# *Nutritional aspects of sexual dimorphism in the American mink mustela vison (schreber)*

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# NUTRITIONAL ASPECTS OF SEXUAL DIMORPHISM IN THE AMERICAN MINK MUSTELA VISON (SCHREBER)

by

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B. Sc. (Dunelm)

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of

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Van Mildert College

University of Durham

## DECLARATION

I hereby declare that the work in this thesis is entirely my own and that no part has previously been submitted for a degree in this or any other university.

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Jonathan Gregory

Durham

May 1987

# Contents

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## ABSTRACT

Nutritional aspects of size-related sex differences in the diets of free-living mink were investigated in laboratory-based feeding trials with adult farm-bred mink maintained on 'natural' diets. As preliminary studies had shown that carcase utility was virtually complete, the rations presented comprised the minced whole carcasses of wild rabbit *Oryctolagus cunniculus*, eel *Anguilla anguilla*, laboratory rats and mice, and domestic fowl. Determinations of gross composition revealed significant differences between these diets; the smaller prey types, including rodents, birds and fish, were found to have a higher ratio of Apparent Digestible Energy to Nitrogen than larger items such as rabbits, although comparisons with data presented by other workers demonstrated that the variations between species within these prey groups are as great as, or greater than, those between the diets themselves. From the results of the feeding trials, it was also apparent that such diets do not differ significantly, either in digestibility or biological value and attempts to classify particular prey items in terms of their nutritional value are, therefore, of limited application in analyses of the feeding ecology of a generalist predator.

Nutrient intake was related to diet composition and varied widely between trials, although the mass-specific requirements of females were higher than those of males. Comparisons of gut morphology indicated that, in females, hypertrophy of the alimentary tract may develop in response to increased energy demands. A similar adaptation was evident in both males and females from wild populations, suggesting that the natural diets of free-living mink are generally of a lower quality than the rations fed to commercially raised animals.

Feeding trials were also carried out on growing kits from 56 to 105 days *post partum*. Sex differences in nutrient metabolism were not significant but the growth rates of males were higher than those of females. In both sexes the growth rates of kits feeding on 'natural' diets were lower than those of animals raised on commercial rations. This effect was most pronounced in males, a finding which supports the hypothesis that the degree of sexual dimorphism in this species is dependent on the extent to which the growth potential of males is constrained by dietary regime during the early phases of development.

# List of Materials

All reagents were of analytical grade unless stated otherwise. Materials were obtained from the following suppliers:-

Alltech Associates/Applied Science, Carnforth, Lancashire., U.K.

Alltech CS-5, on chromasorb WAW support (100 - 120 mesh) GLC fatty acid methyl esters (12:0 - 22:6)

BDH Chemicals, Poole, Dorset, U.K.

Acetic acid, glacial
Ammonium sulphate
Amyl alcohol
Calcium chloride
Chloroform
Cupric sulphate
Diethyl ether
Dimethyldichlorosilane (G.P.R.)
Di-Sodium Hydrogen orthophosphate
n-Hexane
Hydrochloric acid (G.P.R.)
Hydrogen peroxide
Iodine
Kieselgel 60H (Merck)
Nitric acid

Petroleum spirit (B.P. 60-80°C fraction)

Petroleum spirit (B.P. 40-60°C fraction)

Potassium di-hydrogen orthophosphate

Potassium hydroxide

Silica gel

Sodium hydroxide

Sodium hypochlorite

Sodium nitroprusside

Sulphuric acid (G.P.R.)

Toluene

Tri-Sodium orthophosphate

Urea

Zinc dibenzyldithiocarbamate (G.P.R.)

B.O.C., Ltd., Birtley, Durham, U.K.

Air

Hydrogen

Oxygen

Nitrogen

Farm Feed Formulators, Northallerton, Yorkshire, U.K.

Vitamin supplement MA2

Gallenkamp, Loughborough, Leicestershire, U.K.

Benzoic acid (thermochemical standard)

I.C.I. Ltd., Welwyn Garden City, Hertfordshire, U.K.

Tensol no. 12 plastic cement

J. J. (Chromatography), Ltd., King's Lynn, Norfolk, U.K.

Glass GLC columns

Sigma Chemical Co., Poole, Dorset, U.K.

Boron trifluoride
Ethylenediaminetetra-acetic acid (EDTA)
Palmitic acid
Phenol
Triethanolamine
Triolein
Urease

Special Diet Services, Ltd., Witham, Essex, U.K.

Solkafloc

Thompson and Capper, Ltd., Runcorn, Cheshire, U.K.

Kjeltabs AAOAC

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# Chapter One

# GENERAL INTRODUCTION

## 1:1 The American Mink - an Introduction to the Species

### 1:1.1 Distribution and Habitat

A member of the family Mustelidae (Order Carnivora), the American Mink Mustela vison (Schreber) is a predatory, semi-aquatic mammal originally native to the Nearctic, with a wide-ranging distibution extending throughout North America and most of Canada, except the high arctic. It is less common in the more arid south-western areas of the continent, and is absent from Mexico. Hatler (1976) presented a thorough account of the taxonomy and distribution of the species, documenting the extent of it's range in North America.

In recent times, this range has been extended to the Palearctic region through introductions associated with the fur trade. During the 1920's and 1930's fur farms were established in most countries in northern Europe and the small numbers of animals escaping led to the growth of sizeable feral populations. In the U.S.S.R., commercial farming was combined with deliberate release of animals into the wild, to provide a population available for hunting and trapping (Pavlov, 1970). The country has now been extensively colonised, the species being present throughout most of the temperate forest zones of the north and extending into the tundra. In western Europe the mink is widespread in Finland and Scandinavia, Germany, France, Spain, Britain and Ireland, and Iceland.

The mink is generally associated with aquatic habitats, particularly eutrophic streams and lakes which provide dense cover and a selection of potential den sites (Erlinge, 1972; Allen, 1983). It is also common in marshes and wetlands (Sargeant, Swanson and Doty, 1973; Eberhardt and Sargeant, 1975), and in reedbanks or carr (Chanin, 1976, 1981; Birks 1981; Birks and Linn, 1982). Gerell (1967, 1968, 1970, 1971) and Erlinge (1972) recorded the presence of mink in a variety of habitats in Sweden including coniferous woodland, farmland and off-shore islands. Though less common in oligotrophic environments where waterside cover is sparse (Chanin 1981), the mink may occupy stretches of rocky coastline, provided that sufficient prey is available and suitable cover close by, as reported by Cuthbert (1979) and Dunstone and Birks (1983) of populations in Scotland, by Hatler (1976) in British Columbia, and Gerell (1968) in Sweden.

The species is tolerant of man, and is found in urban areas close to rivers and canals, allotments and other secluded situations affording shelter and a plentiful supply of food (Birks and Linn, 1982). Habitat requirements have been extensively reviewed and evaluated by Allen (1983).

### 1:1.2 Diet

The mink is an opportunistic predator, taking a wide variety of mammal, fish, bird and invertebrate prey. The composition of the diet varies with season and habitat, according to changing availability of prey.

#### (i) Variation with habitat

In riparian habitats, coarse fish, frogs and crayfish constitute the highest proportion of the diet, with small birds and rodents also being taken, together with beetles, molluscs and, occasionally, earthworms (Chanin and Linn, 1980; Burgess and Bider, 1980; Chanin, 1981; Wise, Linn and Kennedy, 1981; Gilbert and Nancekivell, 1982). Ground-nesting birds such as waterfowl are selectively predated where available, as in marshes and wetland areas (Gerell, 1968; Sargeant _et al_, 1973; Eberhardt and Sargeant, 1975; Chanin and Linn, 1980; Wise _et al_, 1981), while in coastal habitats gulls, taken as carrion or juveniles, often make up a significant proportion of the diet (Cuthbert, 1979; Birks and Dunstone, 1984, 1985). For coastal-living mink foraging in the littoral zone, shore crabs form an important food source (Hatler, 1976; Cuthbert, 1979; Birks and Dunstone, 1985; Dunstone and Birks, 1987), particularly in late summer when the crabs migrate inshore to breed. Rockpool fish are also taken (Hatler, 1976; Birks and Dunstone, 1984, 1985).

Regional differences in the composition of the diet are related, in part, to the distribution of the prey species. For instance, rabbits are heavily predated where present, and in the British Isles may form the most important prey item (Wise et al 1981; Birks and Dunstone, 1985). In Sweden, Gerell (1967, 1968) and Erlinge (1969, 1972) found crayfish to be the major component, while in North America, muskrats and other small rodents are reported to be the species most frequently taken (Sealander, 1943; Korschgen, 1958; Hamilton, 1959).

### (ii) Variation with season

The occurrence of different prey types in the diet varies with the seasonal changes in their abundance. In the winter months, fish are frequently found to be the major food source (Wise et al, 1981; Birks and Dunstone, 1985; Dunstone and Birks, 1987). Gerell (1968) suggests that this is due to their greater susceptibility to capture in colder waters. In summer, crayfish become increasingly important as their mobility (and hence vulnerability) increases with the rising water temperature (Gerell, 1967, 1968). Similarly, predation upon rodents varies with their abundance, peaking in autumn, while lagomorph predation is maximal in summer when juvenile rabbits are most numerous. Dunstone and Birks (1987) contend that in coastal-living mink, increases in predation of rockpool fish in winter derive in part from the decreased availability of alternative prey types, principally lagomorphs, at that time.

The diets of wild mink and the variations in their composition are reviewed in greater detail in Chapter Two.

### 1:1.3 Social Organisation and Breeding System

Social organisation is based upon individual territories contiguous with those of animals of the same sex, but often overlapping to a great extent with those of opposite sex (Gerell, 1970; Birks, 1981; Birks and Linn, 1982). The territories have well-defined boundaries which are patrolled regularly and delineated by scent-marking, usually in the form of scats or droppings deposited in prominent places.

As is true of many carnivores, home ranges vary in size, depending on habitat, prey density and season (Rosenzweig, 1968; Dunstone and Birks, 1985). They are generally linear in shape since activity is usually concentrated along water margins. Ranges of 1.5 - 6.0 Km in length have been described for males (Gerell, 1970; Chanin, 1976; Birks and Linn, 1982; Dunstone and Birks, 1985), while those of females are slightly smaller, with 1 - 3 Km being more common. Dens are situated throughout the range and several may be in use at any one time (Birks and Linn, 1982). Located in rabbit burrows, amongst rocks and dense vegetation or beneath waterside trees, their use is related to foraging activity, which is often localised and centred upon areas in which prey is seasonally abundant.

During January and February the territorial system begins to break down with the onset of the spring mating season, when resident males may vacate their territories in search of females and transient non-residents become more numerous (Gerell, 1970, 1971). The mating system is promiscuous and polygynous, with strong intra-sexual competition between males for access to receptive females (Marshall, 1936; Birks, 1981). Successful males may mate with several females in the course of the season and individual females may be mated by several males (Hatler, 1971, 1976). There is no evidence of pair-bonding or male parental care.

Mating takes place in February to March, ovulation being induced by coitus. A second mating is necessary for fertilisation, which may be by the same or a different male. In instances of multiple matings by different males, litters of mixed paternity can result, a phenomenon known as superfetation. Implantation may be delayed by a variable period of time, such that gestation may last from 39 - 76 days, with females bred in early March having the longest gestation period (Enders, 1952). The kits are usually born in May, approximately 28 days after implantation, the average litter size being 4 - 6. Lactation lasts 6 - 8 weeks, and the kits are weaned at about 6 weeks old (Pilbeam, Concannon and Travis, 1979).

Adult size is attained in approximately 4 months, with mean weights being around 1400 g and 720 g for males and females, respectively (see Table 1:1). The young are sexually mature at 10 months and can breed the following year.

## 1:2 Sexual Dimorphism in Mustelids

Sexual dimorphism is a characteristic feature of mustelids, particularly of the Mustelinae, the sub-family to which the mink belongs and which includes the stoats, weasels and martens.

Males are always the larger sex and may be more than twice the size of females, though the ratio varies with species and geographically within

Table 1:1 Sexual Dimorphism in the Body Size of the American Mink

| Location | Author | | n | Body Wt. (Kg) | S.E | Range | Dimorphism | Range |
|---|---|---|---|---|---|---|---|---|
| Oligotrophic stream, England | Chanin, 1983 | Male | 48 | 1.153 | ( - ) | 0.850-1.805 | 1.86 | 1.1-4.0 |
| | | Female | 32 | 0.619 | ( - ) | 0.450-0.810 | | |
| Coastal, Scotland | Birks and Dunstone, 1983 | Male | 15 | 1.196 | (0.045) | 0.930-1.530 | 1.74 | 1.2-2.7 |
| | | Female | 11 | 0.688 | (0.020) | 0.560-0.770 | | |
| Farm-bred, England | This study | Male | 14 | 1.823 | (0.051) | 1.130-2.040 | 2.05 | 0.9-3.3 |
| | | Female | 16 | 0.861 | (0.022) | 0.660-1.020 | | |

species (McNab, 1971; Ralls and Harvey, 1985). Dimorphism is evident not only in size but in shape also. The practice of measuring skulls for taxonomic purposes has afforded details of sex differences in the mink (Kruska, 1980; Wiig and Rolf, 1980; Wiig, 1980, 1982a, 1982b, 1985; Wallace Park and Nowosielski- Slepowron, 1981) as well as polecats Mustela putorius (Ashton and Thomson, 1955; Buchalczyk and Ruprecht, 1977; Ruprecht, 1978), weasels M. nivalis (King, 1980), martens Martes spp. (Hagmeier, 1961; Leach, 1977; Gerasimov, 1985), badgers Meles meles (Wiig, 1986), and otters Lutra lutra (Hysing-Dahl, 1959; van Bree, Jensen and Kleijn, 1966; Wiig, 1986). Birney and Fleharty (1966) and Ondrias (1966) have reported secondary sexual variation in the post-cranial skeleton too. For the purpose of the present work, however, the term 'dimorphism' will be used only in reference to dimorphism in body size (expressed as weight), unless stated otherwise.

The sexual dimorphism of mustelids is striking in both it's magnitude and universality. Several theories, falling into two main groups, have been advanced to explain this phenomenon. Those in the first group are based on the premise that sexual dimorphism has arisen as a means of increasing niche separation between males and females, while those included in the second seek to explain dimorphism in terms of selective pressures for promoting large size in males and/or small size in females.

### (i) Sexual dimorphism and the reduction of niche overlap

The theories belonging to the first catagory invoke the concept of differential niche utility and draw upon data from a number of vertebrate groups, particularly birds, in which dimorphism appears to have arisen as a means of reducing competition for food between the sexes. Selander (1966) and Holyoak (1970) noted that, in some species of birds, there are differences in the feeding apparatus as well as size, which enable males and females to exploit different food resources. Feduccia and Slaughter (1974) found similar developments in various species of skate (Chondrichthyes: Rajidae); sexual dimorphism in the mouthparts is related to the depths at which each species is normally found. At greater depths, with fewer prey items available, intra-specific competition increases, with the concomitant development of the observed sex differences.

The dimorphism in the skull shape of mustelids lends support to this theory, as noted by Erlinge (1979). All the differences recorded are related to increases in the size of the canine and carnassial teeth in the male, and the greater development of those anatomical features by which larger and more powerful jaw muscles, particularly the temporalis, might be accommodated. Functional analyses of these features have been presented by Maynard Smith (1959); Herring and Herring (1974); Emmerson and Radinsky (1979) and Greaves (1984).

The absence of consistent differences in the diet of some markedly dimorphic carnivores however, may mean that such comparisons are misleading. For instance, there is marked sexual dimorphism in foxes Vulpes spp. (Churcher, 1960) and in otters and badgers (Wiig, 1986), even though in each of these species the diet is comprised mainly of items smaller than themselves. That the observed dimorphism indicates specialisation for a particular size class is neither likely nor evident. Wiig (1985) found a similar pattern in the hooded seal Cystophora cristata, a species to which the same considerations apply. As in mustelids, the changes seem to be related to age rather than diet (Birney and Fleharty, 1966; van Bree, 1966; King, 1980; Kruska, 1980; Wiig, 1982), though it is possible that the pronounced dimorphism seen today is a relict of former times, when different ecological conditions pertained (MacKinnon, 1974).

Mustelids have evolved a disproportionately long, slender body shape, presumably as an adaptation enabling pursuit of fossorial prey into their burrows. Brown and Lasiewski (1972) have postulated that having such a shape incurs a penalty in the form of higher energy demands, since their greater heat losses require an elevated metabolic rate. Sexual dimorphism might therefore be selected for as a means of reducing the competition for available food resources.

Similar considerations are thought to apply to birds, particularly predators such as hawks and owls, for which prey density may be a limiting factor (Storer, 1966; Earheart and Johnson, 1970). In these groups reversed sexual dimorphism is the norm i.e., females are larger than males. Reynolds (1972) argued that small size in one member of a breeding pair should be selected for to alleviate competition and to reduce the net energy requirement of the pair. Females should always be the larger of the two, given consideration to egg quality and the aerodynamic limitations placed upon an avian predator during the breeding period (Greenwood and Wheeler, 1983). In accordance with the predictions made by Elton (1927) regarding the 'pyramid of numbers', prey density is inversely related to size. Thus predators specialising in such small prey types, by a reduction in their own size, would accrue the benefits of having a more abundant food supply. Hence the evolution of smaller males (Storer, 1966).

In the case of mammals, the constraints imposed upon female body size by reproductive considerations do not apply and there is no necessity for maintaining a large size. Small size may be selected for if, as Storer (1966) suggested, an advantage were to be gained in so doing, through specialising in a different feeding niche. However, the theory that differential niche utility developed through size dimorphism, as proposed by

Brown and Lasiewski (1972), has been criticised by several authors (Erlinge, 1979; Moors, 1980; Ralls and Harvey, 1985) who cite a number of features of mustelid ecology which are incompatible with the theory.

