some differences exist between these two species.

In the dog, the pancreatic duct and common bile duct are distinct, but enter the duodenum together, whereas in the rat the pancreatic duct ends in the common bile duct (Hallenbeck, 1968). The gallbladder is absent in the rat. As the rat is an intermittent feeder, it requires a continuous flow of bile, so there is no need for a gallbladder. A direct quantitative relationship exists between bile flow and bile salt secretion rate. In the rat and the dog, bile salt in the enterohepatic circulation has a significant influence on bile flow, whereas in some species such as human interruption of enterohepatic circulation appears to have no obvious effect (Weiner and Lack, 1968). Bile acids are conjugated at position 24 with either glycine or taurine. The relative amounts of glycine and taurine conjugates are determined by the availability of these amino acids and their affinities for the enzyme system. In some species the system is specific for either taurine or glycine, whereas in the dog and rat conjugation can occur either with glycine or taurine (Weiner and Lack, 1968).

Release of gastrointestinal hormones in both species is qualitatively the same with small differences in quantity. Gastrointestinal regulatory peptides have been isolated and purified and species similarities have been demonstrated in mammals (Adrian and Bloom, 1981).

1.3.2.5 Large intestine

The large intestine, consisting of caecum, colon and rectum, plays a major role in the absorption of water and electrolytes.

In both animals the caecum is relatively small and only slightly differentiated. However, the caeco appendix is more pronounced in the rat indicating that in the rat there is a considerable amount of carbohydrate and fibre digestion occurring in the large intestine. In the dog the caecum is smaller than the rat which would indicate that this animal has ingested smaller amount of carbohydrate or fibre throughout evolution.

1.3.2.6 Conclusions

Overall, evidence based on the anatomy, histology and physiology of digestion suggests that there is a close similarity between rats and dogs. This indicates that the rat may be a suitable model animal for studying digestion in the dog. However, it must be stressed that there are some differences between the rat and the dog, especially the practice of coprophagy. Furthermore, vomiting in the dog seems to be relatively common while this is very rare in rats. The dog, unlike the rat, is a meal feeder and has a functional gall bladder, which stores bile acids until needed. Under strict experimental condition, however, the influence of these differences can be minimised.

1.4 ENDOGENOUS GUT SECRETION OF PROTEIN IN THE DOG

Considerable quantities of protein, peptides, amino acids and other nitrogen-containing compounds diffuse into and are secreted into the lumen of the gut during the digestion of food (Fauconneau and Michel, 1970). The salivary glands, pancreas, stomach and small intestine secrete digestive enzymes (Table 1.6), which hydrolyse the nutrients in the food. The liver secrets bile acids which aid the digestion and absorption of fat and fat-soluble compounds. There are glands situated along the entire length of the digestive tract, which secrete mucus that lubricates and protects the mucosa. Furthermore, there is a continuous

turnover of the epithelial mucosa of the intestine while plasma proteins, free amino acids, amines and urea diffuse into the gut from the epithelial cells. All these secretions are regulated by both nervous and hormonal controls and result in sufficient secretion to provide efficient digestion. The following will review the endogenous gut secretions in dogs.

Table 1.6 Essential amino acids in digestive enzymes of monogastric animals

Amino acid	Amylase	Pepsin	Trypsinogen	Chymo trypsinogen	Carboxy peptidase
			(mM/100 g protein)		
Arginine	32.3	7.5	9.5	16.1	26.0
Histidine	25.2	7.1	10.9	7.4	20.6
Isoleucine	87.8	74.1	50.4	40.1	52.7
Leucine	-	76.8	51.9	75.2	64.1
Lysine	-	7.2	63.7	53.4	48.6
Methionine	14.1	12.1	7.1	8.0	2.7
Phenylalanine	61.2	42.3	20.6	23.3	40
Threonine	32.8	84.0	45.3	91.6	68.1
Tryptophan	32.8	11.5	18.1	27.5	16.7
Valine	66.7	62.8	69.2	85.4	41.9

Source: Nasset (1964)

1.4.1 Salivary secretions

The composition of salivary secretions has been extensively reviewed by Ellison (1968), Davenport (1982), Maskell and Jonhnson (1993), Cook *et al.* (1994) and Wright *et al.* (1994). There are a variety of proteins and other nitrogenous compounds present in saliva including enzymes, growth factors and gastrointestinal regulatory peptides. Esterase, ribonuclease, acid phosphates, kallikrein, rennin, galactosidase and amylase are the common enzymes present in saliva. Albumin, transferrin, globulin, lactoperoxidase, transaminase, ATP-ase, secretory IgA, blood group substances, antigen, free amino acids, urea, uric acid and mucus are some other nitrogen-containing compounds present in saliva. The protein components of saliva are mainly secreted by the end piece cells, although some proteins enter saliva from the interstitium and the duct cells.

The dog's parotid and submaxillary salivary glands secret approximately 0.55 and 1.31 ml of saliva per minute, respectively (Ellison, 1968). Furthermore, canine salivary mucin contains 24.2 % of protein and 0-9 % of uric acid (Ellison, 1968).

1.4.2 Gastric secretions

The composition and function of gastric secretions are described by Davenport (1982), Maskell and Johnson (1993), Hamosh (1994), Wright *et al.* (1994) and Strombeck and Guilford (1996). There are a variety of substances secreted by different types of cells: proteases (pepsin, chymosin mainly in neonates and cathepsin D) by chief cells and mucous cells, lipase from chief cells and mucous cells, intrinsic factor by fundic glands, the hormone gastrin by G cells, and mucus from mucous cells and surface epithelial cells. In addition the gastric mucosa secretes small quantities of other enzymes, such as esterase, gelatinase, lysozyme, urease, neuraminidase and carbonic anhydrase. The origin and location of gastric lipase differs among species.

In the dog, pepsinogen seems to be localised in zymogen granules of the peptic cells at the base of fundic glands, whereas lipase is found in foveolar mucous type cells in the pit of the gastric glands. Furthermore, in the dog lipase activity is present throughout the entire stomach including the antrum, whereas in some animals (e.g. humans) lipase action at the antrum is low (Hamosh, 1994). Cardiac and pyloric glands in dogs secrete approximately 1.5 ml of fluid per hour and the rate of secretion of non parietal secretions is over 1.7 ml/min with the rate for parietal components being 2.6 ml/min (Conway, 1953). The dog's gastric surface epithelial secretions contain 10-11 g of protein per litre of fluid (Davenport, 1982). Nasset and Davenport (1955) demonstrated that 15 different free amino acids are secreted from the stomach of dogs, after visual and olfactory stimulation.

1.4.3 Pancreatic secretions

The pancreas secretes a variety of enzymes and bioactive peptides. Rhodes (1968), Davenport (1982), Wright *et al.* (1994), Strombeck (1996) and Williams (1996) have extensively reviewed the composition and the function of the pancreatic secretions. Enzymes in the pancreatic secretions include carbohydrases (amylase and chitinase), endopeptidases (trypsin 1,2 & 3, chymotrypsin 1, 2 & 3, elastase 1 & 2, collaginase and enterokinase), exopeptidases (carboxypeptidase A_1, A_2, A_3 & B), nucleases (ribonuclease

and deoxyribonuclease) and lipases (glycerol ester hydrolase, phospholipase A_2 and carboxyl ester hydrolase). The pancreatic secretion also contains some enzyme cofactors (colipase), enzyme inhibitors (trypsin inhibitor), kallikrein, kinin, lactoferrin, intrinsic factor and plasma proteins.

The canine pancreas secretes approximately 2.3 ml of fluid per minute (Janowitz, 1968). As the dog is an intermittent feeder the volume of pancreatic fluid secreted is low compared to other animals in which secretion is continuous (Davenport, 1982). The protein contents in canine pancreatic juice varies from 0.1-10 % (Davenport, 1982).

1.4.4 Bile secretions

Secretion of bile and its protein content are extensively reviewed by Hofmann (1968), Smith (1973), Davenport (1982) and Wright *et al.* (1994). Major components of bile are water, bile pigment, bile acids, cholesterol, phospholipids, neutral fats, protein, and inorganic ions. Bile acids are conjugated with amino acids, glycine and taurine. Besides these two amino acids, other amino acids and small amounts of urea, uric acids and creatinine are also present at similar levels as those found in blood plasma. Bile also contains numerous metabolites arising from the metabolism of steroids. Glycoprotein makes up the largest percentage of the total bile protein. Albumin is the most abundant protein followed by transferrin, gamma globulin, ceruloplasmin, apolipoproteins, haptoglobin, secretary IgA, IgM, IgG, insulin, epithelial growth factor and cholecystokinin. Concentrations of protein in canine bile range from 6-40 mg per 100 ml (Wheeler, 1968)

1.4.5 Intestinal secretions

Burnner's gland, goblet cells, and crypts of Lieberkuhn, which are present in the intestinal mucosa, secrete mucus, amylase, lysozyme and brush border enzymes. Brush border enzymes include glycosidases, enterokinase, neutral endopeptidase, various aminopeptidases, dipeptidyl peptidase, various carboxypeptidases, glutamyl transpeptidase, membrane dipeptidase, folate conjugase, angiotensin converting enzyme, nucleotidase, nucleosidase and alkaline phosphatase (Rhodes, 1968; Malagelada, 1981; Davenport, 1982; Strombeck, 1996). Sucrase-isomaltase, trehalase, lactase-glycosyl-ceraminidase and glucoamylase-maltase are the most common brush border glycosidases

(Wright *et al.*, 1994). Except for enterokinase most of the duodenal enzymes are derived from desqaumated cells (Davenport, 1982).

The gastrointestinal mucosa has the most rapid turnover rate of any tissue in the mammalian body. The replacement time of the total mucosal cell population is estimated to be approximately 4-6 days in the dog (Johnson, 1981b). In humans it is estimated that 287 g of cells are lost in every 24 hours (Groft and Cotton, 1973), containing 180 g of nitrogen (Da Costa, 1971). Secretion of plasma proteins into the gastrointestinal tract has been extensively described by Jeffries and Sleisenger (1968).

From the above it is apparent that there is a large quantity of endogenous protein and other nitrogen-containing compounds secreted in the intestine of dogs. However, from a nutritional point of view the important implication in practise is the amount of endogenous nitrogen and amino acids remaining unabsorbed at the end of terminal ileum or at the end of the entire digestive tract. Several studies have been conducted in dogs pertaining to endogenous secretion. However, none of these studies allows estimation of endogenous protein and amino acids excreted at the terminal ileum or at the end of the entire digestive tract of adult dogs. Nasset and Ju (1961) fed adult jejunostomised dogs a diet containing C^{14} labelled casein and measured the percentage of endogenous nitrogen in total digesta collected at the jejunum at different times after feeding. In another approach Nasset *et al.* (1963) measured amino acid concentrations in digesta collected from jejunostomised dogs after feeding three types of diets (egg albumin, zein and protein-free diet). In these studies endogenous secretion was determined in jejunal digesta. Nasset *et al.* (1955) and Nasset and Rochester (1957), in similar studies, fed three groups of dogs either with egg albumin, zein or a protein-free diet, sacrificed the animals 1½ after feeding and measured nitrogen and amino acids present in different parts of the intestine. In the latter two studies, however, endogenous nitrogen secretions were measured 1½ hours after feeding. The data obtained in these studies, therefore, may not be accurate estimates of endogenous nitrogen remaining unabsorbed at the terminal ileum. Nevertheless, from studies in other animals (Butts *et al.*, 1991; Butts *et al.*, 1993; Rowan *et al.*, 1993; Hendriks *et al.*, 1996), it can be considered that a significant amount of endogenous secretions remained unabsorbed at the terminal ileum of dogs too.

1.5 INFERENCES FROM THE LITERATURE REVIEW

From the review of the literature it is logical to infer that accurate data on the digestibility of amino acids in feeds is needed for the optimal formulation of diet. Measuring amino acid digestibility at the faecal level is a relatively easy method. However, it has been shown that the amino acids in the hind gut are metabolised by microorganisms resulting in an inaccurate estimate of the digestibility of amino acids. In most species of monogastric animals there is no absorption of amino acids in the hind gut and the nitrogen absorbed from the hind gut is of no nutritional value to the animal. Consequently, it is accepted that measuring digestibility at the faecal level is misleading and measuring amino acid digestibility at the end of the ileum gives a more reliable estimate of the amino acids absorbed from the gut. However, the digesta collected at the terminal ileum contains, not only, unabsorbed dietary amino acids but also a significant amount of endogenous amino acids. To determine a true estimate of amino acid absorbed from the small intestine, corrections have to be made for endogenous excretions. With the removal of the effect of the confounding variable of endogenous excretion, the true digestibility coefficient can be determined, which is independent of assay condition. Overall, the true digestibility coefficients give more reliable estimates of the amino acids absorbed from a feedstuff. Different methods have been developed for the collection of digesta at the terminal ileum all with their unique advantages and disadvantages. High ethical and practical cost, however, hamper that many of these methods can not be used for the routine determination of amino acid digestibility in companion animal diets. A model animal, therefore, would be highly valuable as an alternative to studying digestion in the dog. The comparative anatomy and physiology of the rat and the dog offer much promise for the use of the rat as a model animal for studying digestion of the dog. However, before the latter can happen, the rat has to be validated.

CHAPTER 2

ENDOGENOUS AMINO ACID AND NITROGEN FLOW AT THE TERMINAL ILEUM AND AT THE END OF THE DIGESTIVE TRACT OF THE ADULT RAT AND DOG DETERMINED BY FEEDING A PROTEIN-FREE AND ENZYME HYDROLYSED CASEIN-BASED DIET

2.1 INTRODUCTION

Considerable amounts of protein, peptides, amino acids and other nitrogen-containing compounds are secreted or diffuse into the lumen of the gastrointestinal tract during the digestion of food (Fauconneau and Michel, 1970; Low, 1982). This nitrogen mainly originates from enzymes, mucoprotein, desquamated cells, plasma protein, free amino acids, amines and urea. The majority of these nitrogen-containing endogenous compounds are digested and reabsorbed but a smaller significant amount is excreted in the faeces.

The measurement of endogenous nitrogen and amino acids at the terminal ileum is of fundamental importance in nutritional science and provides a better understanding of aspects of digestive physiology. Endogenous gut amino acid losses, furthermore, are an important component in the factorial approach to calculate amino acid requirements. It is also used to correct apparent ileal digestibility coefficients to true ileal digestibility coefficients.

Endogenous amino acid losses from the gastrointestinal tract can be determined by collecting digesta at the terminal ileum or over the entire digestive tract. The method of choice will depend on the purpose for which the data is to be used. Traditionally endogenous losses of nitrogen and amino acids from the small intestine of animals have been determined following protein-free alimentation. This method has been criticised, however, due to the physiologically abnormal nature of the protein-free state (Low, 1980). Furthermore, Darragh *et al.* (1990) and Moughan and Rutherfurd (1990) showed that dietary protein or peptides in the gut stimulate endogenous secretions. Moreover,

bioactive peptides formed during the digestion of food have been isolated and may play a role in gut secretory process (Schlimme *et al.*, 1989). Nowadays the enzyme hydrolysed casein method seems to be a more appropriate approach to determine endogenous excretions in the gut.

Studies by Hendriks *et al.* (1996) show that in the cat, endogenous gut excretions of amino acids and nitrogen are higher than in other species such as the rat and the pig. There is little information available on nitrogen and amino acid excretions from the digestive tract of dogs.

The present study was undertaken to obtain information on endogenous excretions of nitrogen and amino acids from the gastrointestinal tract of adult dogs and to compare endogenous excretions of amino acids and nitrogen between the dog and the rat at the end of the ileum and over the entire digestive tract. Endogenous losses were determined by feeding a protein-free and an enzyme hydrolysed casein-based diet.

2.2 MATERIAL AND METHODS

2.2.1 Animals, housing and diets

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

2.2.2.1 Study 1

Two groups of five healthy adult dogs (three males and two females for each group) of mixed breeds with an initial body weight range of 19-36 kg (mean ± SEM, 26 ± 1.4 kg) were selected from a group of dogs at the Animal Health Services Centre (Jenners Mead Farm, Fielding, New Zealand). The dogs were housed individually outdoors, in concrete kennels. One group received an enzyme hydrolysed casein (EHC) based diet, while the other group was fed a protein-free (PF) diet (Table 2.1). Chromic oxide was included in both diets as an indigestible marker to allow calculation of digesta flows. Diets were formulated according to the nutritional requirements of adult dogs (NRC, 1985), with the exception of protein. Each day's total food allowance was given in ten equal portions, beginning hourly at 0800. At each meal time food was available for one hour. Fresh water was available at all times. The hourly feeding regime was employed in an attempt

to ensure a constant flow of digesta at the terminal ileum on the day of digesta sampling. After each meal, food intake was recorded to allow calculation of intake. The dogs were exercised daily outdoors for one hour. Care was taken to prevent animals from ingesting other material during the period of exercise. The dogs were weighed on the first and last day of the study. Faecal samples of dogs fed the PF diet were collected from the concrete floor one day before sampling of ileal digesta.

Table 2.1 Ingredient composition of the experimental diets

Ingredient	Protein-free	Enzyme hydrolysed casein
	(g/kg as is)	
EHC[1]	-	230.0
Starch[2]	470.0	240.0
Sucrose	200.0	200.0
Tallow[3]	200.0	200.0
Cellulose[4]	50.0	50.0
Vitamins/mineral mixture[5]	50.0	50.0
Soya bean oil	24.8	24.8
Choline chloride [6]	2.7	2.7
Chromic oxide	2.5	2.5

[1]New Zealand Pharmaceutical Ltd, Palmerston North, New Zealand.

[2]Glacier wheaten cornflour, N. B. Love Starches, NSW, Australia.

[3]Chelsea natural cane sugars, N.Z. Sugar Company Ltd., Auckland, New Zealand.

[4]Avicel microcrystalline cellulose, Asahi Chemical Industry Co. Ltd., Osaka, Japan.

[5]Unitech Industry Ltd, Auckland, New Zealand.

[6]Unitech Industry Ltd, Auckland, New Zealand (60 % Choline chloride).

On day ten, 4½ h after the start of the hourly feeding regime, the dogs were euthanased with an intravenous injection of pentobarbitone (0.5 ml/kg body weight, Chemstock Animal Health Limited, Christchurch, New Zealand). The body cavity was immediately opened and 20 cm of ileum anterior to the ileocaecal junction was dissected out. The outside of the dissected ileum was washed with distilled deionised water to remove any blood and hair and then carefully blotted using an absorbent paper towel.