Erlinge (1979) pointed out that sexual dimorphism is a characteristic of all the solitary mustelids whether or not they possess the elongate body form, though Powell (1979a) found evidence that sexual dimorphism was positively correlated with the degree of elongation. This relationship has since been shown to be apparent rather than real (Ralls and Harvey, 1985).

If dimorphism arose as an adaptation by one sex to the predation of a particular size of prey, there should be a discernable relationship between the body sizes of predator and prey. No such relationship has been found in any of the mustelid species studied (Rosenzweig, 1968; Ralls and Harvey, 1985), though in the stoat Mustela erminea, in which the female is considered to be a vole specialist, female size is more closely correlated with vole size than is that of the male (Erlinge, 1979; Simms, 1979). Ralls and Harvey (1985) demonstrated that the degree of sexual dimorphism is not related to either the number of vole species present or their size range, and concluded that while a positive relationship existed between the two, prey size was not a good, nor even the best, predictor of predator size. Rosenzweig (1968) noted that analyses of this kind were confounded by the problem of defining prey size in a biologically meaningful way.

Predator size is governed not only by the size relationship between predator and prey but also by that between sympatric predators. Rosenzweig (1966, 1968) introduced the concept of 'hunting sets' to describe groups of closely related predator species sharing the same habitats and employing similar hunting strategies. Their coexistence, he asserted, was made possible because of the differences in size between the species and between the sexes within each species. This minimised exploitation competition for food resources, a phenomenon known as character displacement.

In North America, five mustelid species may be included in such a set, the (least) weasel *Mustela nivalis*, the stoat *M. erminea*, the long-tailed weasel *M. frenata*, the American marten *Martes americana*, and the fisher *M. pennanti*. McNab (1971) found a uniform gradation in size from the smallest to the largest, with body weight increasing by a factor of approximately 2.00 at each step. This suggested that an even spacing of ecological niches had arisen; a finding predictable from the ideas of Hutchinson (1959), elaborated upon by Schoener (1974).

McNab concluded from his analysis that the maximum and minimum size attained by a predator was constrained by the occupation of adjacent niches by other members of the set. In those parts of a species' range in which one or more of these potential competitors was absent McNab found that body size increased, such that the adjacent niche could be filled. Thus *M. erminea* was held to grow larger in the north, in the absence of it's larger competitor *M. frenata*, while *M. nivalis* showed an increase in size with latitude beyond the western limits of distribution for *M. erminea*. Similar size increases were recorded for *Martes americana* to the north of the range of the larger *M. pennanti*. However, Ralls and Harvey (1985) demonstrated that the observed geographical variations in the size of *M. erminea* were not correlated with the presence or absence of either *M. nivalis* or *M. frenata*. Furthermore, neither of the latter covaried in size or degree of dimorphism with *M. erminea*, thus confounding the argument for character displacement as the means by which dimorphism in competing predators might arise. Moors (1980) argued that exploitation competition between the sexes could be reduced without evolving dimorphism, and suggested the use of different hunting strategies, activity patterns or foraging habitats as alternatives. In addition, he reasoned that sexual dimorphism could lead to an increase in interference competition between the sexes, since social dominance in mustelids appears to be closely correlated with body size in both intrasexual and interspecific confrontations (Lockie, 1966; Erlinge, 1977; King and Moors, 1979; Simms, 1979). Wilson (1975) demonstrated that while 'larger animals eat things which are unavailable to smaller competitors, the reverse is much less true', implying that there would be a severe selective disadvantage in being the smaller of a dimorphic pair. As Moors (1980) pointed out, the benefits of sexual dimorphism cannot be related to reducing competition for limited resources, since it is the scarcity of prey which creates the conditions in which such competition arises.

Finally, as described above, sexual dimorphism is evident in a number of mustelids in which no differences in diet or niche utility are apparent, for example the otter and the badger. It may be concluded therefore, that sexual dimorphism is not primarily an adaptation for avoiding intersexual competition for food, though the niche separation resulting from such large differences in size may accrue secondary advantages which would serve to augment the selective pressures favouring the trait.

The theories which seek to explain sexual dimorphism in terms of differential niche utility not only fail to account for a number of important aspects of mustelid biology, but also do not explain the direction of the dimorphism. Consequently, the alternatives which have been proposed attempt to explain the phenomenon with reference to those factors selecting for large males and small females.

### (ii) Selection for large size in males

Gittleman (1984) observed that sexual dimorphism was most pronounced in polygynous carnivores. Large size is selected for in the male, with strong intrasexual competition between males for access to breeding females. Fighting between males during the breeding season has been recorded in stoats (Lockie, 1966; Erlinge, 1977), weasels (King and Moors, 1979), polecats (Poole, 1972, 1974) and mink (Maclennan and Bailey, 1969), with dominance usually being dependent on body size.

Concomitant with increased body size is greater mobility and a proportionately higher encounter rate with females, which are generally more sedentary in habit (Gerell, 1970, 1971). Large body size would also be an advantage to a male in subduing females (Simms, 1979). In addition, maintaining a greater body size necessitates an increase in home range, which in carnivores is directly related to metabolic requirements (Harestad and Bunnell, 1979; Gittleman and Harvey, 1982; Mace, Harvey and Clutton-Brock, 1983). Since larger home ranges would be likely to contain more females, the possibility of more frequent matings would be an advantage against which the energetic cost of maintaining a large body size, and the home range needed to sustain it, might be offset.

Though Erlinge (1979) and Moors (1980) have outlined the factors selecting for large size in males, these alone may not be sufficient to account for the magnitude of the sexual dimorphism in mustelids (Ralls and Harvey, 1985). The observed size differences between the sexes may result from the combined effect of selective pressures acting separately upon each sex, for larger males on the one hand, and smaller females on the other.

(iii) Selection for small size in females

The arguments for reduced body size as an adaptation for predation of a certain prey species or size class have already been discussed. Early sexual maturity in smaller females has been suggested as being of selective advantage (Erlinge, 1979), though no consistent pattern in the reproductive biology of mustelids has emerged to suggest that this is of primary importance.

Powell (1979b) and Moors (1980) favour the theory that smaller females are selected for because of their reduced energy demands, particularly during the breeding season. The cost of reproduction in mammals is high; during gestation the female's energy needs increase by approximately a quarter and double during lactation (Kaczmarski, 1966; Migula, 1969). East and Lockie (1964) reported an increase in food consumption of approximately 127% during the lactation of a weasel with a litter of four. Reduction of the total energy requirement through smaller body size would therefore be of very great benefit, especially in carnivores which must continue providing food for their litters even after weaning, until the young can hunt and kill for themselves. Moors (1980) calculated that a male-sized female weasel would need to catch an extra half a vole per day simply to meet her own increased metabolic costs. Powell and Leonard (1983) similarly estimated the extra energy expenditure in the female fisher <u>Martes pennanti</u> to be 300 - 500 KJ/day.

The idea that smaller females make a saving on their own energy requirements which can be channelled into reproduction has been met with scepticism by Ralls and Harvey (1985), who argue that this would only be the case if hunting efficiency remained constant for predators of different sizes. As has already been indicated, niche breadth may in fact decrease with size (Morse, 1974; Wilson, 1975; Moors, 1980), and Erlinge (1975) observed that male weasels averaged only 12.5 minutes per successful capture compared with 50 minutes for females, though females were able to quadruple their efficiency when supporting a litter. Conversely, Powell and Leonard (1983) contended that the foraging strategy employed by lactating female fishers differed very little from that normally used by male fishers at that time of year, indicating that both males and females were foraging optimally. The maximum daily energy expenditure of both sexes were estimated to be about the same (2000 - 2500 KJ), which suggested that the reduced maintenance requirement of females almost exactly compensated for the higher costs of reproduction.

The findings of Ralls and Harvey (1985) indicate that sexual dimorphism is probably not directly related to energy requirements. The smallest of the North American mustelids, *M. nivalis* is less dimorphic than either of the next largest species, *M. erminea* and *M. frenata*, yet it has the highest surface area: volume ratio and weight specific costs. Moreover, while it is possible that the advantages of lower energy demands or specialisation in certain prey types have led to a reduction in female size in some species of mustelid, it is unlikely that selective pressure for small females is the major cause of extreme dimorphism, as the characteristic is not always associated with small female size.

The large regional variations in body size and sexual dimorphism that occur within species led Ralls and Harvey (1985) to conclude that local environmental conditions were primarily responsible for the patterns observed. They proposed that differences in adult body size were determined mainly by variations in prey abundance during the period when the young were growing.

East and Lockie (1964) provided striking evidence of this in their study of a weasel litter raised in captivity. Fed ad libitum, females attained an adult weight of 85 g, compared with an average of 60 g for wild females from the same population. Males reached 300 g, nearly twice the weight of wild males, which averaged only 160 g.

Laboratory studies have shown that adult size in males is much more dependent on the plane of nutrition than in females, and may be greatly reduced if food resources are restricted during the initial growth period (Widdowson, 1976). Sinclair, Sibbald and Evans (1962) monitored the performance of male and female mink kits of similar birth weights on four different diets for the first five months after weaning. Females were largely unaffected by experimental regime, but the growth of males on the same diets was depressed to varying degrees, depending on the levels of energy and protein. Tauson and Alden (1985), also working with mink, found that limiting energy intake for the first few weeks after weaning significantly affected adult body size. After this period, similar restrictions had no detrimental effect. It has also been shown that energy restriction affects kits of a higher initial weight to a greater extent than smaller animals (Jørgensen, Clausen and Haagen-Petersen, 1962). Males, being larger, have higher maintenance requirements and thus such a restriction is of greater consequence in males than in females.

In the light of these findings, Ralls and Harvey (1985) have postulated that sexual dimorphism is likely to be greatest in areas where prey is abundant and male growth rates maximal. Conversely, it will be lowest in those areas in which food resources are limiting and male body size is reduced. Since dimorphism is already pronounced by the time the kits are weaned (Sinclair et al, 1962; pers. obs.), the extent to which it continues to increase may depend both on the efficiency with which males predate those species for which the females are the optimally-sized predator, and the availability of larger sized prey. In this case, species differences in diet and size of prey taken could account for much of the variation in sexual dimorphism both within and between species.

Supporting evidence for this explanation comes from Powell's (1979a) study of two fisher populations in North America. Animals taken from Minnesota were released in Michigan into an area having abundant prey resources not exploited by other predators. After fifteen years, male fishers in the introduced population were found to be significantly heavier than the males of the parent population in Minnesota. Female weights had not changed. Powell attributed these changes to the large number of porcupines Erethizon dorsatum, the fisher's main prey, which were to be found in Michigan. Such an abundant food supply would enable their predators to achieve maximal growth rates during their early development.

The theories advanced in explanation of sexual dimorphism in the body size of mustelids are many and varied, and it is unlikely that any one of them can fully account for the diversity of patterns within the group.

Sex differences in the diets of most of the smaller species are well-documented; in the weasel (Day, 1968; Erlinge, 1975; Brugge, 1977; King, 1977); stoat (Day, 1968; Brugge, 1977; Erlinge, 1979); polecat (Brugge, 1977); American marten (Murie, 1961; Buskirk and MacDonald, 1984); pine marten Martes martes (Pullianen, 1980); and mink (Sealander, 1943; Birks and Dunstone, 1985; Dunstone and Birks, 1987). Arguments in favour of differential niche utility as the basis for dimorphism are, however, confounded by inconsistencies in the relationship between predator and prey sizes, and the theoretical difficulties in justifying the evolution of size dimorphism as the most effective way of reducing intersexual competition, particularly for the smaller of the dimorphic pair (Moors, 1980). Moreover, the absence of any firm evidence of character displacement in sympatric populations of carnivores indicates that niche separation is achieved by other means and that it is probably not the mechanism by which size differences between males and females would arise (Ralls and Harvey, 1985).

The Moors-Powell hypothesis identifies a selective advantage in small size for females because of their reduced energy requirements. There is no doubt that the total cost of reproduction is substantially lower for a small female than for a hypothetical male-sized female (Moors, 1980; Powell and Leonard, 1983), but it remains to be proven that these costs are any easier to meet in a smaller animal. Since the differences in hunting efficiency and prey utilisation contingent upon body size have yet to be quantified it is difficult to determine the extent to which small body size is truly advantageous in the context of a predator's feeding ecology.

If body size has no appreciable effect on hunting efficiency then, as Ralls and Harvey (1985) point out, it is necessary to seek an explanation for increased body size in males, to account for their growing so large and energetically inefficient. Intrasexual competition for females provides the necessary selective pressure, and has been presented as the explanation of extreme size dimorphism in groups as diverse in their ecology as primates (Harvey, Kavanagh and Clutton-Brock, 1978) and turtles (Berry and Shine, 1980).

The extent to which males are larger than females is thought to depend on the quality and amount of food available to young males during their early development (East and Lockie, 1964; Powell, 1979a). It has also been postulated (Emlen and Oring, 1977; Ralls, 1977) that in those areas where prey abundance is low, female home ranges should be larger. As a result the potential for polygyny, and hence male competition, would be diminished, thereby further reducing the potential for increased male size.

It is clear from the above that a knowledge of the relationship between nutrition and the energy requirements for growth and maintenance is central to an understanding of sexual dimorphism in the body size of mustelids. Diet quality governs the extent to which males achieve their maximum growth potential and is basic to those arguments for a selective advantage in small female size. It is pertinent then, to review briefly what is known about the effect of nutrition on the growth and development of carnivores, the degree to which the sexes differ in their requirements, and the capacity of different types of diet to meet those requirements.

## 1:3 Studies in the Nutrition and Energetics of Carnivores

A vast amount of material has been published on the food habits of wild carnivores, mostly dealing with diet content and preferred prey species. Relatively few studies have been devoted to the chemical composition, nutritional quality or digestibility of these diets.

With the domestication of several types of herbivore, research in animal nutrition has been directed towards those species which are of economic importance. In cattle, sheep and swine, therefore, the relationship between nutrition and production has been the subject of extensive and detailed investigation. Conversely, for most species of carnivore the requirements for growth and maintenance are still largely unknown, though the increasing use of the domestic cat *Felis catus* and certain breeds of dog *Canis familiaris* in scientific research has meant that the situation has begun to change.

Rosenthal and Allison (1951) reported on the effect of caloric intake on Nitrogen balance in dogs, Miller and Allison (1958) and Greaves and Scott (1960) carried out similar studies on dietary protein and energy requirements of the cat. More recently, Barbiers, Vosburgh, Ku and Ullrey (1982) have calculated the digestive efficiency and maintenance requirements for some of the larger felids, including the puma Felis concolor, leopard Panthera pardus, lion P. leo, and tiger P. tigris. Though studies on captive wild carnivores are still relatively rare, data have been collected for a number of species.

Inman (1941), Inman and Smith (1941) and Smith (1942) determined the digestibility of a variety of artificial, meat-based diets fed to North American red foxes Vulpes fulva. Natural diets (composed of prey species known to be taken in the wild) were used by Vogtsberger and Barrett (1973) to estimate energy utilisation in growing cubs, while Litvaitis and Mautz (1976) made similar determinations for adults. Energy balance and prey digestibility in the bobcat Lynx rufus have been investigated by Golley, Petrides, Rauber and Jenkins (1965) and Johnson and Aldred (1982), and in the Polar bear Ursus maritimus by Best (1985). Feeding trials and energy balance studies have also been carried out on grey seals Halichoerus grypus by Ronald, Keiver, Beamish and Frank (1984), and on harp seals Phoca groenlandica by Keiver, Ronald and Beamish (1984). Despite the interesting issues raised by the peculiarities of their ecology, the nutrition and energetics of mustelids have received little attention.

Golley (1960) conducted feeding trials on a single male weasel Mustela nivalis fed a vole diet, while Moors (1977) investigated the metabolism, food consumption and assimilation efficiency of both male and female weasels on natural diets comprising rodents, lagomorphs and birds. Significant sex differences were found, with females having substantially lower energy requirements for maintenance and a higher assimilation efficiency on each diet. These observations later formed the basis of the theory propounding increased energy efficiency as the explanation of small size in female mustelids (Moors, 1980). Similar work undertaken on the fisher Martes pennanti (Davison, Mautz, Hayes and Holter (1978); Powell, 1979a, 1979b; Powell and Leonard, 1983), provided corroborative evidence for the hypothesis. The results presented by these workers, though confined to only two species, complete what is presently known of the metabolism of mustelids feeding on natural diets.

While academic interest in carnivore nutrition has been limited, an expanding market for the pelts of some species, notably the American mink, has nevertheless ensured that it has been the subject of keen commercial interest.

A substantial trade in furs has existed since the late 17th. century, but throughout the 18th. and 19th. centuries intensive commercial trapping in North America led to the serious depletion of many wild populations, and for one species, the sea mink Mustela macrodon, over-exploitation led to extinction (Waters and Ray, 1961). It was soon found, though, that many of the smaller members of the mustelidae and canidae were relatively easy to rear in captivity. A surge in the demand for fur in the years after the First World War, together with the high prices of wild-caught pelts quickly led to the establishment of 'fur farms', and the husbandry of fur-bearing carnivores became a profitable and widespread business. The American mink, together with the Silver or Black fox Vulpes fulva , the Arctic or Blue fox Alopex lagopus and their hybrids, now form the mainstay of a world-wide fur industry. For these species, as for others bred for profit, the economics of nutrition and production came to be of increasing importance.

Feedstuffs for animals reared on a commercial basis must meet three main criteria, they should be: 1) comparatively cheap, 2) available all year round, and 3) of a consistent composition which promotes fast growth rates and high adult body weight, thus giving the breeder maximum returns for a minimal financial outlay.

The food requirements of carnivores make them unique among animals domesticated for man's use; all the fur-bearers have very high protein and energy demands, with mink having the highest of all in proportion to it's size. Protein is the most expensive component of the ration, and consideration of criteria 1) to 3) has led to the development of feeds which in composition are far removed from the natural diet.