The ileal content was gently flushed into a plastic bag using a syringe containing distilled deionised water. The samples were frozen (-20^0 C) immediately after collection and subsequently freeze-dried.

2.2.1.2 Study 2

Two groups of eight-week-old Sprague Dawley rats (three females and three males) were selected at random from the Small Animal Production Unit, Massey University, Palmerston North. The body weights of the female rats ranged from 207 to 244 g (mean ± SEM, 226 ± 5.9 g) and the body weights of male rats ranged from 350 to 430 g (mean ± SEM, 399 ± 37.5 g). The animals were kept individually in raised stainless steel cages with wire mesh floors at 22 ± 2^0 C and with a 12 h reverse light/dark cycle. During the period of darkness a low intensity lamp was used to provide minimal background lighting. Before the start of the study, the rats were fed hourly with a basal diet for 4 d. During the experimental period the two groups of rats were fed either the EHC-based diet or PF diet (Table 2.1). The diets were offered hourly between 0800 and 1700 for 10 min. Food intake was recorded after each meal. Water was available all the time. The animals were weighed at the start and end of the study. On day eight, 4½ h after the start of feeding, the animals were asphyxiated with carbon dioxide, decapitated and the digesta was collected from the final 20 cm of the ileum as described previously. A faecal sample was collected at d 7 of the study.

2.2.2 Chemical analysis

Freeze-dried samples were manually crushed and dehaired. Seven volumes of water were added to the ileal digesta collected from the animals fed EHC-based diet. The samples were centrifuged at 7000×g for 10 min at 4^0 C. The supernatant was decanted into Centriprep-10 tubes (Amicon, Beverly, MA) whereafter a small volume of water was added to the precipitate, which was then recentrifuged. The supernatant from the recentrifugation was transferred into the Centriprep-10 tube. The precipitate was stored (-20^0 C) and the supernatant in the Centriprep tubes was ultrafiltrated again according to the manufacturer's instruction. The high molecular weight fraction was added to the precipitate, which was then freeze-dried, ground and stored at -20^0 C until chemical analysis

The faecal and ileal samples from the animals fed the PF diet and the ultrafiltered ileal sample from the animals fed the EHC-based diet were analysed for dry matter, chromium, nitrogen and amino acids. Diet samples were analysed for dry matter, chromium and nitrogen.

Dry matter was determined in duplicate by drying samples at 105^0 C for 16 h. Chromium in diet (quadruplicates) and digesta samples (duplicates) was determined on an Instrumentation Laboratory Atomic Absorption Spectrophotometer (GBC 904, GBC Scientific Equipment Pty. Ltd., Dandenong, Victoria, Australia) using the method of Costigan and Ellis (1987). Total nitrogen was determined in duplicate using the Kjeldahl method. Protein (±5 mg sample) was hydrolysed using 1 ml of 6M glass-distilled HCl containing 0.1 % phenol for 24 h at 110 ± 2^0 C in glass tubes sealed under vacuum. Amino acids were determined in duplicate using ion exchanged HPLC system (Waters, Millipore, Milford, MA) employing postcolumn derivatisation with ninhydrin. Proline was detected at 440 nm while the other amino acids were detected at 570 nm. Tryptophan and cystine were not determined. No corrections were made for the loss of amino acids during hydrolysis, and amino acid weights were calculated using free amino acid molecular weights.

2.2.3 Data analysis

Endogenous flows of amino acids and nitrogen at the terminal ileum and at the end of the digestive tract, relating to the ingestion of 1 g of dry matter, were calculated using the following equation (units are μg):

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

The endogenous excretion data for nitrogen and each amino acid of the rat and the dog were tested for outliers using the Dixon method (Snedecor and Cochran, 1980). There were no outliers found. Then the endogenous excretion data for nitrogen and each amino acid were tested for homogeneity of variance using Bartlett'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 analysed using ANOVA in the Minitab package (Ryan and Joiner, 1994), with dietary treatment, species and the interaction between dietary treatment and species as variables.

2.3 RESULTS

All animals remained healthy throughout the trial period. Except for two dogs (one from each group), all dogs consumed all of their respective diet readily and hourly food intake was 100 %. After day six, all dogs consumed all the diet provided. Feed intake remained constant in both groups of rats after day two of the test period. The average food intake of the rats receiving the PF and the EHC-based diets were 14.7 ± 2.34 g/d and 16.3 ± 1.95 g/d, respectively. The rats and dogs fed the EHC-based diet gained weight (0.12 %/d and 0.13 %/d, respectively) whereas the rats and dogs fed the PF diet lost weight (0.87 %/d and 0.61 %/d, respectively). There was no evidence at slaughter that coprophagy had occurred in either animal species.

The mean endogenous amino acid and nitrogen excretion at the terminal ileum of the adult rats and dogs determined under the condition of PF feeding and peptide alimentation are given in Table 2.2. The most abundant amino acids in the endogenous excretion of both the rat and the dog were glutamic acid and aspartic acid followed by serine, leucine, proline, threonine, valine and isoleucine. A higher variation in endogenous excretion was found for EHC-fed animals compared to those fed the PF diet. The mean endogenous amino acid excretions of the dogs fed the PF diet were, on average, 1.8 times higher than the endogenous amino acid excretion of the rats fed the PF diet. Under the condition of peptide alimentation the dogs excreted on average 2.7 times higher endogenous amino acids compare to the rats. Endogenous amino acid excretions under the condition of peptide alimentation were higher than the corresponding protein-free values, with the dogs having, on average, 2.6 times higher excretion and the rats having, on average, 1.7 times higher excretion when the diet contained peptides.

The mean endogenous amino acid and nitrogen excretions as measured over the entire digestive tract of the adult rat and dog determined using the protein-free diet are given in the Table 2.3. In general in both species, endogenous excretions of nitrogen and amino acids were higher over the entire digestive tract than at the terminal ileum. The dogs excreted approximately twice the amount of endogenous amino acids over the entire digestive tract compare to the rats. In both species glutamic acid, aspartic acid, serine, leucine, proline, threonine, valine, isoleucine and glycine were the most abundant amino acids excreted at the end of the digestive tract.

Table 2.2 Mean (± SEM) endogenous amino acid and nitrogen excretion at the terminal ileum of the adult rat and dog fed either a protein-free (PF) or enzyme hydrolysed casein (EHC) based diet

Amino acid	Endogenous excretion			
	Rat (n=6)		Dog (n=5)	
	PF	EHC	PF	EHC
	(*μ g/g dry matter intake*)			
Aspartic acid	660 (75)	1095 (107)	960 (130)	2428 (890)
Threonine	450 (51)	659 (48)	1168 (189)	2015 (724)
Serine	407 (53)	887 (157)	925 (154)	3386 (1354)
Glutamic acid	696 (106)	1513 (246)	1089 (156)	5993 (2283)
Proline	442 (19)	691 (59)	643 (99)	1814 (656)
Glycine	513 (72)	435 (45)	600 (85)	1001 (362)
Alanine	269 (31)	474 (85)	515 (70)	951 (343)
Valine	273 (33)	599 (71)	536 (65)	1448 (551)
Methionine	75 (8)	157 (38)	119 (15)	323 (124)
Isoleucine	249 (34)	575 (86)	362 (56)	1192 (466)
Leucine	378 (50)	706 (111)	560 (60)	1180 (438)
Tyrosine	180 (20)	361 (49)	444 (39)	648 (218)
Phenylalanine	171 (20)	316 (60)	465 (52)	624 (222)
Histidine	136 (17)	218 (17)	268 (29)	700 (235)
Lysine	193 (24)	390 (69)	441 (50)	866 (287)
Arginine	159 (20)	328 (41)	370 (46)	728 (250)
Amino acid nitrogen	705 (78)	1227 (134)	1271 (167)	3329 (1217)
Nitrogen	1914 (56)	1631 (199)	2271 (303)	4117 (1425)

Table 2.3 Mean (±SEM) endogenous amino acid and nitrogen excretion at the end of the digestive tract of the adult rat and dog fed a protein-free diet

Amino acid	Endogenous excretion	
	Rat (n=6)	Dog (n=5)
	(μ g/g dry matter intake)	
Aspartic acid	900 (81)	1538 (309)
Threonine	488 (41)	1258 (347)
Serine	532 (44)	1086 (226)
Glutamic acid	1069 (94)	1859 (353)
Proline	450 (33)	679 (160)
Glycine	420 (32)	846 (174)
Alanine	525 (50)	961 (220)
Valine	485 (43)	854 (162)
Methionine	201 (20)	260 (50)
Isoleucine	446 (34)	661 (144)
Leucine	739 (69)	961 (198)
Tyrosine	383 (28)	604 (136)
Phenylalanine	345 (34)	660 (136)
Histidine	177 (16)	382 (77)
Lysine	431 (55)	972 (189)
Arginine	364 (51)	643 (131)
Amino acid nitrogen	1056 (89)	1926 (393)
Nitrogen	1689 (67)	3007 (600)

Table 2.4 shows the statistical significance of the effect of protein, animal and the interaction between protein and animal on endogenous amino acid and nitrogen excretion at the faecal and ileal level. There was no interaction ($P > 0.05$) between protein and species on the ileal endogenous excretion of any amino acid. Ileal endogenous excretion determined by the ultrafiltration method was significantly ($P < 0.05$) higher than those determined by the protein-free method for all amino acid, except for aspartic acid, threonine, glycine and phenylalanine. For total nitrogen, ileal endogenous excretions determined under the condition of peptide alimentation were not significantly ($P > 0.05$)

different from the PF method. Ileal endogenous excretions of nitrogen and most amino acids, with the exception of glycine, isoleucine, leucine and amino acid nitrogen, were significantly ($P < 0.05$) higher in dogs. Faecal endogenous losses were significantly ($P < 0.05$) higher in dogs than rats for threonine, serine, glycine, phenylalanine, histidine, lysine and total nitrogen.

Table 2.4 Statistical significance of ileal (protein, species and interaction between protein and species as variables) and faecal (species as variable) endogenous excretion of amino acids and nitrogen

	Ileal			Faecal
Amino acid	Protein	Species	Interaction[1]	Species
Aspartic acid	N.S.	*	N.S.	N.S.
Threonine	N.S.	***	N.S.	*
Serine	**	***	N.S.	*
Glutamic acid	***	**	N.S.	N.S.
Proline	**	*	N.S.	N.S.
Glycine	N.S.	N.S.	N.S.	*
Alanine	*	*	N.S.	N.S.
Valine	**	**	N.S.	N.S.
Methionine	**	*	N.S.	N.S.
Isoleucine	**	N.S.	N.S.	N.S.
Leucine	*	N.S.	N.S.	N.S.
Tyrosine	*	**	N.S.	N.S.
Phenylalanine	N.S.	**	N.S.	*
Histidine	**	***	N.S.	*
Lysine	*	**	N.S.	**
Arginine	**	**	N.S.	N.S.
Amino acid nitrogen	N.S.	N.S.	N.S.	N.S.
Total nitrogen	N.S.	*	*	*

N.S. = Non significant, * = P< 0.05, ** = P< 0.01, *** = P< 0.001

[1]between protein and species

2.4 DISCUSSION

The 4½ h sampling time and 20 cm length of terminal ileum was considered optimal for the determination of endogenous excretions, based on studies by Skilton *et al.* (1988) and Donkoh *et al.* (1995). In this study the dogs used for the determination of endogenous amino acid flows were of a wide range of body weights and variety of breeds. Kendall *et al.* (1982), however, showed that endogenous nitrogen losses are not affected by body weight or breed of dog and, therefore, the effect of breed or body weight on endogenous excretions can be expected to be minimal.

The endogenous ileal amino acid excretion of the rat in the present study determined by feeding the PF and EHC-based diet closely agreed with values reported in other studies (Skilton *et al.*, 1988; Darragh *et al.*, 1990; Moughan and Rutherfurd, 1990; Butts *et al.*, 1991; Donkoh *et al.*, 1995). There are no previous data regarding ileal endogenous excretion in dog. Kendall *et al.* (1982) and Meyer *et al.* (1987) reported faecal nitrogen excretion under the condition of protein-free feeding in adult dogs of 63 and 54 mg/kg$^{0.75}$/d, respectively. In the present study faecal nitrogen excretions under the condition of protein-free feeding in dogs was 73 mg/kg$^{0.75}$/d. The endogenous ileal nitrogen and amino acid excretions in the dogs in the present study were higher than those of the rats. Hendriks *et al.* (1996) found endogenous ileal amino acid excretions in cats (carnivore) which were similar in magnitude to those found for the dogs in the present study. In general it seems that carnivores (cat and dog) have higher endogenous excretion of amino acids at the end of the ileum than omnivores (rats and pigs) when similar diets are fed, as endogenous ileal amino acid excretion in pigs have been shown to be similar to values found for rats (Butts *et al.*, 1991; Butts *et al.*, 1993).

The present results show a higher variation in endogenous ileal nitrogen and amino acid excretions obtained with the ultrafiltration method than with the protein-free method. Endogenous ileal nitrogen and amino acid excretions in the rats and the dogs were elevated due to the inclusion of peptides in the diet. Latter findings have also been reported to occur in pigs, humans and cats (Butts *et al.*, 1993; Rowan *et al.*, 1993; Hendriks *et al.*, 1996). The present study presents evidence that the dog is comparable to other animals with respect to increasing gut endogenous nitrogen and amino acid excretions in the presence of dietary protein. Furthermore, the endogenous ileal nitrogen excretion determined by the ultrafiltration method was not significantly different from

those reported by the protein-free method, in the rat and dog. A possible reason for this is that small nitrogen-containing compounds, like ammonia, creatine and urea, are removed during the ultrafiltration process whereas these compounds will be present in the digesta of animals fed a protein-free diet. In the present study, the endogenous ileal nitrogen excretion determined by the protein-free method in the rat was higher than the corresponding values found with the ultrafiltration method. Amino acid nitrogen in the rats fed the EHC-based diet was higher and in line with the increase in endogenous excretion of amino acids normally seen between rats fed a protein-free and EHC-based diet. A possible explanation for the lower endogenous nitrogen excretion of the rats fed the EHC-based diet is that the proportion of small nitrogen-containing compounds removed during the ultrafiltration of the digesta may have been higher.

Generally the endogenous nitrogen and amino acid excretions as measured over the entire digestive tract in the rats and the dogs were higher than the values determined at the terminal ileum. This correspond with the result of Hendriks *et al.* (1996) who made a similar observation in cats. However, in the rat, endogenous nitrogen excretion over the entire digestive tract was lower than the value determined at the terminal ileum. Nitrogen-containing compounds enter the lumen of the large intestine with both the ileal chyme and via the wall of the large intestine in the form of mucus, desquamated cells, urea and other compounds. Qualitatively, mucous protein predominates. Urea appears to be split by bacterial ureases in the mucous coating of the large intestine and only some of the ammonia released enters the lumen of the large intestine, while the remainder seems to diffuse back directly into the bloodstream (Meyer *et al.,* 1987). However, in the large intestine there will be a net catabolism of nitrogen-containing compounds by the proteolytic enzymes of microbial origin. Because of a reduction in acidity, and possibly also because of bacterial breakdown, the activity of endogenous protease from the upper part of the digestive tract will be low in the large intestine. At the same time, nitrogen is absorbed in the large intestine mainly in the form of ammonia and in the adult animals there is no absorption of amino acids through the large intestinal mucosa (Binder, 1970; Schmitz *et al.,* 1991; Darragh *et al.,* 1994). Net nitrogen absorption in the large intestine depends primarily on the level of inflow, but it is also modified by other substances in the food (Meyer *et al.,* 1987). The latter authors also suggest that the absorption decreases or there may be even a net nitrogen secretion in the presence of larger amounts of substances which can easily be broken down by bacteria. Ultimately, the amount of nitrogen fixation

by bacteria, the amount of ammonia absorption, the amount of mucus production and rate of passage of the food determines the amount of faecal nitrogen loss.

Aspartic acid, glutamic acid, threonine, serine, proline and glycine were the predominant endogenous amino acids measured at the terminal ileum and over the entire digestive tract, as determined by either the protein-free or ultrafiltration method, in both the rats and the dogs. These amino acids constitute a large proportion of mucus glycoprotein (Bella and Kim, 1972; Cetta *et al.,* 1972). Furthermore, aspartic acid, glutamic acid and serine are present in a relatively high proportion in pancreatic and intestinal secretions (Corring and Jung, 1972: Buruczewska, 1979), and glycine is the predominant amino acid in bile secretion (Weiner and Lack, 1968; Smith, 1973).

In the present study, except for glycine and proline, the pattern of amino acid excretion in the rats and the dogs under the condition of PF-feeding and peptide alimentation was similar and in accordance with other studies in rats, pigs, cats and humans (Skilton *et al.,* 1988; Darragh *et al.,* 1990; Moughan and Rutherfurd, 1990; Butts *et al.,* 1991; Butts *et al.,* 1993; Rowan *et al.,* 1993; Donkoh *et al.,* 1995; Hendriks *et al.,* 1996). Endogenous excretion of proline is often found to be highly variable between studies (Taverner *et al.,* 1981; Skilton *et al.,* 1988; De Lange *et al.,* 1989; Moughan and Rutherfurd, 1990; Butts *et al.,* 1991; Hendriks *et al.,* 1996). De Lange *et al.* (1989) cited evidence for the finding of higher endogenous proline excretions in pigs under the condition of protein-free feeding. These authors hypothesised that the endogenous excretion of proline is variable due to large quantities of glutamine from muscle breakdown entering the intestine. The latter phenomenon, however, varies with the animals' previous body status and the duration of the experimental period. Hendriks *et al.* (1996) found that in the cat, the proline level is not elevated under the condition of protein-free feeding, because in the cat the activity of pyrroline-5-carboxylate synthase, which is required for the conversion of glutamine to proline in the small intestine, is low. The findings of non elevated proline excretion under the condition of protein-free feeding in the cat is line with the present finding in dogs, because in the dog the activity of pyrroline-5-carboxylate synthase, although higher than in cats, is lower than omnivorous animals (Burns and Milner, 1981; Baker, 1991; Morris, 1994).

There was no interaction between protein and animal on ileal endogenous amino acid and nitrogen excretion in the present study. The latter finding indicates that the dog

reacts similarly to the inclusion of peptides in the diet as the rat, namely by increasing endogenous excretion of amino acids.