Initially, 'wet-feed' mixtures were used, comprising poultry offal, slaughterhouse by-products and fish waste, together with cereal and milk products. In Britain and much of western Europe this is still the practice on most farms but, over the last twenty years, artificial dry-pelleted feeds have come into increasing use, especially in the U.S.A. and Scandinavia. These generally consist of a cereal base, with fishmeal or livermeal and milk powder as the protein source, and additional fats and oils to raise the energy content of the ration. Existing information on the nutritional energetics of mink derives almost entirely from research into the formulation of such feeds; through studies of the growth and development of kits on different types of ration, and estimations of the requirements for maintenance in adults.

The biological utility of a diet depends on the digestibility of it's constituent proteins, fats and carbohydrates. In the case of protein this varies with the source; some types of animal and vegetable protein are more digestible than others (Leoschke, 1959; Roberts and Kirk, 1964; Glem-Hansen and Eggum and Christensen, 1974; Skrede, 1978a, 1978b, 1978c; Kiiskinen, Huida, Pastuszewska and Berg, 1985). To a lesser extent this is also true of fats (Leoschke, 1959), which differ in utility depending on the composition of their constituent fatty acids (Jørgensen, 1985). Feeds must therefore include these ingredients in amounts calculated to meet the needs of the animal.

Farrell and Wood (1968b) estimated the energy requirement for maintenance in adult mink, though the needs of an individual vary with age, season and reproductive status. During the moult, for instance, food consumption and Nitrogen retention are reported to increase, as high levels of both energy and protein are required to support the metabolic cost of new fur growth and provide material for the synthesis of new protein (Seier, Kirk and Bragg, 1970).

In addition, while quantity is clearly important, formulation of a suitable ration requires that attention also be given to qualitative considerations. The effectiveness of the protein complement in promoting fur production is dependent not only on it's digestibility, but also it's amino acid composition (Moustgaard and Riis, 1957). Ideally the latter should approximate that of the proteins to be synthesised. Those of fur, the B-keratins, have a very high proportion of the Sulphur-containing amino acids, such as methionine and cystine. The feed of moulting animals should therefore contain these amino acids in increased amounts. The importance of their inclusion in the diet was recognised by Leoschke and Elvehjem (1959), and it has been shown that proteins of low biological value, in which these amino acids are limiting, have a deleterious effect on fur development (Skrede, 1978a; Tauson, 1985b).

The requirements for reproduction can be evaluated on a similar basis. Though such requirements are comparatively low in males, it has been found that a positive relationship exists between the plane of nutrition and litter size (Tauson, 1985a), presumably indicating increased sperm viability. In females, as has already been described, the costs of reproduction are high and sustained for long periods. The constitution of feed protein influences both the development of the foetuses _in utero_ (Moustgaard and Riis, 1957), which is sometimes reflected in the birth-weights of the kits and, during lactation, affects the composition and nutritional value of the mother's milk (Glem-Hansen, 1979).

Optimising the nutrition of the kits is of critical importance to the breeder, and numerous studies have been carried out on all aspects of their growth and development. The young differ considerably from adults in their requirements, as the demands for tissue deposition and fur development have to be met simultaneously. Glem-Hansen (1980c), and Glem-Hansen and Enggaard Hansen (1981) have reported on the changing rates of amino acid assimilation and deposition during the growth period.

For all these processes to take place, the diet must provide both the substrates and the energy needed for their metabolism and synthesis. As in adults, the efficiency with which constituents of a diet are assimilated is related to their source. Kits have a lower digestive efficiency than adults (Skrede, 1978c), and their performance on different types of diet and dietary regime has been extensively documented (Seier, Kirk, Devlin and Parker, 1970; Harper, Travis and Glinsky, 1978; Skrede, 1978c; Chwalibog, Glem-Hansen, Thorbek, 1980, 1982; Kiiskinen _et al_, 1985; Tauson, 1985b). Males have been found to have a higher growth rate than females (Sinclair _et al_, 1962; Allen, Evans and Sibbald, 1964; Skrede, 1978a), and also a higher digestive efficiency (Allen _et al_, 1964). Because of their greater demand for energy and protein their performance is dependent on dietary regime to a much greater extent than is that of females, especially over the first five months, the initial growth period (Sinclair _et al_, 1962; Allen _et al_, 1964;

Glem-Hansen, 1980a, 1980b; Tauson and Alden, 1985).

## 1:4 The Purpose of the Present Study

In the preceding sections, the hypotheses seeking to account for extreme sexual dimorphism have been reviewed, together with the evidence, both direct and indirect, for the mechanisms by which it is thought to have arisen.

In the light of existing data on mustelid biology, it has been reasoned (Moors, 1980; Ralls and Harvey, 1985) that dimorphism did not develop primarily as a means of increasing niche separation, though the sex differences in diet are evidence of differential niche utility. That these differences are always mutually advantageous has been questioned by some authors (Wilson, 1975; Moors, 1980) and arguments have recently been advanced for separate selective pressures in each sex, which are independent of niche utility (Moors, 1980; Powell and Leonard, 1983; Ralls and Harvey, 1985).

It is pertinent, therefore, to consider in greater detail the consequences of these contrasting dietary specialisations in terms of the nutritional demands of the individual. The sex differences in the diets of wild mink are notable in view of recent research into the effect of nutrition on the growth and development of mustelids, particularly the disparity between the responses of males and females to various dietary regimes. Moreover, it is interesting to speculate upon the extent to which the diets of each sex in the wild accord with the estimates made of their requirements by nutritionists. The purpose of the present study is to address four main questions:-

1). How does the composition of prey items consumed by wild mink compare with that of diets used in laboratory studies?

The validity of any assumptions made about the feeding energetics of wild mink based on such studies depends upon the degree to which the diets of free-living and farm-bred animals are comparable. Therefore, it is essential to carry out quantitative analyses on prey items representative of those of which the natural diet is typically composed.

2). How do the different types of prey compare in their digestibility and nutritional value?

Gross intake can readily be estimated from biochemical analyses of the diet, but the utility of ingested material is contingent upon it's digestibility, which can only be determined by feeding to animals under carefully controlled conditions. From such feeding trials, the relative value of items of various size from each of the major prey groups can be quantified, and estimates made of the needs of both males and females feeding on a particular prey type.

3). Are there any sex differences in the digestion and metabolism of ingested material?

Selection for large size in males may have favoured the development of a more efficient digestive metabolism to promote and sustain high growth rates in young animals. Females, having higher mass-specific maintenance costs and restricted by virtue of their size to predating smaller prey of relatively low nutritional value, may have undergone similar changes in response to selective pressures for increased energy efficiency. The validity of these two hypotheses might be established by comparing assimilation efficiencies on different diets.

4). To what extent is the growth of kits raised on a 'natural' diet comparable with the performance of similar animals on commercial diets?

The magnitude of sexual dimorphism is thought to depend on the quality and amount of food available to young males during their early development. Since it has been shown (Tauson and Alden, 1985) that the first few weeks of growth are critical in the determination of adult body weight, it is relevant to compare and contrast the performance of male and female kits fed a 'natural' diet during this critical initial period. The validity of the supposition (Ralls and Harvey, 1985) that adult male size would be substantially reduced by restriction to feeding on low-value prey items might also be tested in this way.

In Chapter Two, an attempt is made to answer the first of these questions.

# Chapter Two

# DIET ANALYSIS

## 2:1 Introduction

### 2:1.1 Sex Differences in the Diet

The mink is an opportunistic predator of catholic tastes and reference has already been made to the variable composition of the diet of free-living animals. Table 2:1 gives some indication of the regional differences between populations from North America and Europe, respectively, and highlight the relative importance of the major prey species in different habitats.

It is evident that, in North America, the muskrat *Ondatra zibethicus*, cottontail rabbits *Sylvilagus floridanus* and microtine rodents predominate (Sealander, 1943; Burgess and Bider, 1980; Gilbert and Nancekivell, 1982; Casson and Klimstra, 1983) with crayfish and cyprinids such as carp *Cyprinus carpio* becoming more important in riparian habitats (Hamilton, 1959; Burgess and Bider, 1980; Melquist *et al*, 1981 ). In Europe the pattern is less consistent. Avian prey are taken more often, especially the larger species of the Orders Ralliformes and Anseriformes (Gerell, 1968; Day and Linn, 1972; Chanin and Linn, 1980), but again lagomorphs, principally the rabbit *Oryctolagus cunniculus*, voles and fish are recorded in the diet of most populations (Erlinge, 1969, 1972; Akande, 1972; Rosser, 1980). As in North America, shore crabs *Carcinus maenas* and *Cancer* spp. are a major component of the diet in coastal habitats (Hatler, 1976; Dunstone and Birks, 1987).

In reference to sex differences in the diets of mustelids, Shubin and Shubin (1975) remarked that 'the ecological differences between males and females are so great in these animals that particularly as regards their obtaining food, their behaviour and their activity, they are as different from each other as absolutely different species'. Moors (1980) reviewed the evidence for this assertion in seven species of mustelid and found a

Table 2:1 Summary of Published Information on Food Habits of Free-living Mink

Prey items have been allocated to one of eight major classes, breakdown of diets carried out on the basis of percentage occurrence (of all prey items). Main prey families and genera are indicated.

Key:-

Season Sp = spring, Su = summer, A = autumn,
W = winter, Y = all year round

Sample size a = stomach content, b = intestine content

Prey genera:-

| | | |
|---|---|---|
| Esox | Esox lucius | Northern pike |
| Lota | Lota lota | Burbot |
| Pugettia | Pugettia producta | Kelp crab |
| Anguilla | Anguilla anguilla | Eel |
| Cambarus | Cambarus bartoni | Crayfish |
| Oryctolagus | Oryctolagus cuniculus | European rabbit |
| Culaea | Culaea inconstans | Brook stickleback |
| Ondatra | Ondatra zibethicus | Muskrat |
| Sylvilagus | Sylvilagus floridanus | Cottontail rabbit |
| Ciliata | Ciliata mustela | Five-bearded rockling |
| Lipophrys | Lipophrys pholis | Blenny |
| Peromyscus | Peromyscus maniculatus | Deer mouse |

Table 2:1 Summary of Published Information on Food Habits of Free-living Mink

| Author and Region | Habitat | Season | Sample Size | Large Bird | Small Bird | Large Mammal | Small Mammal | Fish | Crust. | Amphib./ Reptile | Insect/ Invert. | Main Prey Items (% Occurrence) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sealander, 1943 Michigan | various | W | 102a | 13.0 | - | 52.0 | 18.0 | 11.0 | 6.0 | 25.0 | - | Ondatra (36.0), Anura (23.0) |
| Sealander, 1943 Michigan | various | W | 101b | 4.0 | - | 42.0 | 21.0 | 16.0 | 18.0 | 10.0 | 3.0 | Ondatra (24.0), Cambarus (18.0), Sylvilagus (16.0) |
| Hamilton, 1959 New York | tidal flats, streams, marshes | Sp,Su | 561a,b | 9.3 | | 44.0 | | 32.4 | 12.7 | 23.0 | 29.9 | Anura (18.9), Cambarus (14.1), Cyprinidae (14.0), Microtus (12.4) |
| | | A,W | 630a,b | 1.0 | 1.3 | 24.9 | 19.6 | 34.1 | 14.4 | 23.3 | 10.8 | |
| Korschgen, 1959 Missouri | various | Y | 372a | 5.9 | | 10.2 | 20.7 | 19.9 | 9.3 | 24.9 | 0.4 | fish (30.9), Anura (25.5), Muridae (22.6) |
| Gerell, 1968 Sweden | bog | Sp | - | 4.0 | | 14.5 | | 49.5 | 22.0 | 10.5 | - | Cyprinidae, Astacus, Microtus, Anura |
| | | Su | - | 10.0 | | 6.0 | | 19.0 | 65.0 | - | - | |
| | | A | - | 9.3 | | 25.7 | | 35.6 | 18.0 | 11.6 | - | |
| | | W | - | 6.0 | | 8.3 | | 79.3 | 2.7 | 3.7 | - | |
| | eutrophic lake | Sp | - | 11.6 | | 32.6 | | 39.7 | 11.7 | 4.7 | - | Microtus, Anseriformes, Rana |
| | | Su | - | 30.3 | | 37.6 | | 11.6 | 18.6 | 2.0 | - | |
| | | A | - | 24.3 | | 55.7 | | 12.3 | 5.7 | 2.0 | - | |
| | | W | - | 10.5 | | 29.5 | | 54.0 | 6.0 | 0.5 | - | |
| | eutrophic river | Sp | - | 1.0 | | 40.0 | | 39.0 | 1.0 | 20.0 | - | Microtus, Salmonidae, Cottidae |
| | | Su | - | 6.0 | | 72.0 | | 15.0 | 1.0 | 6.0 | - | |
| | | A | - | 10.0 | | 78.0 | | 13.0 | - | 2.0 | - | |
| | | W | - | - | | - | | - | - | - | - | |
| Erlinge, 1969 Sweden | river | Sp | 32 | 12.5 | | 12.5 | | 96.7 | - | - | - | Lota (40.6), Cyprinidae (18.7), Esox (18.7) |
| | | Su | 18 | - | | 66.6 | | 33.3 | - | - | - | birds (66.6), Lota (22.2) |
| | | A | 45 | 17.8 | | 20.0 | | 62.2 | - | - | - | Lota (44.4), mammals (20.0), birds (17.8) |
| | | W | 27 | 14.8 | | 11.1 | | 95.5 | - | - | - | Lota (44.4), Esox (25.2), Salmo (18.5) |
| | | Y | 122 | 22.9 | | 8.9 | | 62.5 | - | 1.9 | 3.8 | Lota (31.1), birds (22.9), Esox (11.3) |
| Akande, 1972 Scotland | various | Y | 88 | 15.7 | 5.2 | 7.8 | 24.3 | 52.0 | - | - | - | Salmonidae (52.0), voles (13.9), Saricidae (7.8) |
| Day and Linn, 1972 England and Wales | various | Y | 204 | 26.6 | 6.4 | 20.0 | 12.4 | 12.7 | 4.9 | 3.0 | 2.5 | Ralliformes, lagomorphs |

... continued

Table 2.1 Summary of Published Information on Food Habits of Free-living Mink

| Author and Region | Habitat | Season | Sample Size | Large Bird | Small Bird | Large Mammal | Small Mammal | Fish | Crust. | Amphib./ Reptile | Insect/ Invert. | Main Prey Item (% Occurrence) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Erlinge, 1972 | stream | W | 67 | 5.0 | | 47.5 | | 52.5 | - | 40.5 | 2.5 | mammals (47.5), Anura (40.5), Esox (27.5) |
| Sweden | river | W | 41 | 17.5 | | 20.0 | | 85.0 | - | 7.5 | 5.0 | Percidae (55.0), Cyprinidae (25.0), mammals (20.0) |
| | lake | W | 32 | 7.5 | | 5.0 | | 92.5 | 12.5 | 17.5 | 5.0 | Percidae (47.5), Lota (22.5), Cyprinidae (17.5) |
| Hatler, 1976 | coastal | Sp | 588 | 11.1 | | 0.5 | | 49.5 | 66.4 | - | - | Pugettia (41.2), Cancer (20.4), Anatidae (6.0) |
| British Columbia | | Su | 504 | 1.4 | | 0.7 | | 30.6 | 85.4 | - | - | Cancer (27.8) |
| | | A | 324 | 9.9 | | 2.6 | | 52.4 | 69.4 | - | 1.3 | Cancer (37.8) |
| Cuthbert, 1979<br>Scotland | rivers | Y | 722 | 7.0 | | 16.0 | | 67.0 | - | - | 8.0 | Salmonidae (31.0), Anguilla (23.0) |
| Burgess and Bider, 1980<br>Quebec | stream | Su,A | 40 | - | - | 17.5 | 32.5 | 10.0 | 20.0 | 12.5 | 15.0 | Microtus (32.5), Cambarus (20.0), insects (15.0) |
| Chanin and Linn, | oligotrophic stream | Su,A,W | 475 | 1.6 | 7.9 | 10.5 | 18.3 | 53.9 | - | 2.7 | 2.5 | Salmonidae (34.2), Anguilla (16.8), Oryctolagus (6.3) |
| 1980 | eutrophic stream | Su,A,W | 153 | 21.4 | 1.6 | 9.3 | 21.3 | 34.4 | - | 1.0 | 9.3 | Ralliformes (16.4), Microtus (10.9), Anguilla (7.7) |
| British Isles | eutrophic lake | Su,A,W | 57 | 20.9 | 1.4 | 12.5 | 2.8 | 52.8 | - | - | 1.4 | Anguilla (26.4), Ralliformes (15.3), Oryctolagus (9.7) |
| Rosser, 1980 | eutrophic stream | Sp,Su | - | 4.8 | - | 14.3 | 14.4 | 28.7 | 9.5 | 9.5 | 19.1 | insects (19.1), lagomorph (14.3), Salmonidae (14.3) |
| England | oligotrophic stream | Sp,Su | - | 16.2 | 0.7 | 45.5 | 3.9 | 26.7 | - | 0.7 | 2.1 | lagomorph (43.7), Anseriformes (12.0), Salmonidae (9.9) |
| Melquist et al., | eutrophic stream | Y | 659 | 19.0 | - | 3.0 | 39.0 | 59.0 | - | 2.0 | 24.0 | Cyprinidae (30.0), Cricetidae (37.0), insects (24.0) |
| 1981<br>Idaho | eutrophic stream | A | 66 | 19.0 | 6.0 | 7.0 | 11.0 | 77.0 | - | 2.0 | 14.0 | Salmonidae (39.0), Cricetidae (17.0), insects (14.0) |
| Gilbert and | lake | Sp,Su,A | 140 | 25.7 | 1.4 | 41.4 | 17.8 | 31.4 | 0.4 | - | 3.9 | Culaea (27.9), Esox (21.0), Ondatra (21.4) |
| Nancekivell, 1982<br>Alberta | stream | Sp,Su,A | 61 | 16.4 | - | 50.8 | 45.9 | 6.6 | - | - | - | lagomorph (42.6), Microtus (31.2), Saricidae (13.1) |
| Casson and Klimstra, 1983<br>Illinois | various | W | 280a,b | - | 4.0 | 22.0 | 55.0 | 23.0 | 14.0 | 22.0 | 8.0 | Microtus (15.0), Sylvilagus (15.0), Cambaridae (14.0) |
| Dunstone and Birks, | coastal | Sp | 537 | 7.0 | | 51.0 | | 22.4 | 9.6 | - | - | lagomorph, Charadriiformes |
| 1987 | | Su | 686 | 7.6 | | 44.9 | | 25.6 | 21.9 | - | - | lagomorph |
| Scotland | | Au | 347 | 7.4 | | 41.3 | | 27.6 | 23.6 | - | - | lagomorph |
| | | W | 473 | 13.0 | | 26.5 | | 40.7 | 19.8 | - | - | Ciliata, Lipophrys, Anguilla |

consistent trend for females to consume smaller prey than males, with rodents being the most important prey item. In contrast, males took a significantly higher proportion of large rodents and lagomorphs. The extent to which these generalisations apply to the feeding ecology of mink can be seen from Table 2:2, and it is clear that this species conforms to the pattern observed in the other members of the family. Males appear to specialise in the larger mammals such as muskrats, rabbits and hares, while females take mainly small mammals, fish and frogs.

### 2:1.2 Aspects of Prey Utility

The utility of any particular prey item, as defined in terms of it's biological value to the predator, may be measured in a variety of ways. For free-living carnivores actively predating a prey population (as distinct from carrion-feeders), the biological value of a single prey item is a function not only of it's nutritional quality, but also of it's abundance relative to other prey types, and the energy expended in search, pursuit and capture. With larger prey items, the cost of handling the carcass may also be significant. An additional consideration is the proportion of the carcass which the predator is able to digest. Components of relatively low digestibility, such as bone, fur and feather, comprise varying percentages of the total, depending on the size of the animal.

Since these qualities can only be quantified with respect to a particular predator, ecological studies have generally been directed towards evaluating gross or whole-body composition. This may be limited simply to the determination of energy value (Golley, 1960, 1961; Gorecki, 1965; Cummins and Wuycheck, 1971; Bergeron, 1976; Kaufman and Kaufman, 1976; Kitchell, Magnusson and Neill, 1977; Craig, Kenley and Talliny, 1978;

Table 2:2 Summary of Published Information on Sex Differences in the Diet of Free-living Mink

| Author and Region | Habitat | Season | Sample Size | Large Bird | Small Bird | Large Mammal | Small Mammal |
|---|---|---|---|---|---|---|---|
| 1. Sealander, 1943 | various | W | 101b | 11.0 | 2.0 | 65.0 | 9.0 |
| Michigan | | | | ( 7.0) | ( 7.0) | (35.0) | (28.0) |
| 2. Sealander, 1943 | various | W | 102c | 9.0 | – | 49.0 | 16.0 |
| Michigan | | | | ( 5.0) | ( 3.0) | (26.0) | (35.0) |
| 3. Casson and Klimstra, | various | W | 210b,c | – | 3.0 | 24.0 | 34.0 |
| 1983 | | | 70b,c | ( – ) | ( 6.0) | (13.0) | (60.0) |
| Illinois | | | | | | | |
| 4. Birks and Dunstone, | coastal | Y | 512 | 3.9 | 2.2 | 49.2 | 4.5 |
| 1985 | | | | ( 5.5) | ( 4.2) | (16.8) | ( 4.8) |
| Scotland | | | | | | | |

Table 2:2 Summary of Published Information on Sex Differences in the Diet of Free-living Mink (contd..)

| | Fish | Crust. | Amphib./ Reptile | Insect/ Invert. | Dietary Overlap | Main Prey Items (% Occurrence) |
|---|---|---|---|---|---|---|
| 1. | 16.0 | 5.0 | 18.0 | - | 0.84 | Ondatra (49.0), Sylvilagus (16.0), Anura (16.0) |
| | (26.0) | ( 7.0) | (28.0) | 5.0 | | small mammals (28.0), Anura (26.0), Ondatra (23.0) |
| 2. | 7.0 | 14.0 | 9.0 | - | 0.82 | Ondatra (35.0), Small mammals (16.0) |
| | (14.0) | (22.0) | (14.0) | ( - ) | | small mammals (35.0), fish (27.0), Cambaridae (22.0 |
| 3. | 27.0 | 14.0 | 22.0 | 10.0 | 0.87 | fish (27.0), Anura (21.0), Sylvilagus (17.0), |
| | (11.0) | (14.0) | (19.0) | (13.0) | | Peromyscus (21.0), Anura (19.0), Microtus (17.0) |
| 4. | 20.4 | 18.8 | - | - | 0.75 | lagomorph (48.4), Carcinus (15.6) |
| | (37.5) | (29.2) | ( - ) | ( - ) | | Carcinus (24.3), Lipophrys (11.9), lagomorph (15.2) |

Dolnik, and Gavrilov 1979) but estimations of protein, fat and carbohydrate content have become more common as the facility with which such determinations can be made has increased.

Data have been published on many species of fish (Karrick, Clegg and Stansby, 1956; Thurston, Stansby, Karrick, Miyauchi and Clegg, 1959; Dugal, 1962; Murray and Burt, 1969; Sidwell, Foncannon, Moore and Bonnet, 1974; Craig, 1977) and bird (Turcek, 1958; Brisbin, 1968; Newton, 1968; Myrcha and Pinowski, 1970; Chilgren, 1977), together with several genera of mammal, including deer (Robbins, Moen and Reid, 1974; Torbit, Carpenter, Alldridge and Swift, 1985) and shrews (Myrcha, 1969). Of particular relevance to the present study are the investigations carried out by Hayward (1965) on the body composition of deermice *Peromyscus maniculatus* and Myrcha (1968) on the European hare *Lepus europeus*, both of which are found in the diet of wild mink (Sealander, 1943; Hamilton, 1959; Korschgen, 1959; Burgess and Bider, 1980; Melquist *et al*, 1981; Casson and Klimstra, 1983; Birks and Dunstone, 1984).

Such studies are, however, of limited application in the evaluation of prey utility as defined above. By definition, the determination of gross body composition entails the use of samples from whole-animal preparations, usually in the form of a homogeneous mince to ensure uniformity and comparability between sub-samples. Exceptions to this procedure include the work of Myrcha (1968), Sidwell *et al* (1974) and Dolnik and Gavrilov (1979), who carried out separate analyses on various components of the carcasses.

Ideally the diet should reflect the composition of the material normally ingested by the predator, which may differ substantially from that of the carcass as a whole. The use of whole carcasses in feeding trials with carnivores has theoretical advantages in that it is the closest approximation to the natural situation, but there are several practical considerations which militate against it. The composition of the ingested material would be estimated from comparisons of whole carcasses with those parts remaining at the end of the trial period, necessitating many small-scale determinations with an attendant loss in accuracy. Moreover, with larger prey species, the presentation of a whole carcass is often impractical and, for smaller items, simultaneous provision of the many small carcasses which would be required to meet the subject animal's daily needs may result in a pattern of prey utility entirely different from the natural situation (Heidt, 1972). By employing a minced diet these problems are avoided, while retaining the advantages of consistency between presentations and convenience of storage and cleaning. Estimations of ingested material can be made on the basis of the weight of food consumed. The validity of the results so obtained is assured, provided that the composition of the mince is comparable with those parts of the carcass normally consumed.

The first part of this chapter concerns a series of experiments by which carcass utility was estimated, prior to the preparation of the minced diets used in the feeding trials described in Chapter Three. In the second part, the methods used to determine their nutritional value are described and a breakdown of their composition presented. Comparisons are then made between these various 'natural' diets and rations used in other studies.

## 2:2 Assessment of Carcass Utility

### 2:2.1 Materials and Methods

The carcasses used in this series of experiments were chosen as being representative of those prey types found in the diet of wild mink and included laboratory mice and rats, wild rabbit, perch *Perca fluviatilis*, and domestic fowl *Gallus domesticus* aged one day, four weeks and twelve weeks. These were adjudged to correspond with small, medium and large mammal prey, fish, and small, medium and large bird, respectively. Mean carcass weights are presented in Table 2:3, from which it may be seen that the size classes used were typical of a variety of potential prey species.

The mean weights of laboratory rats and mice (257 g and 32 g) accorded well with those recorded for their wild counterparts; the body weights of brown rats *Rattus norvegicus* range from 200 - 485 g, and those of house mice *Mus musculus* from 18 - 30 g (Walker, 1975). Day-old chicks, which averaged 36 g, were of a similar size to many small ground-nesting birds potentially available to the mink, such as the wheatear *Oenanthe oenanthe* (body weight 17.1 - 41.5 g), corn bunting *Emberiza calandra* (38 - 55 g), skylark *Alauda arvensis* (27 - 52 g) and dunlin *Calidris alpina* (38.9 - 58.0 g). (Data from Hickling, 1983). The mean weight of twelve-week old ckicks (1341 g) was broadly comparable with those recorded by Cramp and Simmons (1980) for several species here classified as 'large bird', including herring gull *Larus argentatus* (body weight 813 - 1385 g) and mallard *Anas platyrhynchos* (750 - 1572 g). The figure of 366 g for four-week old chicks may be compared with those published for medium-sized birds such as pheasant *Perdix perdix* (310 - 455 g), moorhen *Gallinula chloropus* (186 - 493 g), Coot *Fulica atra* (300 - 1200 g) and rock dove *Columba livia* (200 - 370 g) all of which feature in the diet of wild mink.

Table 2:3 Live weights of Prey Items used in Diets

| Prey Item | N | Body Weight (g) |
|---|---|---|
| Laboratory mouse | 20 | 32 ( 1.47) |
| Laboratory rat | 20 | 257 (10.29) |
| Wild Rabbit | 44 | 1638 (43.28) |
| 1-day old chick | 18 | 36 ( 1.09) |
| 4-week old chick | 16 | 366 ( 6.97) |
| 12-week old chick | 14 | 1341 (29.62) |
| Eel | 50 | 290 (16.72) |

Mice and rats were supplied from colonies maintained in the Department, while fowls were bought in from a commercial hatchery as day-old chicks and maintained in the Department until they had grown to the necessary size. Rabbits were obtained as carcasses, either shot or snared by local farmers, and perch were caught by net-fishing in a local reservoir (Hallington) by courtesy of the Northumbrian Water Authority. All carcasses were stored at -20 °C and thawed for 24 hrs. prior to use.

Four adult mink were used in each trial, two males and two females of either the 'pastel' or 'standard' strains. These animals had been acquired from a local mink farmer as eight-week old weanling kits, and subsequently maintained in the Department's animal housing facilities on a diet of fish waste and slaughterhouse by-products identical with that used on the mink farm (for general details of animal housing and management see Chapter Three). None of the mink had any experience of natural whole-animal food prior to the commencement of these experiments.

For the duration of the trials with rabbit and twelve-week old chick, the mink were kept singly in a room 2.8 x 1.9 m, with access to a standard wooden nest-box measuring 0.28 x 0.26 x 0.26 m. For all the other trials, animals were housed individually in 'weldmesh' cages of 1.0 x 0.5 x 0.5 m, to which were attached wooden nest-boxes of the standard type. In both situations, a 12:12 light regime obtained throughout. Water was provided ad libitum. Animals were deprived of food for twelve hours preceding a trial.

Each trial commenced with the presentation of a single carcass to which the mink had free access for a period of up to 36 hrs. During this time the animal's response to, and treatment of, the carcass was observed continuously for the first 60 min. and then for 5 min. periods at hourly intervals thereafter. At these times carcass weights were also recorded and corrected for evaporative loss by comparison with similar specimens which had been dissected to expose the viscera. Trials were terminated according to one of three criteria:-

a). The 36 hr. period had elapsed.

b). The carcass had been completely consumed.

c). No discernable change in the appearance of the carcass had been recorded for an interval of 6 hrs. (Based on decreases in weight not attributable to evaporative losses.)

Carcasses of each type were presented in random order to each of the mink on three separate occasions, at intervals of one week. No attempt was made to study the effect on carcass utility of simultaneous presentation of carcasses of different types and sizes.

## 2:2.2 Results and Discussion

All the prey types presented were readily accepted by the mink except for perch, which were consistently refused by every animal, even after alternative diets had been withheld for up to six successive days.

On first presentation of a carcass, all the mink displayed signs of intense excitement. Typically these included arching of the back and stiffening of the tail, together with erection of the body hair, particularly along the spine. A heightening of animation was also noted, usually accompanied by gaping of the jaws and hissing vocalisations, as described by Gilbert (1969) and MacLennan and Bailey (1969). The intensity of these responses, which have been interpreted as behaviour patterns associated with aggression and threat displays when directed towards other mink (MacLennan and Bailey, 1969), declined in later trials as the mink became more familiar with natural prey items.

Within the first 15 min. some action directed towards 'killing' the carcass was usually observed. In males this generally took the form of a single bite at the base of the skull or through the cranium, sometimes maintained for periods of up to a minute. Often such 'killing bites' were followed by frenzied thrashing or a spinning motion along the sagittal axis of the mink's body, possibly an adaptive movement aimed at tearing through the integument of the carcass. Females, in contrast, rarely exhibited this behaviour but, instead, shook or dragged the carcass back and forth around the room or the floor of the cage. The latter activities were adopted by both sexes when dealing with larger prey items.

Though the time taken to finish with a carcass varied both with it's size and with individual differences between the mink, it was noted that all prey types were consumed without any evidence of selectivity in the portions eaten being apparent. Mice and day-old chicks were consumed in their entirety, usually without pause. Rats were generally eaten within 12 - 18 hrs., beginning with the tail and hind-quarters then working up through the abdomen, picking flesh and bone from the skin, and leaving the head and pelt until last. With four- and twelve-week old chicks, it was more usual for the mink to commence with the head or breast muscles or, occasionally, the feet.

Sex-differences were observed only in the treatment of rabbit carcasses, though these were not consistent. There was a tendency, however, for males to enter the carcass through the soft underbelly or beneath the base of the tail, with the fleshy hind-quarters being consumed first (four presentations out of six). Females usually started with the head, around the eye, then down the neck and into the thorax (three presentations out of five). No explanation is offered as to why these differences should exist, other than the possibility that individual variations may have assumed unusual prominence through the use of such a small sample size.

Despite slight differences in the handling of the various prey types, a consistency in carcass utility was evident throughout. It was further noted that no portion of a carcass seemed to be unpalatable except, in the case of rabbit, the content of the lower alimentary tract. This was of particular relevance to the present study. Fur, feathers and teeth were all consumed, even in trials with the largest prey items, rabbit and twelve-week old chick, of which virtually nothing remained at the end of the trial period. Although feet, wings or shreds of skin were occasionally left behind, this was not taken as evidence that these parts were not normally eaten by the mink.

It might be argued that their relative lack of experience in handling natural prey could invalidate comparisons between the behaviour of these farm-bred animals and that of their wild counterparts. The establishment of feral populations in such a wide variety of habitats, however, is an indication that the 'domesticated' animals quickly develop the skills necessary for a successful predator. As described above, certain behaviour patterns, such as the 'killing bite', appear to be innate and are exhibited in animals not previously exposed to natural prey (Rasa, 1973). Heidt (1972) found similar responses to live prey in young weasels which had been separated from mother and littermates while still blind, demonstrating that the skill is not acquired through parental instruction, or learned from siblings, as asserted by Eibl-Eibesfeldt (1970).

Birks and Dunstone (1984) have reported on the prey remains collected from the dens of feral mink, including skulls, feet, wings and tufts of fur and feathers from a variety of prey species. The results of this short series of experiments suggest that these remains should be seen as an indication of the extent to which prey of a given size (usually the larger species) are utilised before another foraging bout begins, rather than the palatability of their constituent parts. Powell (1984, pers. comm.) provided corroborative evidence of this kind of feeding behaviour in a number of a mustelids including fisher, marten, mink, stoat, weasel, long-tailed weasel, ferret, and Siberian polecat M. eversmanni. All of these species were observed to consume the whole of a carcass, often at one sitting, if all the parts were edible. Powell suggested that fur, feathers and bone provided a source of roughage or 'fibre' which assisted in the passage and digestion of food. Consumption of semi-natural diets which lacked these constituents was found to lead to constipation and other bowel disorders.

In conclusion, the interpretation placed upon these results is that, for the types of prey used in this study, minced whole-animal preparations are comparable in their composition to those parts of a carcass normally consumed by wild mink, and therefore their use in laboratory feeding trials is a valid procedure.

## 2:3 Determination of Gross Composition of Diets

### 2:3.1 Materials and Methods

### 2:3.1.1 Preparation of the Diets

For all diets except rabbit, carcasses were stored intact at -20 $^{o}$C and thawed at room temperature for 24 hrs. before mincing. Because of heavy infestation with tapeworm (Cittotaenia spp.) in the wild rabbit used, the alimentary tracts were removed from these before freezing.

Eel Anguilla anguilla replaced perch as the species chosen for the fish diet. Eels had been used in the Department for several years as a 'reward' food item in behaviour studies, and were known to be palatable to mink. The eels were bought in live from commercial dealers and killed by freezing. Slime was removed after thawing by washing in cold water.

Mincing operations were carried out at a local mink farm using large-capacity commercial machines, either a Wolfking 160 or Palmia 200, with sieve plates having a 13 mm pore size. Diets were prepared in batches of 50 - 100 Kg which were minced twice to give a mix of uniform consistency, then packed in polythene bags in portions of 1 Kg and re-frozen until required.