The present study provides values for endogenous amino acid and nitrogen excretion at the faecal and ileal level of the rat and the dog under the condition of protein-free and peptide alimentation. The values determined by the protein-free method were lower than those determined under the condition of peptide alimentation, indicating that the protein-free method underestimates gut endogenous amino acid and nitrogen excretions. It was, furthermore, found that dogs have higher endogenous amino acid and nitrogen excretions than rats but are similar to amounts of endogenous excretion seen in cats. In general, the pattern of endogenous amino acid excretions in the dog and the rat are similar. The present data can be used to correct apparent digestibility coefficients of amino acids to true values and allow comparison of the suitability of the rat as a model animal for the digestion of protein in the dog.

CHAPTER 3

COMPARISON OF THE AMINO ACID DIGESTIBILITY OF A COMMERCIAL DRY DOG FOOD BETWEEN THE ADULT RAT AND DOG

3.1 INTRODUCTION

Digestibility is a measure of the proportion of an ingested nutrient which has been absorbed. Accurate data on the digestibility of amino acids in feeds is required to determine if the diet meets the animal's requirement for, among others, individuals amino acids. Digestibility can be measured relatively easily by collection of faeces. However, this approach has been criticised after the establishment of the extent of microbial activity in the large intestine (McNeil, 1988). Nowadays it is generally accepted that measuring amino acid digestibilities at the terminal ileum gives a more reliable estimate of the amount of amino acids absorbed by the animal, particularly if the diet contains protein of low quality (Moughan and Donkoh, 1991). But at the same time, other than the dietary unabsorbed amino acids, considerable amounts of endogenous amino acids are present in the digesta collected at the terminal ileum. So, measuring the flow of amino acids at the terminal ileum is not a true measure of the uptake of amino acids from the diet. Furthermore, apparent digestibility has been shown to increase with increasing protein level in the diet as a result of endogenous protein, which when expressed to the ingestion of dry matter intake, remains constant at the end of the ileum (Sauer *et al.*, 1980; Furuya and Kaji, 1989; Donkoh, 1993). On the other hand, the true digestibility assay is not only independent of assay methodology (Furuya and Kaji, 1989; Donkoh, 1993) but also true digestibility values are more additive than the apparent digestibility values (Furuya and Kaji, 1991). Collectively, the true digestibility value is the fundamental property of the diet and it should be more accurate in detecting the digestibility of various protein sources in a feedstuff. However, the technical problem in the determination of endogenous excretion leads some authors to use the apparent digestibility values.

Finding true or apparent ileal digestibility values for a feed or feed ingredient, however, is an invasive technique. Practical and ethical reasons hamper the use of this

method in routine determination of protein digestibility in companion animal diets. A model animal, therefore, may provide a useful alternative to accurately predict the protein and amino acid digestibility in diets for companion animals.

The aim of the present study was to compare the true digestibility of amino acids and nitrogen, and the apparent digestibility of amino acids, nitrogen, dry matter and organic matter, of a dog food at the faecal and ileal level for the adult rat and dog.

3.2 MATERIAL AND METHODS

3.2.1 Animals, housing and diet

The following study was approved by the Massey University Animal Ethics Committee. Five healthy adult dogs (three females and two males) of mixed breed with an initial body weight range of 15-25 kg (mean ± SEM, 20 ± 2.4 kg) were selected from a group of dogs from the Animal Health Services Centre, at Jenners Mead Farm (Fielding, New Zealand). A group of eight-week-old Sprague Dawley rats (three females and three males) were also selected at random from the Small Animal Production Unit, Massey University, (Palmerston North, New Zealand). The body weight of the female rats ranged from 210 to 234 g (mean ± SEM, 226 ± 7.6 g) and the male rats ranged from 378 to 434 g (mean ± SEM, 408 ± 16.4 g). The dogs were housed individually outdoors, in concrete kennels. The rats were kept individually in raised stainless steel cages with wire mesh floors at 22 $\pm 2^0$ C and with a 12 h reverse light or dark cycle, the period of light was from 1800 to 0600 h. The dogs and rats were fed a commercial available dry dog food (Table 3.1) with added chromic oxide to allow calculation of digesta flows. The dogs were fed according to their daily energy requirements (132 kcal/$kg^{0.75}$ body weight; NRC, 1985). Each day's total food allowance was given in 10 equal portions, beginning hourly at 0800 h. The rats were fed hourly between 0800 and 1700 h with the food available at each mealtime for 10 min. Fresh water was available to the dogs and rats at all times. The hourly feeding regime was employed in an attempt to ensure a constant flow of digesta at the terminal ileum on the day of digesta sampling. After each meal, feed intake was recorded. The dogs were weighed on the day one and nine of the study. The rats were weight on day one, four and seven of the experiment. The dogs were exercised outdoors for one hour

each day. Care was taken to prevent the dogs from consuming other material during this time.

Table 3.1 Ingredient composition of the dog food

Ingredient	Amount
	(g/kg dry matter)
Crude protein	266
Ash	50.6
Aspartic acid	20.2
Threonine	8.5
Serine	9.8
Glutamic acid	32.7
Proline	13.9
Glycine	14.6
Alanine	13.0
Valine	9.9
Methionine	3.8
Isoleucine	8.3
Leucine	17.4
Tyrosine	7.5
Phenylalanine	9.3
Histidine	5.5
Lysine	11.2
Arginine	28.1

On day 10, 4½ h after the start of hourly feeding, the dogs were euthanased with an intravenous injection of pentobarbitone (0.5ml/kg body weight, Chemstock Animal Health Limited, Christchurch). On day 8 the rats were asphyxiated with carbon dioxide and decapitated. The body cavity of the rats and dogs was opened and 20 cm of ileum immediately anterior to the ileocaecal junction was dissected out. The outside of the dissected ileum was washed with distilled deionised water to remove any blood and hair and then carefully blotted using an absorbent paper towel. The ileal contents were gently

flushed out with distilled deionised water into a plastic bag, using a syringe. The samples were frozen (-20^0 C) immediately after collection and subsequently freeze-dried. A faecal sample of each rat and dog was collected on the day before the sampling of ileal digesta.

3.2.2 Chemical analysis

Prior to analysis, the freeze-dried faecal and ileal samples were ground and manually dehaired. The faecal and ileal samples and the diet were subjected to analysis of dry matter, organic matter, chromium, nitrogen and amino acids.

Dry matter was determined in duplicate by drying samples at 105^0 C for 16 h, while organic matter was determined by heating the samples at 550^0 C for 16 h. Chromium content of quadruplicate diet samples and duplicate ileal and faecal samples was determined on an Instrumentation Laboratory Atomic Absorption Spectrophotometer (GBC 904, GBC Scientific Equipment Pty. Ltd., Dandenong, Victoria, Australia) using the method of Costigan and Ellis (1987). Total nitrogen was determined in duplicate using the Kjeldahl method. Duplicate protein (±5 mg) samples were hydrolysed in 1 ml of 6M glass-distilled HCl containing 0.1 % phenol for 24 h at 110 ± 2^0 C in glass tubes sealed under vacuum. Amino acids were determined using ion exchanged HPLC system (Waters, Millipore, Milford, MA) employing postcolumn derivatisation with ninhydrin. Proline was detected at 440 nm while other amino acids were detected at 570 nm. Cystine and tryptophan were not determined. No correction was made for loss of amino acids during hydrolysis, and amino acids weights were calculated using free amino acids molecular weights.

3.2.3 Data analysis

The apparent ileal and faecal digestibility of nutrients (amino acids, nitrogen, dry matter and organic matter) were calculated from the dietary ratio of nutrients to the corresponding ratio in the ileal flow or faecal flow, respectively using the following equation (units are mg/g dry matter):

$$\text{Apparent nutrient digestibility} = \frac{\text{Nutrient in the diet} - \text{Nutrient in the digesta}}{\text{Nutrient in the diet}}$$

Nutrient in the digesta were calculated using the following equation (units are mg/g dry matter):

$$\text{Nutrient in the digesta} = \text{Nutrient concentration in the digesta} \times \frac{\text{Diet chromium}}{\text{Digesta chromium}}$$

True ileal digestibility of amino acids and nitrogen were calculated using the following equation (units are mg/g dry matter):

$$\text{True amino acid or nitrogen (AA/N) digestibility} = \frac{\text{(AA/N) in the diet} - \text{(AA/N) in the digesta} - \text{Endogenous (AA/N))}}{\text{(AA/N) in the diet}}$$

The endogenous ileal amino acid and nitrogen flows that determined by feeding a protein-free diet were used to correct the ileal apparent digestibilities. Digestibility data of the rat and the dog were tested for outliers using the Dixon method (Snedecor and Cochran, 1980). There were no outliers found. Then digestibility values were tested for homogeneity of variance using Bartlett's test (Snedecor and Cochran, 1980) and found to be homogenous. The data were then analysed using ANOVA using Minitab (Ryan and Joiner, 1994). Regression analysis was performed between ileal amino acid digestibility values of the rats and the dogs using the least square method.

3.3 RESULTS

All the animals remained healthy throughout the trial. The dogs consumed all their hourly food allowance readily. The food intake of the rats stabilised at day two of the study. The average food intake of the rats was 18.7 ± 3.47 g/d and the rats gained weight (0.20 %/d), whereas the weight of the dogs decreased slightly (0.09 %/d). No signs of coprophagy were observed in either species.