### 2:3.1.2 Determination of Dry Weight and Ash-free Dry Weight

Samples of 10 - 20 g wet weight were placed in glass petri dishes and dried in an oven for 24 hrs. to constant weight. To minimise volatilization of energy-rich organic components such as lipid, the temperature was maintained at 80 °C (Paine, 1964). Water content was obtained by difference. Samples retained for subsequent analyses were stored in a desiccator over either Calcium chloride or silica gel.

Ash-free dry (AFD) weight was determined by further combustion of samples placed in lidded porcelain crucibles in a muffle-furnace for 3 hrs. at 550 °C, as advocated by Cummins and Wuycheck (1971). The mineral or ash content of the samples was obtained by difference.

### 2:3.1.3 Determination of Caloric Value

Samples of each diet were dried by following the above procedure and the material was then ground in a micro hammer mill (Fisons Scientific Apparatus) using a 0.8 mm mesh screen, to yield a fine powder. Approximately 0.5 ml of plastic cement (Tensol no. 12, ICI Ltd.) was added to each sample to act as a binding agent and pellets of 0.3 - 0.8 g were then prepared in a pellet press. The solvent, a dichloromethane mixture, was evaporated off by heating the pellets in an oven at 40 $^{o}$C for a further 24 hrs. Pellets were stored at -20 $^{o}$C over a desiccant of silica gel for up to 48 hrs., until required.

Energy content was determined using a GallenKamp ballistic bomb calorimeter, callibrated by direct proportion using benzoic acid (26.45 KJ/g in air) as a thermochemical standard. Pellets were placed in tared Nickel-Chromium crucibles and weighed in a balance chamber containing silica gel immediately prior to combustion. Galvanometer deflections were recorded at a standard interval of 45 sec. after ignition, at which time the reading was assumed to have reached a maximum.

An alternative procedure was adopted for analyses of eel and twelve-week old chick (CK12) diets. These diets had a relatively high lipid content, and after drying to constant weight, samples were still 'wet' due to the presence of an oily residue which made grinding and pelleting impracticable. Therefore, these stages were omitted and determinations carried out on small samples of 0.5 - 1 g. which were dried down in the crucibles and ignited directly.

### 2:3.1.4 Determination of Protein Nitrogen

(i) Digestion of samples

A variant of the Kjeldahl procedure was used, in a modification of the method of Fleck and Munro (1965). Samples of 1 - 2 g wet weight were placed in Kjeldahl flasks of 300 ml capacity, to which were then added equal volumes (65 ml) of concentrated sulphuric acid and hydrogen peroxide (100 volumes), and two catalyst tablets each containing 5 g Potassium sulphate and 0.35 g Mercuric oxide (Kjeltabs AAOAC, Thomson and Capper, Ltd.)

The flasks were then heated gently in a fume cupboard using a Soxhlet heating unit, until effervescence ceased and the contents became clear and colourless. They were then heated more vigorously for a further 2 hrs., until digestion was complete.

(ii) Assay of Nitrogen

Assays of total Nitrogen were performed using a procedure based on the method of Mitchell (1972). The technique utilised the phenol-hypochlorite or Bertholet reaction, in which a coloured complex, indophenol blue, is formed from ammonia and phenol in the presence of alkaline Sodium hypochlorite.

Samples of 1 ml were transferred from the digestion flasks to small beakers and neutralised to pH 7 using a solution of 10-M Sodium hydroxide in the presence of a buffer consisting of Potassium hydroxide in 1-M Potassium di-hydrogen orthophosphate. After dilution to 500 ml, 1 ml aliquots were placed in tubes containing 0.1 ml of EDTA (1%, w/v, pH 10), 6.9 ml of water and 1 ml of phenol reagent comprising 0.11-M phenol and 0.17-µM Sodium nitroprusside. Colour was developed by the addition of 1 ml of hypochlorite reagent comprising 26-mM anhydrous di-Sodium hydrogen orthophosphate, 84-mM tri-Sodium orthophosphate, 0.125-M Sodium hydroxide and 10-mM Sodium hypochlorite solution (approximately normal in 0.1-N Sodium hydroxide). Absorbance was measured at 625 nm after 2 hrs.

Standard curves, prepared using a 0.1-M solution of ammonium sulphate were found to be linear for concentrations from 0.007 - 0.035-mM and determinations of dietary Nitrogen made on the basis of direct proportionality. Protein content was estimated using the standard multiplication factor of 6.25. The effect of increasing digestion time on Nitrogen yield was tested using internal standards of ammonium sulphate, and the percentage recovery of known quantities of Nitrogen determined by direct assay, as described above.

De-ionised water was used throughout in the preparation and execution of all assays. Reagents were stored in the dark at 4 $^{o}$C and replaced as necessary at intervals of not more than 14 days.

### 2:3.1.5 <u>Determination of Total Lipid</u>

(i). Saponification and extraction

Total lipid was assayed in the form of free fatty acid. Saponification of dietary fats was carried out according to the procedure devised by van der Kamer, Bokkel Huinink and Weyers (1949).

Samples of 1 - 4 g wet weight were placed in 'quick-fit' flasks of 250 ml capacity, to which were added 10 ml of 5.88-M Potassium hydroxide solution and 40 ml of 96% ethanol containing 0.4% amyl alcohol. Each flask was then attached to a condenser (400 mm effective length) and the joint sealed with silicone lubricant before boiling under reflux for 30 min. Heat was supplied from a water bath, and coolant water was drawn from the mains supply. After refluxing, the water bath was drained and refilled with water and crushed ice, and the digests allowed to cool for a period of 20 min. Free fatty acids were liberated by the addition of 17 ml of 1.15-M hydrochloric acid, and the digests again cooled before extraction of the fatty acids into petroleum spirit (b.p. 60 - 80 °C fraction).

Measured volumes of petroleum ether (50 - 100 ml, depending on sample weight and estimated lipid content) were introduced into the flasks using a bulb pipette of 25 ml capacity and the necks sealed with ground glass stoppers. The flasks were then shaken vigorously for 1 min. Separation of organic and aqueous layers was complete after approximately 5 min., and the extraction after a further 25 min.

### (ii). Assay of free fatty acids

Fatty acids were assayed using a modification of the method described by Antonis (1965). This entailed the production of their copper salts in chloroform, followed by estimation of the copper content of the organic phase by reaction with Zinc dibenzyldithiocarbamate (ZnDDC).

Aliquots of 100 ul were transferred from the digestion flasks to glass centrifuge tubes which had previously been treated with a solution of dimethyldichlorosilane (1%, v/v), and evaporated to dryness under a stream of Nitrogen. The lipid residue was then re-dissolved in 5 ml of chloroform, to which was added 2.5 ml of Copper reagent comprising 3.8% (v/v) cupric sulphate pentahydrate, 0.45-M triethanolamine and 50-mM glacial acetic acid. The preparations were mixed thoroughly for 12 - 15 sec. on a 'whirlimixer' (Fisons Scientific Apparatus), then centrifuged at low speed (16000 G) for 10 min.

Following centrifugation the aqueous upper phase was removed by aspiration with a Pasteur pipette, and a 3 ml aliquot of the lower organic phase transferred to another tube. Colour was developed by the addition of 0.5 ml of ZnDDC (0.3%, v/v) in chloroform, and the absorbance of each tube measured after 15 min. at 440 nm using a Pye-Unicam SP8-100 spectrophotometer.

Standard curves, prepared with a 0.78-M solution of palmitic acid in chloroform, were found to be linear for concentrations of 15 - 150-uM and calculations were performed on the basis of direct proportionality. All assays were carried out in triplicate, with blanks and standards included in each series. Stock standard solutions were stored under Nitrogen at -20 $^{o}$C in sealed glass receptacles. Copper reagent was replaced every 10 days.

Extraction efficiency was estimated using triolein (glycerol trioleate) as an internal standard. Measured quantities of a 3.39-mM solution of triolein in anhydrous isopropanol were boiled under reflux and saponified as described in (i) above. The resulting oleic acid residues were then assayed using the method described above.

### 2:3.1.6 Gas-liquid Chromatographic Analysis of Dietary Fatty Acid Composition

(i) Thin-layer chromatographic separation of lipids

TLC separation of lipid classes was carried out to ensure that only fatty acid methyl esters derived from the methanolysis of fatty acid would be present in subsequent GLC analyses. Samples of lipid, extracted into petroleum ether as described in 2:3.1.3 above, were evaporated to dryness under Nitrogen and re-dissolved in a small volume of chloroform. Aliquots of 25 ul were then applied in duplicate as 1 cm 'streaks' to activated plates of Kieselgel 60 H. A solvent system of petroleum ether (b.p. 40 - 60 °C fraction)/ diethyl ether/ acetic acid (60:40:1, by volume) was used to separate lipid classes. Plates were run in chromatography tanks which had been flushed with Nitrogen immediately prior to use.

After development and drying, one set of each pair of duplicate sets was visualised by exposure of the appropriate portion of the plate to Iodine vapour. Silica gel was scraped off the non-exposed portions of the plate from those areas corresponding to the position of the visualised samples, and the scrapings stored under Nitrogen at -20 °C in tightly sealed bijou bottles, until required. Subsequent visualisation of entire plates was carried out to ensure that scrapings had been taken from the appropiate areas.

### (ii) Preparation of fatty acid methyl esters

Fatty acid methyl esters were prepared using the method of Morrison and Smith (1964). Boron trifluoride in methanol (14%, w/v) was added to the bijou bottles in sufficient quantity to 'wet' the silica gel and, after placing 'Tuf-bond' teflon seals in the screw caps, the bottles were tightly sealed and heated at 100 °C for 15 min.

After cooling, a stream of Nitrogen was passed over the samples to volatilise residual Boron trifluoride reagent and other contaminants. Fatty acid methyl esters were eluted from the dried samples with n-hexane and each suspension was filtered through a Pasteur pipette plugged with glass wool. After evaporation of the filtrates to minimal volumes (approximately 20 µl), aliquots of 0.5-1.0 µl were injected directly onto the GLC column.

### (iii) Gas-liquid chromatography of fatty acid methyl esters.

Separations were carried out using a Shimadzu GC-9A gas chromatograph fitted with glass columns 2 m in length, of internal diameter 2.0 mm and external diameter 6.0 mm (J.J. (Chromatography), Ltd.). The columns were packed with a cyano-silicone stationary phase, 10% Alltech CS-5, on a chromasorb WAW 100-120 mesh support. Nitrogen was used as the carrier gas, with a flow rate of 55 ml/min and resolved components were detected by a flame ionisation combustion system (hydrogen/air). Pilot studies using pentadecanoate methyl ester had shown the response to be linear over the range $10^{-8}$ g - $10^{-4}$ g (Talbot, 1986). During each separation the two columns (sample and reference) were run in a temperature program having a single temperature ramp, described by the parameters listed below:-

| | |
|---|---|
| Initial temperature | 210 $^{o}$C |
| Initial time | 10 min. |
| Program rate | 4 $^{o}$C/min. |
| Final temperature | 250 $^{o}$C |
| Final time | 7 min. |

Peaks were identified by comparing their retention times with a series of fatty acid methyl ester standards. All retention times were expressed relative to palmitic acid (C16:0) and stearic acid (C18:0), to facilitate comparison between samples having different absolute retention times.

Determinations of the percentage mass contribution of individual fatty acids to the total fatty acid present were made with the aid of a Trilab 2 computing integrator (Trivector Scientific, Ltd.) connected to the flame ionisation detector. Calculations were carried out on the basis of peak area.

## 2:4 Results

The gross composition of the diets analysed is depicted in Fig. 2:1. Sample means of each of the main constituents are presented in Table 2:4, together with the standard errors of the means. Comparisons between diets were made using 'Student's ' t-test, and the results are tabulated in Tables 2:5 and 2:6, and in Tables 2:9 - 2:11. Values given therein are the levels of significance, expressed as maximum values of 'p'.

### 2:4.1 Variations in Gross Composition

Dry-matter' was calculated by difference from determinations of water content. As can be seen from Table 2:4, the proportion of dry-matter varied over a relatively narrow range. In EEL, dry-matter constituted on average 41.16% of the wet weight, in RBBT only 30.7%. Values for the other diets were intermediate between these two. Comparison of the means revealed that there were significant differences between the diets, and the results are presented in Table 2:5. The dry-matter content of EEL was significantly higher than for each of the other diets, and that of RAT (34.04%) higher than the remaining three, RBBT, CK04 and CK12, which were not significantly different from each other.

Ash content was found to be more variable, ranging from 2.43% of wet weight in CK12 to 4.99% in RAT (see Table 2:4). Both RAT and CK04 contained a high proportion of ash, and were not significantly different from each other, though in each the value was higher than in the other diets. As

Fig. 2:1 Composition of Diets Fed to Mink
(As Percentage of Wet Weight)

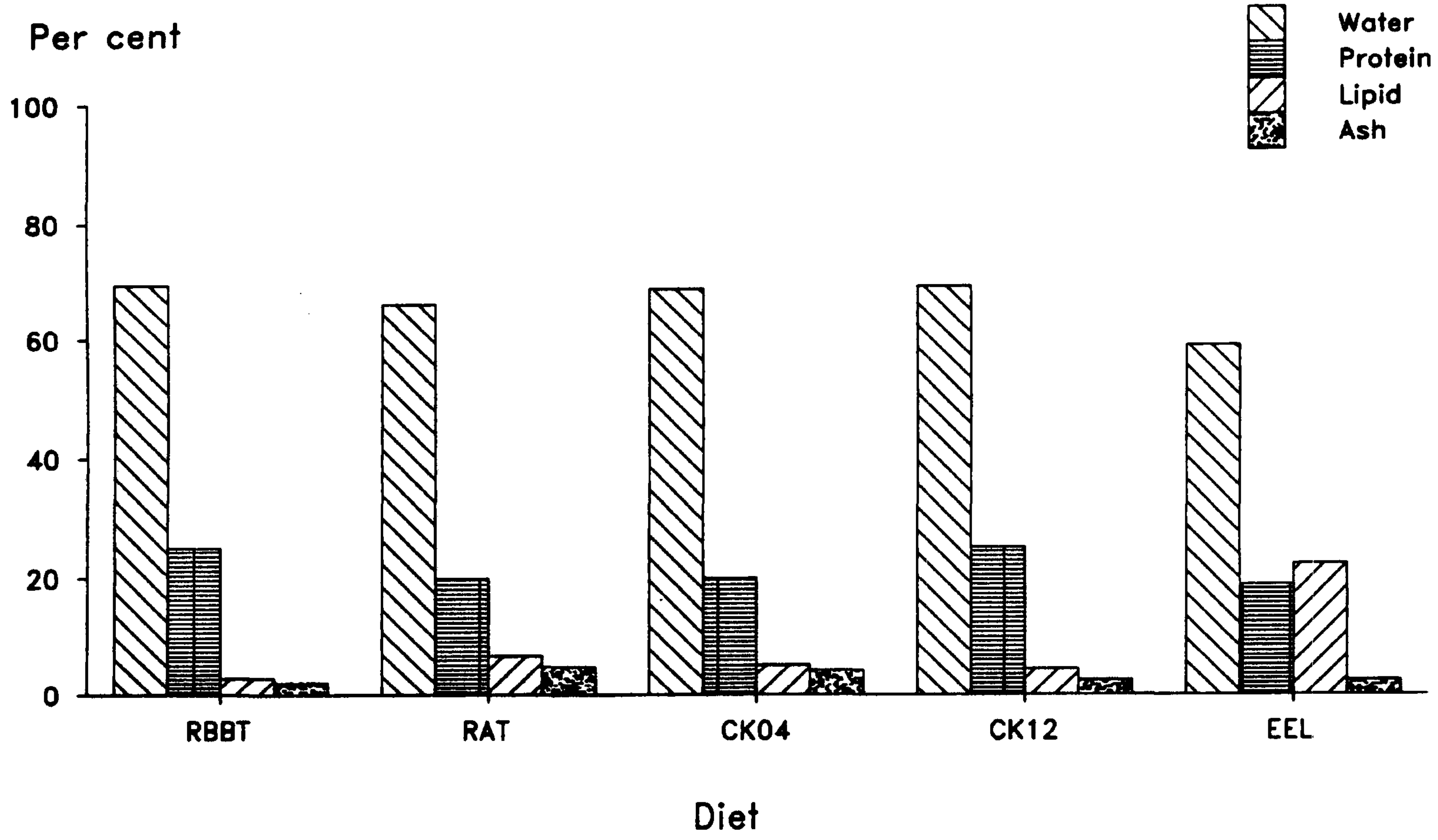


Table 2:4 Proximate Composition and Energy Content of Diets

| | RBBT | RAT | CK04 | CK12 | EEL |
|---|---|---|---|---|---|
| Protein | 24.61 (0.56) | 19.82 (0.40) | 21.36 (0.57) | 24.95 (0.68) | 18.76 (0.59) |
| Lipid | 2.84 (0.26) | 6.71 (0.74) | 5.15 (0.32) | 4.41 (2.92) | 22.22 (1.25) |
| Ash | 2.53 (0.02) | 4.89 (0.56) | 4.22 (0.12) | 2.43 (0.02) | 2.50 (0.28) |
| Water | 69.30 (0.26) | 65.96 (0.20) | 68.50 (0.19) | 68.93 (0.22) | 58.84 (0.29) |
| TOTAL | 99.28 | 97.37 | 99.22 | 100.70 | 102.32 |
| Dry-Matter | 30.70 | 34.04 | 31.50 | 31.07 | 41.16 |
| Energy (KJ/g) | 6.27 (0.11) | 8.56 (0.09) | 7.69 (0.09) | 7.22 (0.06) | 12.95 (0.22) |

Table 2:5 Comparison of Dietary Dry Matter Content

| Diet | N | RBBT | RAT | CK04 | CK12 | EEL |
|---|---|---|---|---|---|---|
| RBBT | 10 | * | 0.001 | 0.05 | N.S. | 0.001 |
| RAT | 9 | | * | 0.001 | 0.001 | 0.001 |
| CK04 | 10 | | | * | N.S. | 0.001 |
| CK12 | 12 | | | | * | 0.001 |
| EEL | 10 | | | | | * |

Table 2:6 Comparison of Dietary Ash Content

| Diet | N | RBBT | RAT | CK04 | CK12 | EEL |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | 0.01 | 0.001 | N.S. | N.S. |
| RAT | 8 | | * | N.S. | 0.01 | 0.001 |
| CK04 | 8 | | | * | 0.001 | 0.001 |
| CK12 | 7 | | | | * | N.S. |
| EEL | 5 | | | | | * |

shown in Table 2:6, no significant differences were found between RBBT, CK12 and EEL.