Table 3.2 shows the mean apparent ileal and faecal digestibility of amino acids, nitrogen, dry matter and organic matter in the rats and the dogs fed the dry dog food. The apparent ileal digestibility values of the nutrients measured were higher in the dogs whereas the apparent faecal digestibility values were higher in the rat. The highest and lowest digestibility coefficients were found for arginine and aspartic acid, respectively in both species. Generally the basic amino acids (histidine, lysine and arginine) were digested more efficiently than the acidic and neutral amino acids in both the dog and the

rat. Table 3.2, furthermore, shows that amino acid nitrogen had a higher digestibility value than total nitrogen. In general the essential amino acids were digested more efficiently than total nitrogen.

Table 3.2 Mean (±SEM) apparent ileal and faecal amino acid, nitrogen, dry matter and organic matter digestibility coefficients in rats and dogs fed a commercial dry dog food

Amino acid	Apparent digestibility coefficient			
	Rat (n=6)		Dog (n=5)	
	Ileal	Faecal	Ileal	Faecal
	(%)			
Aspartic acid	66.5 (1.75)	84.2 (0.51)	74.2 (1.78)	82.1 (0.70)
Threonine	71.2 (1.22)	83.0 (0.06)	73.5 (2.37)	80.1 (1.00)
Serine	72.3 (1.43)	85.0 (0.59)	77.1 (2.30)	82.9 (0.83)
Glutamic acid	80.5 (0.87)	89.2 (0.39)	85.3 (1.08)	87.5 (0.58)
Proline	70.9 (1.67)	89.6 (0.70)	81.8 (1.23)	87.2 (0.68)
Glycine	71.6 (1.06)	89.2 (0.37)	78.6 (1.46)	84.7 (0.61)
Alanine	77.3 (1.18)	84.6 (0.51)	84.4 (1.12)	84.8 (0.73)
Valine	76.2 (1.08)	82.7 (0.54)	81.2 (1.52)	81.9 (0.78)
Methionine	79.0 (1.39)	80.0 (0.69)	86.3 (1.04)	82.7(0.61)
Isoleucine	75.8 (1.47)	79.4 (0.56)	84.1 (1.19)	83.2 (0.73)
Leucine	79.8 (1.17)	83.9 (0.47)	85.4 (1.11)	86.3 (0.70)
Tyrosine	80.9 (0.80)	84.6 (0.55)	85.2 (1.50)	84.7 (0.72)
Phenylalanine	83.0 (1.69)	86.1 (0.37)	86.8 (1.04)	85.7 (0.69)
Histidine	81.7 (1.03)	90.5 (0.22)	80.9 (1.38)	80.2 (4.91)
Lysine	82.2 (0.83)	86.6 (0.31)	83.6 (1.37)	78.7 (3.53)
Arginine	88.0 (0.41)	91.6 (0.29)	89.3 (0.91)	84.6 (5.51)
Amino acid nitrogen	77.3 (1.11)	87.4 (0.51)	83.9 (1.50)	85.9 (0.63)
Nitrogen	75.0 (0.84)	84.5 (0.33)	76.9 (1.17)	85.4 (0.41)
Dry matter	75.8 (0.72)	86.8 (0.16)	78.7 (1.22)	86.8 (0.16)
Organic matter	76.1 (0.77)	88.0 (0.18)	81.8 (1.11)	85.4 (0.32)

The statistical significance of the mean difference in the apparent digestibility values as measured at the terminal ileum and at the faecal level in the dogs are shown in Table 3.3. The apparent faecal digestibility value of aspartic acid, threonine, serine, proline, glycine, dry matter, organic matter, and total nitrogen was significantly ($P < 0.05$) higher, while the apparent ileal digestibility value of methionine was significantly ($P < 0.05$) lower than the corresponding apparent faecal digestibility value.

Table 3.3 Statistical significance between apparent ileal and faecal digestibility of amino acids, dry matter, organic matter and nitrogen of dogs fed a commercial dry dog food

Aspartic acid	**	Leucine	N.S.
Threonine	*	Tyrosine	N.S.
Serine	*	Phenylalanine	N.S.
Glutamic acid	N.S.	Histidine	N.S.
Proline	**	Lysine	N.S.
Glycine	**	Arginine	N.S.
Alanine	N.S.	Amino acid nitrogen	N.S.
Valine	N.S.	Nitrogen	***
Methionine	**	Dry matter	**
Isoleucine	N.S.	Organic matter	*

N.S. = Non significant, * = P< 0.05, ** = P< 0.01, *** = P< 0.001.

Table 3.4 shows that the apparent ileal digestibility values of aspartic acid, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, amino acid nitrogen and organic matter were significantly ($P < 0.05$) different between the rat and the dog.

Table 3.4 Statistical significance of the apparent ileal digestibility of amino acids, dry matter, organic matter and nitrogen between the rat and the dog fed a commercial dry dog food

Aspartic acid	*	Leucine	**
Threonine	N.S.	Tyrosine	*
Serine	N.S.	Phenylalanine	N.S.
Glutamic acid	**	Histidine	N.S.
Proline	***	Lysine	N.S.
Glycine	**	Arginine	N.S.
Alanine	**	Amino acid nitrogen	**
Valine	*	Nitrogen	N.S.
Methionine	**	Dry matter	N.S.
Isoleucine	**	Organic matter	**

N.S. = Non significant, * = P< 0.05, ** = P< 0.01, *** = P< 0.001.

The mean true ileal amino acid and nitrogen digestibility coefficients in the rat and the dog and the statistical significance of the true digestibility coefficients between the rat and the dog are given in Table 3.5. The mean true ileal digestibility values of nitrogen and all the amino acids, with the exception of histidine and lysine, were significantly ($P < 0.05$) higher in the dogs than the rats. Furthermore, similar to the apparent digestibility values the highest and lowest true digestibility values were found for arginine and aspartic acid, respectively

There was a significant ($P < 0.001$) linear regression between the rat apparent and true ileal digestibilities of amino acid and those of the dog. The following regression equation was obtained for the apparent digestibility: $Y = 0.32 (\pm 0.095) + 0.65 (\pm 0.122) X$, ($R^2 = 67$ %), where Y = apparent ileal digestibility coefficient in dogs and X = apparent ileal digestibility coefficient in rats. For the true ileal digestibility, the following regression equation was obtained $Y = 0.45 (\pm 0.090) + 0.53 (\pm 0.112) X$, ($R^2 = 61$ %), where Y = true ileal digestibility coefficient in dogs and X = true ileal digestibility coefficient in rats. The intercept and the slope of both equations were significant at $P < 0.001$.

Table 3.5 Mean (±SEM) true ileal amino acid and nitrogen digestibility coefficients in the rat and the dog fed a commercial dry dog food

Amino acid	Digestibility coefficient		Level of significance
	Rat (n=6)	Dog (n=5)	
	(%)		
Aspartic acid	69.6 (1.75)	78.6 (1.78)	**
Threonine	76.1 (1.22)	86.2 (2.37)	**
Serine	76.2 (1.43)	88.9 (2.30)	**
Glutamic acid	82.5 (0.87)	88.4 (1.08)	**
Proline	73.9 (1.67)	86.1 (1.23)	***
Glycine	74.9 (1.06)	82.5 (1.46)	**
Alanine	79.2 (1.18)	88.1 (1.12)	***
Valine	78.8 (1.08)	86.2 (1.52)	**
Methionine	80.8 (1.39)	89.3 (1.04)	***
Isoleucine	78.6 (1.47)	88.2 (1.19)	***
Leucine	81.9 (1.17)	88.4 (1.11)	**
Tyrosine	83.1 (0.80)	90.7 (1.50)	***
Phenylalanine	84.8 (1.69)	91.1 (1.04)	*
Histidine	84.0 (1.03)	84.6 (1.44)	N.S.
Lysine	83.8 (0.83)	86.5 (1.43)	N.S.
Arginine	89.0 (0.41)	91.2 (0.95)	*
Amino acid nitrogen	79.6 (1.11)	87.4 (1.57)	**
Nitrogen	79.9 (0.84)	82.4 (1.17)	*

N.S. = Non significant * = P< 0.05, ** = P< 0.01, *** = P< 0.001

Table 3.6 presents the actual ileal amino acid digestibility values of the dog and the predicted values using the respective regression equation. Figure 3.1 and 3.2 present the apparent and true ileal digestibility of a commercial dry dog food in the rat and the dog and the linear regression equations, respectively.

Table 3.6 Actual apparent and true ileal digestibility values of amino acids of a dog food in adult dogs and predicted values using the laboratory rat

Amino acid	Digestibility value					
	Apparent			True		
	Actual	Predicted[1]	Difference	Actual	Predicted[2]	Difference
	(%)					
Aspartic acid	74.2	75.3	1.1-	78.6	81.6	3.0
Threonine	73.5	78.3	4.8	86.2	85.0	-1.2
Serine	77.1	79.0	1.9	85.8	85.1	-0.7
Glutamic acid	85.3	84.4	-0.9	88.4	88.4	0.0
Proline	81.8	78.1	-3.7	86.1	83.9	-2.2
Glycine	78.7	78.6	-0.1	82.5	84.4	1.9
Alanine	84.4	82.2	-2.2	88.1	86.7	-1.4
Valine	81.2	81.6	0.4	86.2	86.5	0.3
Methionine	86.4	83.3	-3.1	89.3	87.5	-1.8
Isoleucine	84.1	81.3	-2.8	88.2	86.4	-1.8
Leucine	85.5	83.9	-1.6	88.5	88.1	-0.4
Tyrosine	85.3	84.6	-0.7	90.6	88.7	-1.9
Phenylalanine	86.9	86.0	-0.9	91.5	89.6	-1.9
Histidine	81.0	85.1	4.1	84.6	89.2	4.6
Lysine	83.6	85.4	1.8	86.5	89.1	2.6
Arginine	89.3	89.2	0.1	91.3	91.9	0.6
Amino acid nitrogen	84.0	82.2	-1.8	87.4	86.9	-0.5

[1]Using regression equation $Y = 0.32 + 0.65\ X$.

[2]Using regression equation $Y = 0.45 + 0.53\ X$.

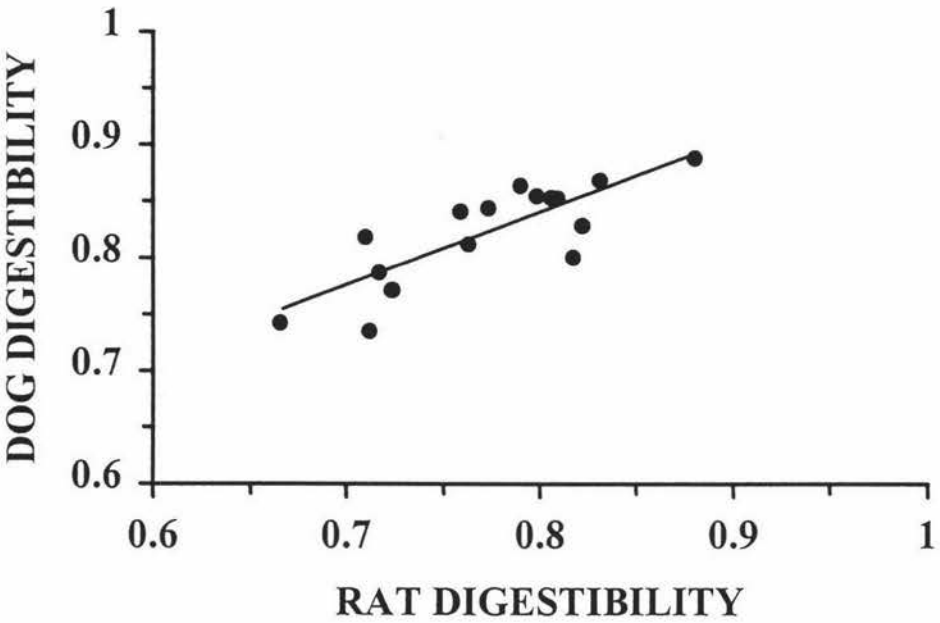

Figure 3.1 Apparent ileal digestibility of a commercial dry dog food in the rat and the dog and the linear regression equation.

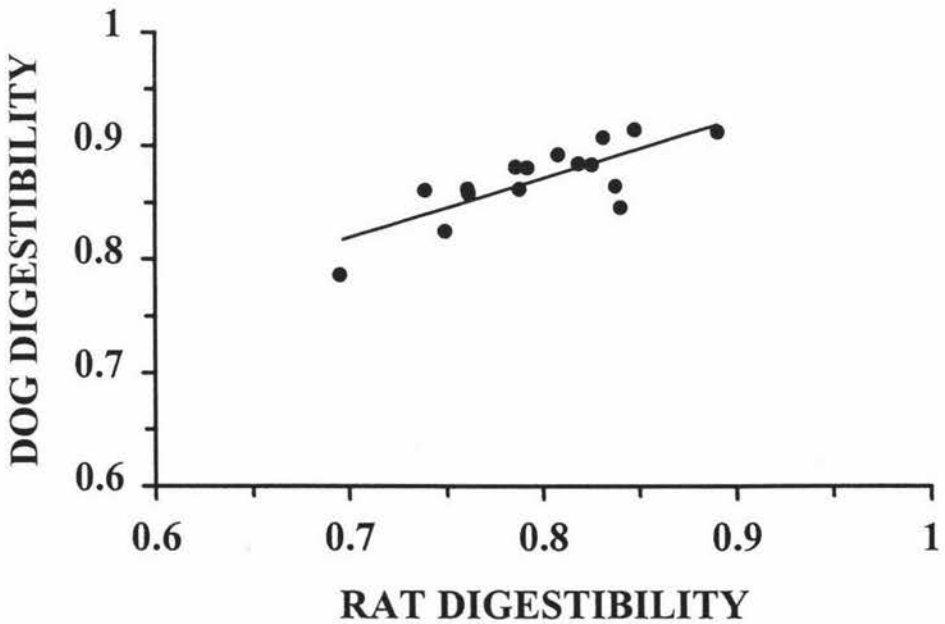

Figure 3.2 True ileal digestibility of a commercial dry dog food in the rat and the dog and the linear regression equation.

3.4 DISCUSSION

To enable a realistic comparison of the digestibility values of nutrients between two species, the experimental conditions must be well defined. The rat and the dog seem to have similarities in their anatomy and physiology of the digestive tract (Chapter 1.3) as well as their nutrient requirements (NRC, 1978 and 1985). The rats and the dogs used in the present study to compare the digestibility of amino acids in a dry dog food were adult animals. In adult animals digestive enzymes have reached a constant maximal level (Cranwell, 1995). Furthermore, similar conditions were maintained for both species throughout the experiment. Time and pattern of feeding, the method of ileal digesta collection employed, time of digesta collection after the start of the hourly feeding regime and the site of digesta collection were kept the same in both species to prevent any possible effect on digestibility. A desirable objective regarding the slaughter technique is to maintain a uniform flow of digesta so that a representative sample can be obtained. To ensure a constant flow of digesta, a frequent feeding regime was adopted in the present study. Digesta was collected in a predetermined optimal time based on previous studies in rats (Skilton *et al.*, 1988; Donkoh *et al.*, 1995). One difference between the dogs and the rats in the present study was that the dogs were fed according to their maintenance energy requirements while the feed intake of the rats was slightly higher than maintenance requirement (as apparent from the 0.20 % body weight gain per day). Nevertheless, it has been shown in other species that dry matter intake has no effect on the digestibility of amino acids (Hayden *et al.*, 1984; Van Leewen *et al.*, 1987; Sauer *et al.*, 1989). The dogs in the present study had a wide body weight range and were of a variety of breeds. James and McCay (1950) showed that protein and dry matter digestibility in dogs does not depend on breed and, therefore, the effect of breed on the digestibility of amino acids in the present study can be expected to be minimal.

Limited data are available on the digestibility of individual amino acids in the dog. Although different diets were fed, the apparent faecal digestibility of dry matter and protein of the dog food in the present study is line with digestibility values recorded for dogs by James and McCay (1950), Hegsted *et al.* (1980) and Burrows *et al.* (1982). There are no data in the literature to compare the true amino acid digestibility values of a dog food.

In the present study the endogenous excretion determined by feeding a PF diet was used to correct the apparent digestibility values to true values. The true digestibility values of some amino acids were over 100 % when the endogenous excretion determined by the ultrafiltration method was used for the correction of apparent digestibility. This may be due to the high variation in endogenous flow determined by the ultrafiltration method and the limited number of dog used in the study.

Table 3.3 shows that in the dogs the faecal digestibility values of dry matter, organic matter, total nitrogen, aspartic acid, threonine, serine, proline and glycine were significantly higher than the corresponding ileal digestibility values. For methionine, however, the ileal digestibility was significantly higher than the faecal digestibility. The digestibility of nitrogen was significantly different between sites with amino acid nitrogen not being significantly different between sites. Amino acid nitrogen digestibility is the result of the digestibility of individual amino acids and, because methionine and lysine digestibility decreased while the digestibility of all other amino acids increased, the digestibility of amino acid nitrogen was not significantly different between the two sites of measurement. Although the dog does not have a well developed large intestine, the microbial population in the large intestine causes the breakdown of unabsorbed amino acids entering from the ileum as is seen in other species such as the rat, pig and human (Moughan *et al.*, 1984; Sauer and Ozimek, 1986; Skilton *et al.*, 1991; Rowan *et al.*, 1994). Due to the microbial action there is often a net gain of methionine and lysine in the large intestine while other amino acids seem to disappear (Low, 1980; Just, 1980; Sauer and Ozimek, 1986; Moughan, 1991). The present study provides evidence that the faecal digestibility index, as in the case of other species, is an inaccurate method for the estimation of amino acids absorbed from the gut in the dog. Consequently the ileal digestibility method more accurately estimates the amount of amino acids absorbed from the gut of dogs.

Table 3.4 shows that for most of the amino acids the apparent ileal digestibility values were significantly ($P < 0.05$) higher in the dogs. This finding is in line with that of Ablstrom and Skrede (1997) who also obtained a higher digestibility values for protein, carbohydrate and fat in the dog compared to the rat. The latter authors, however, did not report whether ileal or faecal digestibilities were measured. The significant difference in apparent ileal digestibility, however, may have been caused by the difference in endogenous excretion. The latter was shown to be different between the two species in

Chapter 2. However, from Table 3.5, it is evident that for most amino acids, especially for the essential amino acids, true ileal digestibility values were higher in dogs than rats.

As the ileal digestibility values of several amino acids were significantly different between the two species, a linear model was fitted to the amino acid digestibility data of the rat and dog. There was a significant linear relationship between the apparent and true ileal digestibility of amino acid between the dog and the rat. The linear models explained 67 % and 61 % of the variation in the dogs' apparent ileal and true ileal digestibility values, respectively. Table 3.6 presents the actual and predicted apparent and true ileal digestibility values using the linear equations. As can be seen from Table 3.6, the true ileal digestibility of amino acids of the dog can be obtained within 2 % units from the ileal digestibility values of the rat. The difference between the actual and predicted true ileal digestibility of the dog exceed 2 % units only for aspartic acid, proline, histidine, and lysine.