The variability in water and ash content as a percentage of wet weight has led several authors (Cummins and Wuycheck, 1971; Paine, 1971) to advocate the use of dry weight or ash-free dry weight as the basis for comparative studies of gross composition. To facilitate comparison of these results with the findings of other workers, the values presented in Table 2:3 have therefore been re-calculated using standard units of gramme dry weight and gramme ash-free dry weight, as shown in Table 2:7. For the purposes of the present study, however, composition 'as fed' is clearly the most appropriate basis for comparison, and statistical analyses were conducted only on data expressed in those terms.

The caloric values of the diets (KJ/g wet weight), which are included in Table 2:4, and corrected to dry weight and ash-free dry weight in Table 2:8. Each one was significantly different in energy content from every other diet, as shown in Table 2:9, with EEL having the highest value of 12.95 KJ/g and RBBT the lowest, at only 6.27 KJ/g.

Protein content was highest in CK12 and RBBT, at 24.95% and 24.61% of wet weight, respectively, and there was no significant difference between these two. The EEL diet was found to have the lowest protein content, with a value of 18.76% wet weight, which was significantly lower than all the other diets except RAT (19.82%) (see Table 2:10).

As expected, the EEL diet contained the highest proportion of lipid which, at 22.22% of wet weight, was far higher than any of the others (see Tables 2.4 and 2.7). Values for RAT, CK04 and CK12 were all similar, though significant differences were found between RBBT (2.84%) and CK12 (4.41%), as shown in Table 2:11. Lipid content was lowest in the RBBT diet, at only 2.84% of wet weight.

Table 2:7 Proximate Composition of Diets

| | | RBBT | RAT | CK04 | CK12 | EEL |
|---|---|---|---|---|---|---|
| Protein | wet | 24.61 | 19.82 | 21.36 | 24.95 | 18.76 |
| | dry | 80.15 | 58.23 | 67.81 | 80.30 | 45.58 |
| | ash-free dry | 87.34 | 67.99 | 78.30 | 87.12 | 48.53 |
| Lipid | wet | 2.84 | 6.71 | 5.15 | 4.41 | 22.22 |
| | dry | 9.25 | 19.71 | 16.35 | 14.19 | 53.98 |
| | ash-free dry | 10.08 | 23.02 | 18.88 | 15.40 | 57.48 |
| Ash | wet | 2.53 | 4.89 | 4.22 | 2.43 | 2.50 |
| | dry | 8.24 | 14.36 | 13.39 | 9.40 | 6.07 |

Table 2:8 Weight-specific Energy Content of Diets

| KJ/g | RBBT | RAT | CK04 | CK12 | EEL |
|---|---|---|---|---|---|
| Wet | 6.27 | 8.56 | 7.69 | 7.22 | 12.95 |
| Dry | 20.42 | 25.16 | 24.43 | 23.07 | 31.47 |
| Ash-free Dry | 22.25 | 29.38 | 28.20 | 25.03 | 33.51 |

Table 2:9 Comparison of Dietary Energy Content

| Diet | N | RBBT | RAT | CK04 | CK12 | EEL |
|---|---|---|---|---|---|---|
| RBBT | 15 | * | 0.001 | 0.001 | 0.001 | 0.001 |
| RAT | 15 | | * | 0.001 | 0.001 | 0.001 |
| CK04 | 15 | | | * | 0.001 | 0.001 |
| CK12 | 21 | | | | * | 0.001 |
| EEL | 11 | | | | | * |

Table 2:10 Comparison of Dietary Protein Content

| Diet | N | RBBT | RAT | CK04 | CK12 | EEL |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | 0.001 | 0.001 | N.S. | 0.001 |
| RAT | 10 | | * | 0.05 | 0.001 | N.S |
| CK04 | 10 | | | * | 0.001 | 0.005 |
| CK12 | 10 | | | | * | 0.001 |
| EEL | 9 | | | | | * |

Table 2:11 Comparison of Dietary Lipid Content

| Diet | N | RBBT | RAT | CK04 | CK12 | EEL |
|---|---|---|---|---|---|---|
| RBBT | 7 | * | 0.001 | 0.001 | 0.005 | 0.001 |
| RAT | 8 | | * | N.S. | 0.05 | 0.001 |
| CK04 | 8 | | | * | N.S. | 0.001 |
| CK12 | 11 | | | | * | 0.001 |
| EEL | 6 | | | | | * |

From Table 2:4 it can be seen that summation of the values obtained from direct assay of each of the individual constituents of the diets yields a total of approximately 100%; the overall mean of 99.78% for all diets indicating that the wet-weight composition of the diets 'as fed' had been accurately determined. As an additional check on the results obtained by calorimetry, data from these analyses were compared with expected values based on proximate composition. A figure of 23.83 KJ/g was used as the caloric equivalent of protein, and 39.71 KJ/g for lipid, to derive an estimate of the gross energy content of each pellet combusted (Kleiber, 1961). The relationship between observed and expected values is depicted in Fig. 2:2, and the means for each diet tabulated in Table 2:12. Again, the reliability of the data discribed above is borne out by the results obtained, there being no significant difference between the two values in any of the diets.

### 2:4.2 Comparison of Fatty Acid Composition

As described in section 2:3.1.6, the constituent fatty acids of the lipid fractions analysed were identified by comparing their retention times with those of a series of fatty acid methyl ester standards. The standards used, together with their mean absolute retention times are listed in Table 2.13. Also given are the retention times relative to palmitate and stearate, which were calculated to enable positive ideentification of individual fatty acids from different samples.

GLC analyses were carried out on two diets, RBBT and EEL. Previous assays of total lipid (section 2:3.1.5) had revealed that RBBT had the lowest lipid content at only 2.84% of wet weight, and EEL the highest at 22.22%, a 7.8-fold difference. As noted in section 2:3.1.3 above, the

# Fig 2:2 Relationship of Observed to Estimated Dietary Energy Content

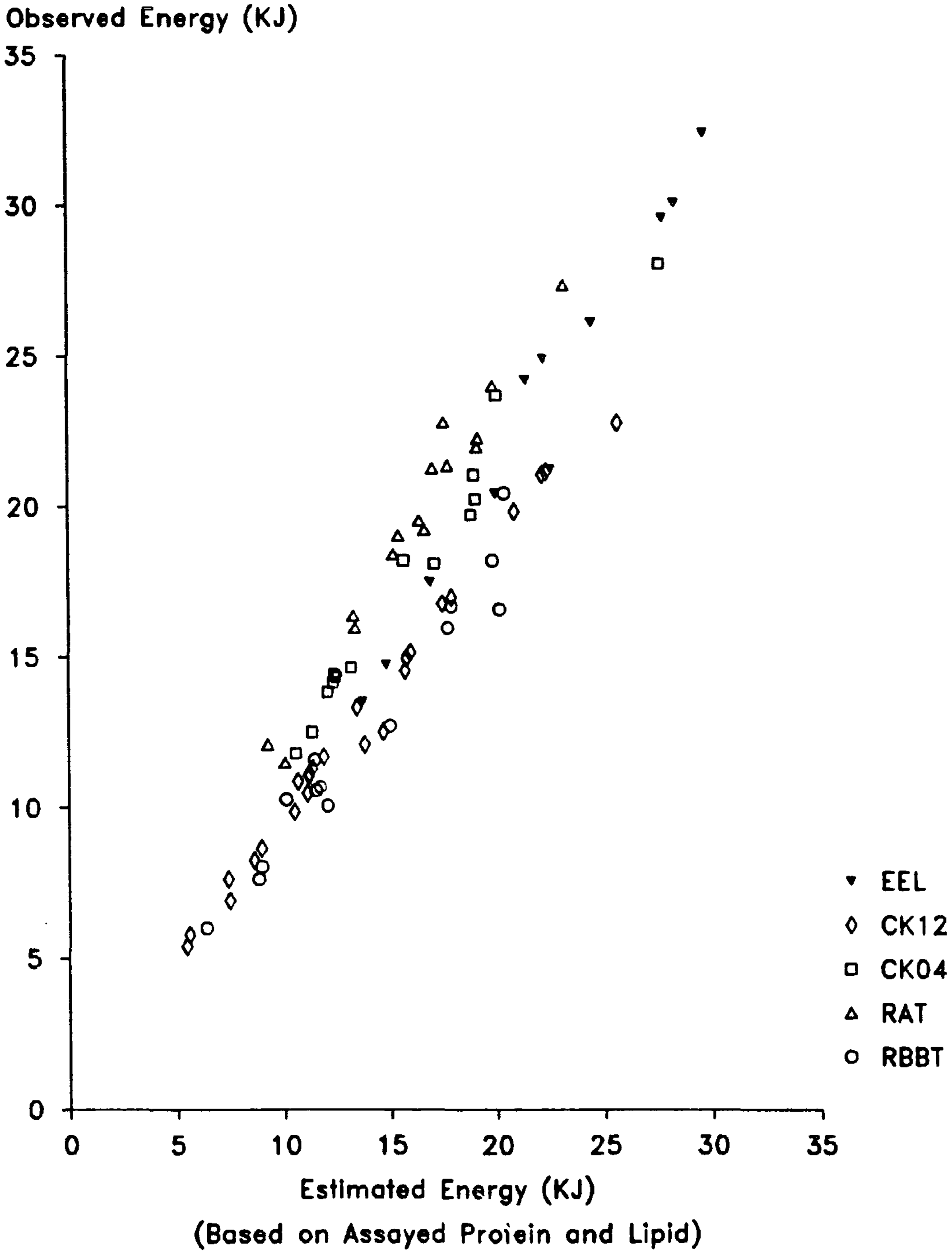


Table 2:12 Comparison of Observed Caloric Value of Diets and Estimates Calculated from Proximate Composition

| Diet | N | Observed Value (KJ/g) | Estimated Value (KJ/g) |
|---|---|---|---|
| RBBT | 15 | 12.40 (1.09) | 13.86 (1.21) |
| RAT | 15 | 19.54 (1.10) | 16.92 (0.99) |
| CK04 | 15 | 17.28 (1.19) | 16.14 (1.24) |
| CK12 | 21 | 12.80 (0.97) | 13.85 (1.10) |
| EEL | 11 | 23.17 (1.90) | 23.61 (1.73) |

Table 2:13 Retention Times of Fatty Acid Methyl Ester Standards

| Peak No. | Retention time (min.) | Rt 16:0 | Rt 18:0 | Formula | Familiar Name | Systematic Name |
|---|---|---|---|---|---|---|
| 1 | 1.08 | 0.33 | 0.18 | 12:0 | Lauric | Dodecanoic |
| 2 | 1.87 | 0.57 | 0.32 | 14:0 | Myristic | Tetradecanoic |
| 3 | 2.17 | 0.66 | 0.36 | 14:1 | Myristoleic | Cis-9-tetradecenoic |
| 4 | 3.30 | 1.00 | 0.56 | 16:0 | Palmitic | Hexadecanoic |
| 5 | 3.73 | 1.13 | 0.63 | 16:1 | Palmitoleic | Cis-9-hexadecenoic |
| 6 | 5.93 | 1.80 | 1.00 | 18:0 | Stearic | Octadecanoic |
| 7 | 6.60 | 2.00 | 1.11 | 18:1 | Oleic | Cis-9-octadecenoic |
| 8 | 7.75 | 2.34 | 1.31 | 18:2 | Linoleic | Cis,cis-9,12-octadecadienoic |
| 9 | 9.37 | 2.84 | 1.58 | 18:3 | Linolenic | All cis-9,12,15-octadecatrienoic |
| 10 | 10.75 | 3.25 | 1.81 | 20:0 | Arachidic | Eicosanoic |
| 11 | 11.82 | 3.58 | 1.99 | 20:1 | Gondoic | Cis-11-eicosenoic |
| 12 | 16.45 | 4.98 | 2.77 | 22:0 | Behenic | Docosanoic |
| 13 | 17.35 | 5.26 | 2.93 | 20:4 | Arachidonic | All Cis-5,8,11,14-eicosatetrienoic |
| 14 | 21.13 | 6.40 | 3.56 | 24:0 | Lignoceric | Tetracosanoic |
| 15 | 22.02 | 6.67 | 3.71 | 22:6 | - | 4,7,10,13,16,19-docosahexaenoic |

physical properties of lipid derived from RBBT differed from those of EEL, which had a lower melting point and was liquid at room temperature. This suggested that there were both quantitative and qualitative differences between the two.

The fatty acid composition of each of the diets is depicted in Table 2:14, from which it can be seen that the most important constituents were myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids. These seven accounted for 91.9% and 89.3% of the total lipid mass in RBBT and EEL, respectively. The lipid moiety of EEL was characterised by relatively high proportions of the long-chain fatty acids, especially the polyunsaturated fatty acids such as oleic and docosahexaenoic acid (22:6), which together made up 35.7% of the total. The percentages of myristic, palmitoleic and behenic (22:0) acids were also significantly higher in EEL than in RBBT ($p < 0.05$).

RBBT lipid comprised a higher percentage of shorter chain and saturated fatty acids. Palmitic, stearic, linoleic and linolenic were the most important, constituting 70.23% of the total. Each was found to be present in significantly higher proportions ($p < 0.05$) in the lipid fractions of the RBBT diet compared with those from EEL.

## 2:5 Discussion

### 2:5.1 Gross Composition of the Diets

Though the diets were all superficially of a similar gross composition, comparison of the proportions of the major constituents revealed significant differences between the diets.

CK12 and RBBT were both characterised by a relatively high proportion of protein and lower levels of lipid than RAT and CK04, a result which may

Table 2:14 Fatty Acid Composition of Rabbit and Eel Diets

| Peak No. | Familiar Name | Formula | Pct. of total fatty acid | | |
|---|---|---|---|---|---|
| | | | RBBT | | EEL |
| 1 | Lauric | 12:0 | 1.04 | | 0.5 |
| 2 | | 12:? | 0.39 | | 0.22 |
| 3 | Myristic | 14:0 | 3.45 | * | 6.67 |
| 4 | Myristoleic | 14:1 | 0.70 | | 0.87 |
| 5 | | 14:2 | 1.03 | * | 0.36 |
| 6 | | 14:3 | 0.17 | | 0.15 |
| 7 | Palmitic | 16:0 | 29.03 | * | 23.75 |
| 8 | Palmitoleic | 16:1 | 4.44 | * | 10.51 |
| 9 | | 16:2 ? | 0.38 | | 0.96 |
| 10 | | 16:3 ? | 0.25 | | 0.57 |
| 11 | Stearic | 18:0 | 7.96 | * | 3.73 |
| 12 | Oleic | 18:1 | 13.78 | * | 33.03 |
| 13 | Linoleic | 18:2 | 12.18 | * | 9.96 |
| 14 | Linolenic | 18:3 | 21.06 | * | 1.66 |
| 15 | Arachidic | 20:0 | 0.05 | | 0.05 |
| 16 | Gondoic | 20:1 | 0.34 | | 1.43 |
| 17 | | 20:2 ? | 0.20 | | 0.24 |
| 18 | | 20:3 ? | 0.17 | | 0.22 |
| 19 | Behenic | 22:0 | 0.14 | * | 0.95 |
| 20 | | 22:1 ? | – | | – |
| 21 | Arachidonic | 20:4 | – | | 0.05 |
| 22 | Lignoceric | 24:0 | 0 06 | | 0.02 |
| 23 | – | 22:6 | 0.17 | * | 2.67 |
| | | Total | 96.99 | | 98.57 |

have been attributable to the increased muscle mass of the larger items. The low lipid content of the RBBT diet may be explained by the fact that, apart from the eels, rabbit was the only one of the five prey types obtained from a wild population; RAT, CK04 and CK12 diets were all composed of animals maintained under artificial conditions, with food provided ad libitum and the opportunity to lay down substantial amounts of depot fat. Hayward (1965) found similar differences in the lipid content of wild-caught and laboratory-raised deer mice P. maniculatus, with animals maintained in the laboratory having a fat content twice that of their free-living counterparts.

The composition of eel was so radically different from that of the other species examined that meaningful comparisons are difficult, though clearly the very high levels of lipid in the body tissue account for most of the differences observed. Recalculation of body composition on a basis of lean or fat-free dry weight is an expediency employed by many workers in studies of species in which fat content is highly variable, for instance certain migratory birds (Newton, 1968; Myrcha and Pinowski, 1970; Chilgren, 1977; Dolnik and Gavrilov, 1979). By applying the technique to the data collected in the present study it can be shown that the basic body composition of eel does not differ greatly from that of other species. The values for protein content as a percentage of fat-free dry weight were 91.89, 80.24, 83.53, 91.19 for RBBT, RAT, CK04, CK12 respectively, and 88.29 for EEL. For ash, the revised figures were 9.32, 19.8, 16.5, 8.89 and 11.76.

The comparatively high proportion of ash in CK04 and RAT is perhaps suprising, given that it is derived mainly from skeletal material. The massiveness of the skeleton is related to age and body weight, with younger animals having a lighter and less robust frame (Gorecki, 1965). Relative increases in the mass of muscle and adipose tissue with age and size, however, may explain the differences observed between these two and the larger and older individuals of which the RBBT and CK12 diets were composed. Progressive pneumatisation of the avian skeleton as development proceeds may also be a contributory factor. Similar findings were reported by Leoschke (1959) in studies of several poultry-based diets fed to mink. Rations comprising chicken heads and feet were found to have the lowest level of protein, due to the very high ash content of these components.

Differences in the energy values of the diets were attributable mainly to variations in protein and lipid content. The use of caloric equivalents of these components to derive estimates of energy content demonstrates the close correlation between these three parameters, and explains the high energy values obtained from the lipid-rich RAT, CK04 and EEL diets.

The carcasses of which the experimental diets were composed were chosen as being representative, in size and composition, of the prey types occurring in the diet of wild mink. With regard to size, rats, rabbits and eels were all assumed to be of a similar weight to the prey types which they were intended to represent, and the two size classes of domestic fowl chosen were thought to provide acceptable substitutes for large and medium-sized avian prey.

For comparisons of gross composition, however, it is also necessary to ensure that the chemical make-up of the animals analysed does not differ from that of their free-living counterparts, and that the species or type of animal used is typical of the group it represents.

This was assured only for the rabbit diet, which comprised individuals of a known prey species, culled from a natural population. Moreover, similar investigations by other workers have yielded results which are in close agreement with those obtained in the present study; Golley et al

(1965) estimated energy content to be 19.44 KJ/g dry weight, while Moors (1977) recorded a value of 20.69 KJ/g, suggesting that these figures are typical of the species.

The importance of lagomorphs in the feeding ecology of mink is well documented (Wise et al, 1981; Casson and Klimstra, 1983; Birks and Dunstone, 1985), and the wild-caught specimens used in the present study were assumed to be of a similar chemical make-up to other members of the group. Comparisons with the findings of other workers, however, indicated that this was not the case. Myrcha (1965) found the caloric value of European hares Lepus europeus to be considerably higher (23.75 KJ/g dry weight), owing to the larger proportion of lipid in this species (19.4% of dry weight). Protein constituted only 33.68% of dry-matter. By comparison, Snowshoe hares Lepus americanus have been shown to contain much less protein and fat (72.2% and 3.7% of dry weight, respectively) than either of the other two species studied (Davison et al, 1978). In fact, variation between species within this group (large mammal, lagomorph) is greater than that between groups for some of the prey types used in this study. These findings indicate that while the composition of the REBT diet may accurately reflect that of rabbits predated by wild mink, it is not necessarily true that the diet itself is representative of that catagory of prey items designated 'large mammal', even when the term is restricted to lagomorphs alone.

Consideration has already been given to the variations in the body composition of small rodents, particularly with regard to fat content. Diet, age, season and habitat have all been found to contribute to observed differences between and within species (Gorecki, 1965; Sawicka-Kapusta, 1968; Myrcha, 1969; Kaufman and Kaufman, 1975; Bergeron, 1976). The relatively large proportion of lipid in the laboratory rats used in these experiments was attributed in part to their nutritional history, as postulated by Hayward (1965), though again there is evidence of considerable variability within the group.

Golley (1960) determined the caloric value of *Microtus* spp. to be 19.44 KJ/g dry weight, while Moors (1977) gave a figure of 21.1 KJ/g and Davison (1975) 21.57 KJ/g. Values ranging from 21.44 to 26.46 KJ/g have been quoted for *P. maniculatus* raised in the laboratory, depending on age and size (Kaufman and Kaufman, 1975), and race (Hayward, 1965). Hayward noted marked differences in the fat content of wild and laboratory bred individuals (12.46% and 34.3% of wet weight, respectively), with concomitant changes in the proportion of protein (21.33% compared with 15.12%). In contrast, Bergeron (1976) reported energy contents of 19.68 - 25.16 KJ/g dry weight for wild-caught individuals of the same species. These results, which are comparable to those obtained from analyses of laboratory rats (Harri and Brockway, 1985; present study), again demonstrate the need for caution in making any generalisations about the body composition of a particular species or group.

Relevant studies on birds have generally been confined to investigations of lipid metabolism in relation to the energetics of moult and migration. Wide variations in lipid content (4.43 - 23.9% of wet weight) have been reported by Chilgren (1977) for the white-crowned sparrow *Zonotrichia leucophrys*, while Myrcha and Pinowski (1970) have quoted similar values for the European tree-sparrow *Passer montanus*, in which lipid accounted for 16.4 - 22.9% of live weight. A great deal of information has been collected on the fat content and caloric value of many species but, in general, detailed determinations of gross composition have only been carried out in the course of nutrition studies of other species. Thus Davison *et al* (1978) reported on the protein, lipid, ash and dry-matter content of the quail *Coturnix coturnix* in the course of feeding trials with fishers *Mustela* pennanti. Caloric values of 27.09 KJ/g dry-matter were recorded for samples consisting of 49.2% protein and 39.2% lipid. Similarly Moors (1977), working with weasels, determined the energy content of starlings Sturnus vulgaris to be 24.33 KJ/g dry weight, while Golley et al (1965), feeding bobcats Lynx rufus a diet of domestic fowl, derived a value of 23.64 KJ/g. These results are very close to the figures of 23.07 and 24.43 KJ/g found for CK04 and CK12 in the present study.

Investigations into the composition of fish are usually of much more limited application, often being confined to analyses of the edible portions of the carcass, and sometimes of the fillet alone (Karrick, Clegg and Stansby, 1956; Dugal, 1962; Murray and Burt, 1969; Sidwell, Foncannon, Moore and Bonnet, 1974). Others are concerned only with the biochemical composition of the oil derived from the fish (Gruger, Nelson and Stansby, 1964; Ackman, 1967; Ackman, Eaton, Bligh and Lantz, 1967; Kinsella, Shimp, Mau and Weihrauch, 1977).

Craig, Kenley and Talling (1978) determined the caloric content of perch Perca fluviatilis somatic tissue to be 5.32 KJ/g wet weight, though Craig (1977) had previously shown body composition in this species to be highly variable in relation to season and breeding status.

Thurston et al (1959) presented data on 21 species of freshwater fish, including Perca, burbot Lota lota, and Northern pike Esox lucius, all species featuring in the diet of the mink. Whole body composition of these species averaged around 0.5 - 2% lipid and 18 - 20% protein, on a wet weight basis. The level of protein was relatively constant between the species and the figure of 18.76% obtained for eel in the present study is in close agreement with these findings. Data on eel have been published by Murray and Burt (1969), and Sidwell et al (1974), though in both cases the analyses were performed only on the edible portion of the fish. Sidwell et al (1974) gave a figure of 18% wet weight for protein, 1.3% ash, 67.2 - 70.1% water and 12.7 - 21.5% lipid, with a caloric value of 9.9 - 10.66 KJ/g. The data of Murray and Burt (1969) were similar, though lipid content was given as 8 - 31% of wet weight.

As with the other groups discussed, variation in the gross composition of fish was related mainly to differences in lipid content. In the eel, a migratory species, this varies within very wide limits and to a much greater extent than in most other inshore and freshwater fish.

### 2:5.2 Nutritional Value: Commercial Rations and the Natural Diet

Determinations of gross composition and caloric value revealed significant differences between the diets, and comparison with data collected by other workers indicates that the chemical make-up of the animals used was similar to that of the natural prey types they were intended to represent. It is also clear, however, that the variations in the protein, lipid and energy content within these different groups are often as great as, or greater than those found between the diets themselves. Thus while it may be said that each of these diets reflects the composition of prey items potentially available to wild mink, none should be considered as typical of any particular prey type or group. Since it is impossible to generalise on the nutritional consequences of feeding on any one prey group rather than another, labels such as 'fish diet' or 'small mammal diet' as descriptors of nutritional quality should be used with caution.

Proximate analyses provide a means by which the composition of any prey type can be estimated, but give no indication of it's nutritional value to the predator; the relative proportions of protein, lipid, ash, etc., are no measure of a diet's suitability for promoting fast growth and large adult body size. Rations used in commercial mink farming are carefully formulated to achieve these objectives, and by reference to Table 2:15 it is possible to compare the basic composition of these artificial feeds with that of natural food sources.

In contrast with the diets analysed in the present study, commercial feeds contain, on average, less than half the protein of most animals, and more than twice the lipid, though energy content is approximately the same. As has been discussed in Chapter 1:3, economic considerations oblige the mink farmer to reduce the protein content to the lowest level consistent with a good growth performance, and therefore the values depicted are an indication of minimal requirements. These are usually expressed as caloric equivalents of digestible protein, as a percentage of metabolisable energy (see Table 2:15). For weanling kits the figure is 45 - 50% of metabolisable energy, declining to 25% for maintenance in adults (Jørgensen, 1985). These values are equivalent to approximately 38% and 22% of dry-matter (Leoschke, 1985).

The amount of protein required depends on the ratio of protein to energy in the diet. Sinclair et al (1962) found that raising the caloric value of the ration by increasing the lipid content had the effect of 'sparing' for growth and metabolic processes, protein which otherwise would have been degraded to provide energy. Since the energetic efficiency with which fat is deposited in the mink averages 80%, compared with 45% for protein, the superiority of the high-fat, low-protein ration over other formulations becomes apparent (Jørgensen, 1985).

Disparities in the composition of natural and artificial feeds are due, then, partly to economic considerations as well as those of nutritional energetics. According to the criteria outlined above, most natural diets would be superoptimal in terms of protein and energy content. Gross

Table 2:15 Proximate Composition of Diets used in Laboratory Studies on Mink

| Author | Dry Matter | Protein | Lipid | Ash | Energy (KJ/g) |
|---|---|---|---|---|---|
| Leoschke 1959 | - | 43.0 | 25.0 | 15.0 | - |
| Sinclair, et al 1962 | 31.5 | 42.3 | 10.5 | - | 21.4 GE |
| Allen, et al 1964 | 29.5-52.8 | 15.3-37.0 | 6.3-49.0 | - | 18.6-28.0 GE |
| Roberts and Kirk 1964 | 26.8 | 57.0 | 21.7 | - | 23.2 GE |
| Seier, et al 1970 | 36.1 | 38.5 | 12.6 | 7.7 | 20.8 GE |
| Seier, et al 1971 | 34.5 | 34.5 | 25.2 | - | 23.5 GE |
| Harper, et al 1978 | 32.2-36.5 | 39.0-41.0 | 19.3-19.6 | 7.7-12.4 | 21.7-23.1 GE |
| Glem-Hansen 1979 | 30.4-35.0 | 26.5-53.9 | 13.2-29.4 | 7.5-10.2 | 19.4-38.7 ME |
| Skrede, 1979b | 24.5-30.1 | 28.9-50.6 | 11.0-19.3 | 6.3-13.5 | 15.2-17.4 ME |
| Glem-Hansen, 1980a | 38.0-63.2 | 15.2-44.5 | 11.8-28.2 | 4.7-10.5 | 15.5-20.7 ME |
| Glem-Hansen and Enggaard-Hansen, 1981 | 30.0 | 34.7 | 13.7 | 14.7 | 14.3 ME |
| Tauson, 1985 | 29.3 | 45.0 | 20.7 | 9.9 | 17.6 ME |

composition is not, however, a reliable indication of nutritional value, as has already been noted.

The formulation of artificial feeds is based on the use of components of high digestibility. That of protein varies with it's source, being low for proteins derived from fur, feathers or other highly keratinised parts of the body, such as birds' heads and feet. Moreover, the composition of the protein must be such that digestion yields a mixture of amino acids appropriate to the needs of the animal: i.e., the protein must have a high biological value, a quality entirely independent of digestibility (Moustgaard and Riis, 1957).

The utility of lipids is related to their fatty acid composition, as the fatty acids themselves vary in digestibility. Evidence has been found of interactions between certain constituent fatty acids which may act either to increase or depress the digestibility of others (Artman, 1964), while Jørgensen and Glem-Hansen (1976) demonstrated an inverse relationship between digestibility and the proportion of stearic acid in the lipid.

Clearly, these are qualities which cannot be assessed using the techniques of proximate analysis. For instance, all specimens of a given type are likely to contain approximately the same proportion of protein Nitrogen, but those of a smaller size might be expected to have a higher percentage of low-digestibility proteins derived from pelage, simply from consideration of their relatively larger surface area. Though sharing the same gross composition, these individuals would be of a lower nutritional quality than the larger items.

Therefore, to extend the evaluation of prey utility to considerations of size and source as well as composition, it is necessary to carry out digestibility trials with each type of diet so that these factors can be accurately quantified. It is with this aspect of the study that the following chapter is principally concerned.

# Chapter Three

# FEEDING TRIALS WITH ADULT MINK

## 3:1 Introduction

As discussed in the preceding chapter, the nutritional value of a diet is defined in terms of it's gross composition and the biological utility of it's constituents. The latter can only be determined through *in vivo* studies carried out under carefully controlled conditions.

Reference has already been made in Chapter One to the large body of literature which now exists, pertaining to the nutrition of captive mink feeding on artificial or semi-natural diets. Investigations directed towards determining the requirements for optimal growth and development, have been made using both adults and growing kits. The requirements of kits, which differ considerably from those of adults, have been extensively documented by a number of workers (Harper *et al*, 1978; Skrede, 1978b; Glem-Hansen, 1980a, 1980b, 1980c; Chwalibog *et al*, 1982) and will be considered in greater detail in Chapter Four.

The present chapter is concerned with studies of adult mink feeding on natural diets, and the comparison of data so obtained with results published by other workers for similar trials in which artificial rations were used. To facilitate such comparisons, however, it is expedient first to define the terms by which studies in animal nutrition are described.

## 3:2 Animal Nutrition: The Rationale of Feeding Trials

The term 'feeding trial' pertains to a wide variety of experimental procedures designed to investigate an animal's response to different types of diet. The most elementary consist of studies of palatability, and estimations of food consumption and nutrient requirements under different conditions, such as temperature, reproductive status, etc. More detailed investigations include the determination of a ration's digestibility, or the digestive efficiency of the subject animal, through comparative analyses of ingested and faecal material. 'Balance' trials incorporate estimates of urinary losses, and aim to provide a complete breakdown of energy and nutrient metabolism.

The energy metabolism of an animal may be described according to the scheme delineated in Fig. 3:1, derived from Robbins (1983). Nitrogen metabolism, which follows the same basic pattern, is illustrated in Fig. 3:2. By referring to these two schematic pathways, it may be seen that studies of an animal's nutritional physiology are directed at four main areas, as follows:-

(i) Ingestion

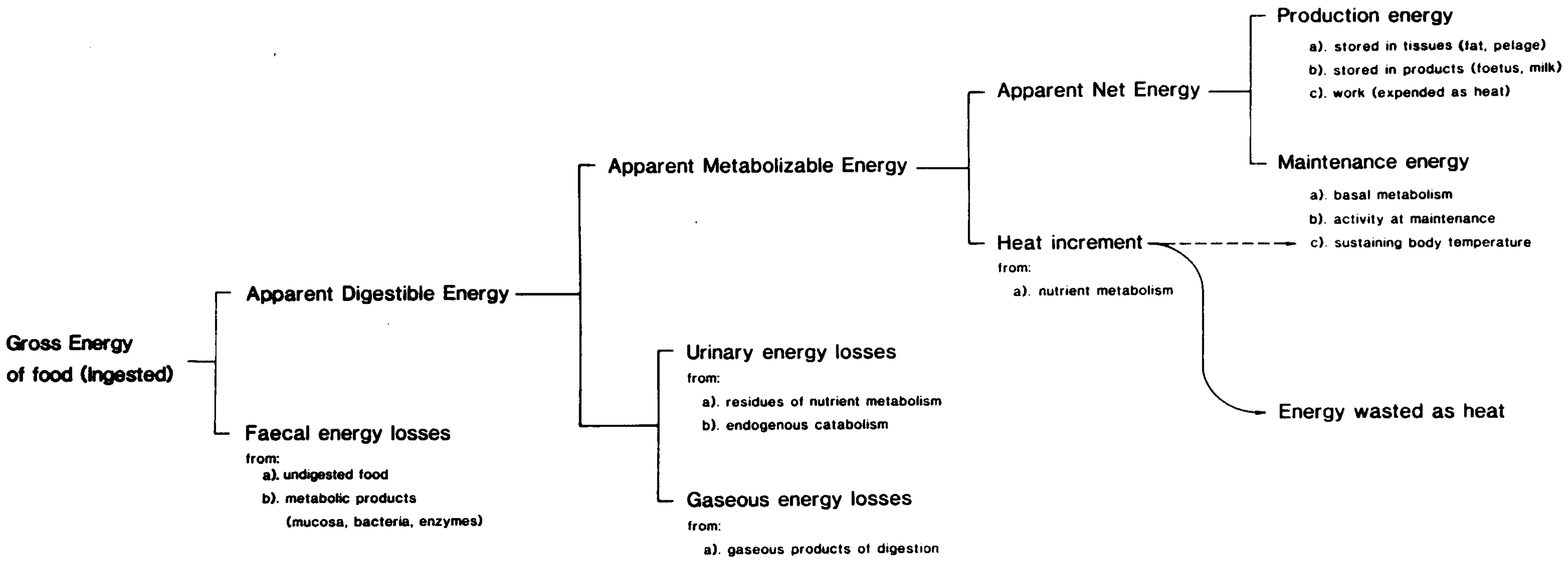
The amount of food ingested is primarily a function of body size. The relationship is non-linear as the weight-specific requirements for maintenance decrease with size; thus metabolic rate and it's covariates, of which food intake is one, are related to body weight by the equation:-

$$M = a.W^{b}$$

(Eqn. 3:1)