In conclusion, the dogs' apparent and true ileal digestibility values can be predicted from respective values of rats using linear regression equations. However, it must be mentioned that the linear models presented in the present study explains 61 % of the variation in true ileal digestibility values and 67 % of the variation in the apparent ileal digestibility values of the dog. The remaining 39 % and 33 % of the variation may be attributed to other factors, which have to be explored in further studies. Furthermore, the present study only investigated one dog food. To make a more general conclusion about the suitability of the rat as a model animal for the digestion of protein in the dog, further studies including a wider range of dog foods need to be investigated to increase the accuracy of the prediction equations. If the relationship presented in the present study is confirmed and strengthened, then the adult laboratory rat can be used as an inexpensive and rapid, routine model for the digestibility of amino acids in the adult dog.

CHAPTER 4

GENERAL DISCUSSION

From the time of domestication, dogs have been part of our society. Dogs serve much more than mere companion animals. They are used to aid the blind and deaf, used in search and rescue work and in farming of sheep. Digestibility is an important criterion to accurately formulate diets. Nowadays it is accepted that, rather than the traditional faecal digestibility measurement, ileal digestibility values more accurately predict the amount of amino acids absorbed from the gut (Sauer and Ozimek, 1986; van Weerden, 1989). However, the ileal digestibility method is an invasive technique and, practical and ethical reasons hamper the use of this method in routine estimation of digestibility in companion animals. Model animals, especially the rat, are often a cheaper choice for assessing the digestibility of compound feeds or feed ingredients. Comparative digestive physiology of the rat and the dog offers much promise that the rat can be used as a model animal for studying digestibility in dogs (Chapter 1). The present study investigated the suitability of the laboratory rat for protein digestibility studies in adult dogs. In this section, the results are discussed more generally and from the view of the findings, the need for some further research is discussed.

There is a large body of literature regarding endogenous excretion of amino acids and nitrogen in monogastric animals such as pigs, humans and rats. Dogs are omnivorous carnivores and Hendriks *et al.* (1996) showed that, when similar diets are fed, gut endogenous amino acid excretions in the carnivorous cat are higher than rats (Butts et al., 1991) and pigs (Butts et al., 1993). Therefore, it is possibility that the endogenous amino acid excretions in the dog may differ from the rat. However, data regarding gut endogenous excretion in dogs are limited. The first aim, therefore, focused on measuring the endogenous amino acid and nitrogen excretion in the dog and the rat at an ileal and faecal level, and under protein-free and peptide alimentation. The present study provides evident that gut endogenous excretion of amino acids and nitrogen are higher in dogs than rats (Chapter 2). The pattern of amino acid excretion, however, were similar in both species and in line with other studies in rats, pigs, humans and the cat (Butts *et al.*, 1991; Butts *et al.*, 1993; Rowan *et al.*, 1993; Hendriks *et al.*, 1996).

The endogenous ileal proline excretion in the dogs in the present study (Chapter 2) was not elevated under the condition of protein-free feeding which is similar to that found in the cat (Hendriks *et al.*, 1996). De Lange *et al.* (1989) hypothesised that the high proline excretion under the condition of protein-free feeding is due to the production of glutamine originating from muscle breakdown. Glutamine is used by the intestine where it is a precursor for the synthesis of proline. The activity of pyrroline-5-carboxylate synthase, which is required for the conversion of glutamine to proline in the intestine, is lower in carnivores compared to omnivores (Barker, 1991; Morris, 1994). Therefore, endogenous ileal proline levels may not be elevated in carnivores due to the low pyrroline-5-carboxylate synthase activity. The endogenous proline excretion of the rats fed the protein-free diet in the present study did also not appear to be elevated. However, the difference between the endogenous proline excretion with the peptide alimentation and the protein-free method was less than the other amino acids which has also been seen in previous studies (Moughan and Rutherfurd, 1990; Butts *et al.*, 1991). The amount of muscle breakdown will vary with the animal's previous body status and experimental period and, therefore, the proline excretion under the condition of protein-free feeding may also vary. The lower values of endogenous amino acid excretion determined under the condition of protein-free feeding compared to peptide alimentation in the present study, indicates that the presence of dietary peptides produces higher endogenous excretion of amino acids both in the dog and the rat.

Although the ultrafiltration method appeared to be a better method for the estimation of gut endogenous excretion, it has some limitations. The main limitation is that during the ultrafiltration step the endogenous free amino acids, small peptides and other non-protein nitrogen-containing compounds are discarded in the low molecular ultrafiltrate. Therefore, this method underestimates the actual endogenous excretions of amino acid and nitrogen. Reducing the molecular size of the dietary peptides in the enzyme hydrolysed casein and then reducing the filter size of the ultrafiltration device to less than 10,000 Da will result in a reduction of the amount of underestimation of this technique. However, when reducing the size of dietary peptides it must be considered that to exert stimulation on endogenous secretion, the molecular weight of peptides cannot be too small. From previous studies in rats (Butts *et al.*, 1992) and pigs (Moughan and Schuttert, 1992) it was concluded that the amount of free amino acids and small peptides in the low molecular ultrafiltrate fraction was low. The present study, like

studies in cats (Hendriks *et al.*, 1996), assumes that the amount of free amino acids and small peptides in the endogenous gut excretion was also low in the dog. However, the latter was not tested in the present work and further studies could focus on determining the degree of underestimation of the EHC/ultrafiltration technique to determine endogenous amino acid excretion in dogs.

Endogenous excretions have been considered to be constant in adult animals in which the levels of enzymes have reached a constant level (Cranwell, 1995). However, the mucus secretion and sloughing of epithelial cells may vary in adults animals with age, especially in old animals these may be higher. Furthermore, there are a variety of breeds of dogs which show a large variation in body weight, and this may affect gut endogenous excretions. Kendall *et al.* (1982), however, showed that there was no significant effect of body weight and breed on endogenous faecal nitrogen excretion in dogs. However, there may be an effect of age on gut endogenous excretion and, therefore, further studies may be aimed at determining the effect of age on gut endogenous nitrogen and amino acid excretions in dogs.

The second part of this work aimed to determine and compare the digestibility of a commercial dry dog food between the rat and the dog. From previous studies (Moughan *et al.*, 1984; Sauer and Ozimek, 1986; Skilton *et al.*, 1991; Rowan *et al.*, 1994) it is evident that in omnivorous animals, because of the microbial population in the large intestine, faecal digestibility measurements are inaccurate for estimating amino acid digestibility. Dogs, however, have a relatively small large intestine and there are no previous data regarding the effect of the large intestine on amino acid digestibility values in dogs. The present work showed that although dogs do not have a large hind gut (large intestine : body length = 0.8, Maskell and Johnson, 1993), like other omnivorous animals, the microbial population in the large intestine causes the breakdown of unabsorbed amino acids while there is a net synthesis of others (methionine and lysine). Therefore, the present work provides evidence that the faecal digestibility method is also an inaccurate method for the estimation of amino acid digestibility in dogs. Consequently, dog ileal digestibility values were compared with those of rats and showed that the apparent ileal digestibility values for most amino acids were higher in the dog. However, the difference in apparent digestibility between the rat and the dog may have been caused by endogenous excretions, which were shown to be different between the rat and the dog (Chapter 2). Therefore, true amino acid digestibility values, using the protein-free

endogenous amino acid excretion estimates, were calculated and compared between the two species. This showed that the true digestibility of amino acids were also different between the rat and the dog for most amino acids. Although there are major similarities in the digestive anatomy and physiology of the digestive tract between both species (Chapter 1), different apparent and true digestibility values were found for the rat and the dog when fed a dry dog food.

Regression analysis, however, showed that there was a significant linear relationship between ileal amino acid digestibility values of the rat and the dog. The regression equations found in Chapter 3 can be used to determine apparent and true ileal amino acid digestibility in dog. These regression equations explained 67 % of the variation in apparent digestibility values and 61 % of the variation in true digestibility value of the dog. To increase the accuracy of the prediction equations, a wider range of dog foods need to be correlated which will also allow relationship for individual amino acids to be generated.

There are some factors which have to be considered when using the rat as a model animal. The first is coprophagy (not only in the rat but also possibly in the dog), which may affect the digestibility of protein. Therefore, the stomach contents should always be checked during sampling of the digesta if the slaughter technique is used. Other feeding habits which may affect digestibility is the selective eating habit of rats. This can be avoided by finely grinding the feed ingredients. However, grinding may affect the digestibility and has some drawbacks regarding, labour, effort and time. Further, particle size separation and/or selection of the test diet may give rise to a design bias and lower the sensitivity and reliability of the assay technique. So further research needs to be conducted regarding the occurrence of those feeding habit and their subsequent effect on digestibility.

A main reason to choose the rat as a model animal is that digesta can be collected easily in rats under anaesthesia. However, desirable objectives regarding the slaughter technique are maintaining a uniform flow of digesta and obtaining representative samples. The latter problems were overcome in the present study by the hourly feeding regime and a pre determined sampling time. Rats are very quick to adapt to this particular feeding regime. However, this is time consuming if this method is to be used routinely.

CHAPTER 5

LITERATURE CITED

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