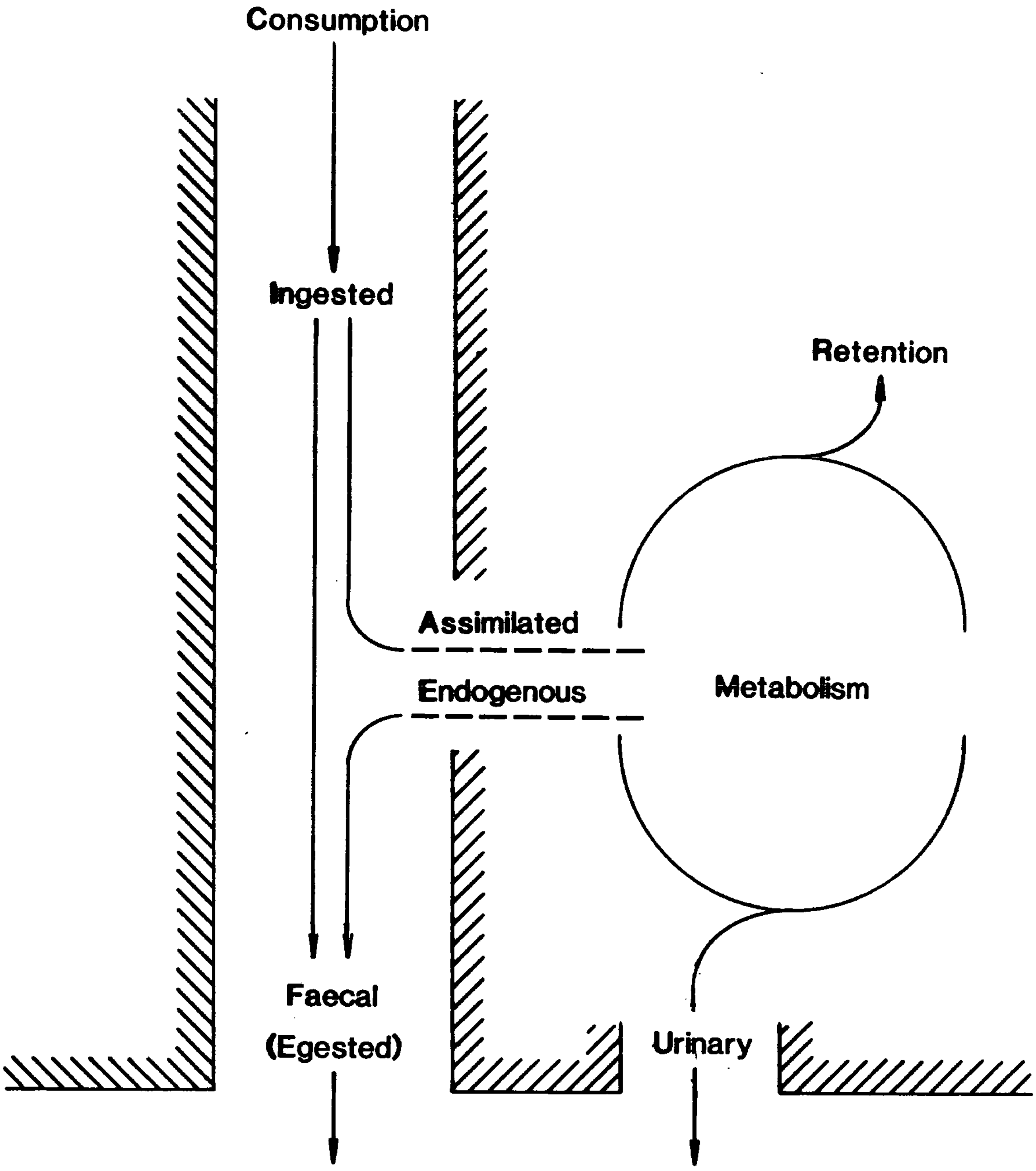
in which 'M' is metabolic rate, 'W' is body weight and 'a' and 'b' are constants. The value of 'b' generally lies between 0.6 and 0.9, and for most species approximates to 0.75 (Kleiber, 1961). 'W ' is known as the Metabolic Weight, and in linearising the relationship between size and metabolic rate provides a standard which forms the basis of most comparative

Fig. 3.1: Apparent Partition of Ingested Gross Energy



(after Robbins, 1983)

Fig.3.2: Nitrogen Metabolism



$$\text{Apparent Digestibility} = 1 - \left[\frac{\text{Faecal}}{\text{Ingested}}\right]$$

studies in animal physiology.

Food intake is known to be correlated with the gross energy content of the ingested material, with intake increasing on rations of low energy value (Sibbald, Bowland, Robblee and Berg, 1957; Miller, 1975; Savory and Gentle, 1976). Intake also varies with metabolic demands. Seier et al, (1971) noted increases in the food consumption of mink during the moult, and a number of workers have reported on the changes associated with pregnancy and lactation (Lockie, 1964; Kaczmarski, 1966; Migula, 1969; Fell, 1972; Cripps and Williams, 1975; Glem-Hansen, 1979).

It is evident that the magnitude of these variations depends largely on the degree to which the composition of the diet meets the animal's needs at any particular time. During the moult, for instance, the requirement is chiefly for protein and in particular, for Sulphur-containing amino acids such as cystine and methionine (Skrede, 1978a; Glem-Hansen and Enggaard-Hansen, 1981; Tauson, 1985b). For diets rich in these substances, the variations in intake are likely to be much less than for those in which amino acid content is limiting (Seier et al, 1971; Glem-Hansen, 1980a).

Ultimately, however, the amount of food which must be ingested depends on the digestive capabilities of the animal, and the proportion of ingested material which is assimilated into the metabolic process. Thus, it is with the determination of digestibility that feeding trials are principally concerned

### (ii) Digestion and Absorption

The 'digestibility' of a substance has been defined as the proportion of the amount ingested which does not appear in the faeces (Robbins, 1983). It is described by a Coefficient of Digestibility, which may be either True (net) or Apparent (gross), though the latter is the most commonly used. Apparent Digestibility is calculated from direct determinations of faecal losses according to the formula:-

$$\text{Apparent Digestibility} = 1 - \frac{(\text{Amount in Faeces})}{(\text{Amount Ingested})}$$

(Eqn. 3:2)

and estimates of Apparent Digestible Energy (ADE), Nitrogen (ADN), Dry Matter (ADDM), etc., are obtained by difference from comparisons of intake with output. They are, consequently, estimates of the absolute amount of a substance absorbed from the lumen of the gut.

An underlying assumption of this method is that there is no endogenous component to the faecal output, the materials assayed being derived solely from the diet. In practice, however, faeces contain not only non-digested feed residues but also metabolic products such as mucus, enzymes and fragments of the intestinal epithelium. Because of this continuous excretion of metabolic faecal Nitrogen and lipid (and their caloric equivalent), Apparent Digestibility coefficients are curvilinear functions of dietary content. Those of True Digestibility include a correction for endogenous faecal losses:-

$$\text{True Digestibility} = \text{Amount Ingested} - \frac{(\text{Total Faecal Loss} - \text{Metabolic Loss})}{\text{Amount Ingested}}$$

(Eqn. 3:3)

and are often used in preference, particularly for comparative studies (Eggum and Christensen, 1974; Kiiskinen et al, 1985). Glem-Hansen and Jørgensen (1973), working on mink, devised a means of estimating metabolic faecal Nitrogen from a knowledge of body weight, age and the dry matter content of the diet, though Farrell and Wood (1968b) had previously shown that, in carnivores, metabolic faecal losses were relatively small and the difference between True and Apparent Digestibility was minimal. Endogenous faecal losses can only be accurately quantified by feeding a protein-free diet or by regression of faecal losses on various levels of intake, both of which procedures necessitate carrying out a further series of trials; coefficients of True Digestibility are, therefore, seldom used for general applications.

Efficient digestion and absorption of foodstuffs results from the interaction of both chemical and physical factors. The biochemical and physiological processes by which digestion is achieved have been comprehensively reviewed elsewhere (Munck, 1981; Thomson and Dietschy, 1981; Carey, Small and Bliss, 1983) and a detailed discussion is beyond the scope of the present work. It is relevant, however, in the context of the feeding trial, to consider the extent to which physical factors affect the digestibility of the rations consumed.

The efficiency with which the animal utilises the ingested material is dependent upon the rate of passage of material along the gut, and the relative magnitude of the surface area across which nutrients are absorbed. Both vary, according to diet quality and nutrient requirements. Rate of passage, for instance, has been found to increase in animals consuming feeds containing a high proportion of non-digestible matter, as gut capacity constitutes a limiting factor on the intake of a sufficient quantity of digestible material to meet the animal's needs (Robbins, 1983).

Rate of passage is usually estimated by the introduction of indigestible markers into the diet and measurement of the time interval between ingestion and first appearance in the faeces, a value known as Bowel Transit Time (BTT) (Kostelecka-Myrcha and Myrcha, 1965; Kotb and Luckey, 1972). The apparent universality of the relationship between rate of passage and the proportion of non-digestible material in the diet has led to the widespread use of BTT as an indicator of diet quality. This is particularly so in trials with animals of economic importance, such as mink, in which the rations fed often contain materials not normally consumed by the animal in it's natural environment (Wood, 1956; Sibbald, Sinclair, Evans and Smith, 1962; Slawinski, Slawon and Bednarz, 1962; Bleavins and Aulerich, 1981).

Diet quality is, however, only one of the determinants of Bowel Transit Time. In studies on the American badger <u>Taxidea taxus</u>, Harlow (1981) reported increases of 18% in the rate of passage in animals which had been fasted. He also noted that Apparent Digestibility was correspondingly higher post-fast than it had been before the fast was begun. Fell (1972) described similar increases in transit time during pregnancy and lactation in a variety of domestic animals, though in contrast with the findings of Harlow (1981), Apparent Digestibility remained relatively constant throughout.

Fell (1972) related these observations to the hypertrophy of the alimentary tract which is known to take place during reproduction. This widely reported phenomenon is believed to be an adaptation permitting the consumption of large quantities of food without loss of digestive efficiency, and may be characterised by increases in the volume of the stomach and intestine, and in the size and density of the mucosal villi. (Myrcha, 1964; Boyne, Fell and Robb, 1968; Cripps and Williams, 1975; Barry, 1976; Karasov and Diamond, 1983).

The adaptive value of gastrointestinal hypertrophy is not confined to meeting the metabolic demands of reproduction; similar developments have been found to occur in animals feeding on low-quality diets, for which the same requirement for a high rate of food intake pertains.

Leopold (1953) examined the alimentary tracts of various species of gallinaceous bird and concluded that the observed differences in gut morphology were attributable mainly to the type and quality of diet consumed; Al-Joborae (1980) found that the dimensions of the digestive tract in the starling *Sturnus vulgaris* changed with seasonal variations in the diet. These changes have been shown to take place over a comparatively small time scale. In the mallard *Anas platyrhynchos* and quail *Coturnix coturnix*, significant increases in the size of the gut were reported after only three weeks on low-quality diets (Miller, 1975; Savory and Gentle, 1976). Gross, Wang and Wunder (1985) noted similarly rapid changes in the digestive tract of cold-stressed meadow voles *Microtus ochrogaster*, in response to increased energy needs. Conversely, Moss (1972) demonstrated that the reverse of these adaptations also took place; in successive generations of red grouse *Lagopus lagopus scoticus*, fed *ad libitum* on a super-optimal diet, the length of the intestine was observed to diminish to only 72% of that typical of wild birds from the same population.

It is evident, then, that neither True nor Apparent Digestibility may be regarded as constant for any particular substance. Both are altered by variations in the absorptive capacity of the animal, effected through changes in the gross morphology of the alimentary tract and the rate of passage of material along it. To some extent these changes are correlated with the nutritional quality of the material ingested, though they arise primarily in response to variations in the animal's metabolic demands.

Comparative investigations of digestive efficiency must, therefore, take into account such factors as the physiological status of the subject animals, their age and nutritional history, and even the duration of the feeding trials themselves, such is the dynamic nature of the processes being studied.

### (iii) Metabolism and the Specific Dynamic Effect

Determination of Apparent Metabolisable Energy (ME) is the next step in the partition of ingested gross energy, and is inextricably linked with Nitrogen metabolism (see Figs. 3:1 and 3:2). It is defined as the ingested energy which is available for the various processes of metabolism, and is equivalent to the Apparent Digestible Energy, less urine losses. Thus, the Coefficient of Metabolisable Energy is calculated according to the general formula given below:-

$$\text{Metabolisable Energy} = \text{ADE} - \frac{\text{Urinary Losses} - \text{Gaseous Losses}}{\text{ADE}} \qquad \text{(Eqn. 3:4)}$$

though in carnivores and other non-ruminants, gaseous losses are negligible and usually ignored.

Urinary losses result mainly from the katabolism of protein and other Nitrogen-containing substances. In the degradation of protein, the constituent amino acids undergo de-amination in the liver, after which the carbon skeletons are usually retained as a source of energy, while the Nitrogen-bearing amine groups are excreted in the form of urea and, to a lesser extent, uric acid, creatine and other associated compounds. The energy contained in the urea so-derived (3.77 KJ/g) constitutes 16% of that which would result from complete oxidation of the original protein (23.83 KJ/g), and thus the amount of energy actually available to the animal is only 20.06 KJ for every gramme of protein ingested. For this reason it has become standard practice for proximate analyses of diet composition to incorporate a correction for Metabolisable Energy content, so that more realistic estimates of the ration's physiological value may be obtained (see Table 2:15).

The coefficient of Metabolisable Energy is highly variable and reflects changes in Nitrogen metabolism in response to diet and metabolic requirements. In animals feeding on diets of relatively high protein content, intake of Apparent Digestible Nitrogen (ADN) may exceed immediate requirements. Most animals lack the capacity to store surplus Nitrogen and therefore, as katabolism of this excess proceeds, urinary Nitrogen (and hence energy) output increases, with a corresponding fall in the level of Metabolisable Energy (Peters and Van Slyke, 1946; Sibbald et al, 1957; Sinclair et al, 1962; Allen et al, 1964; Varley, Gowenlock and Bell, 1980; Keiver et al, 1984; Ronald et al, 1984).

Increases in energy demand also depress Metabolisable Energy retention, as reported by Chwalibog, Glem-Hansen, Henckel and Thorbek (1980) of cold-stressed mink. In these circumstances carbon skeletons derived from amino acid degradation are used to supplement ADE as substrates for oxidative phosphorylation (Sinclair et al, 1962; Allen et al, 1964; Eggum, Chwalibog, Nielsen and Danielsen, 1985).

Conversely, Metabolisable Energy levels rise with increases in Nitrogen retention such as take place during pregnancy (Noblet and Close, 1980) and lactation (Fell, 1972), and the moult (Seier et al, 1971), when urinary losses are minimal. With reference to the last of these three processes, it is interesting to note, however, that on high-protein diets which are deficient in the necessary Sulphur amino acids, compensatory increases in intake lead to higher levels of protein katabolism, and a consequent fall in Metabolisable Energy (Dolnik and Gavrilov, 1979).

A knowledge of Nitrogen balance is clearly of importance in the estimation of Metabolisable Energy. Nitrogen retention may be determined either directly, by carcass analysis (Harper et al, 1978; Glem-Hansen and Enggaard Hansen, 1981), or indirectly, by assay of urine Nitrogen (Sinclair et al, 1962; Glem-Hansen and Jørgensen, 1973; Glem-Hansen, 1980a; Chwalibog et al, 1982). From a knowledge of urine Nitrogen it is also possible to calculate urinary energy losses using a caloric equivalent of urea (Street, Butcher and Harris, 1964; Paladines, Reid, Van Niekerk and Bensadoun, 1964; Eggum et al, 1985), though generally, direct combustion is preferred (Moors, 1977; Chwalibog et al, 1982; Ronald et al, 1984; Kiever et al, 1984; Harri and Brockway, 1985).

As with faecal matter, urinary losses include an endogenous component derived from the normal katabolism of body tissues, and there is thus a parallel dichotomy of True and Apparent Metabolisable Energy. Endogenous urinary Nitrogen is a correlate of Metabolic Weight and may be quantified using regression techniques, as described previously (Glem-Hansen and Jørgensen, 1973), or by the presentation of a Nitrogen-free ration having an energy content high enough to ensure that calorigenic degradation of body protein is minimal. Glem-Hansen and Jørgensen (1973) used the term 'Biological Value' (BV) to describe the efficiency of utilisation of absorbed dietary protein. This index, defined by the formula

$$BV = \frac{N\ Intake - (Faecal\ N - Metabolic\ N) - (Urinary\ N - Endogenous\ N)}{N\ Intake - (Faecal\ N - Metabolic\ N)}$$

(Eqn. 3:5)

incorporates all the parameters mentioned, and constitutes a relative measure of Nitrogen metabolism of widespread application (Glem-Hansen and Eggum, 1974; Glem-Hansen, 1979, 1980a, 1980b).

Though Nitrogen metabolism may be fully described by the five variables combined in the expression above, the partitioning of ingested energy is taken further, with the evaluation of Net Energy. This is the fraction of metabolisable energy which is available to the animal, either for maintenance or production.

Apparent Net Energy coefficients are a measure of the efficiency with which metabolisable energy is utilised, and reflect the energy losses of digestion, absorption and metabolism of the food consumed. The work done in assimilating a given amount of food is indicated by a rise in metabolic rate shortly after ingestion, a phenomenon variously described as the calorigenic effect, heat of nutrient metabolism, or specific dynamic action (SDA). SDA is usually expressed as a percentage of the total caloric value of the digested material. For lipids and carbohydrates, the figure is approximately 5 - 10% (Hill, 1976); in proteins it is much higher, 25 - 30%, due to the processes of degradation and synthesis associated with their metabolism (Ashworth, 1969; Garrow, 1973).

### (iv) Maintenance and Production

Net energy may be apportioned between the tasks of maintenance and production. The energy requirement for maintenance has been defined as the energy needed to sustain basal metabolic rate, body temperature and the normal activities of maintenance, such as feeding, in an animal undergoing zero weight change (Robbins, 1983).

To some extent the costs of thermo-regulation may be met by heat derived from nutrient metabolism; thus, the assumption that the net energy value of lipid and protein may be reduced by 10 - 30% as a result of the work done in assimilating them is not always valid. Nevertheless, the maintanence requirements of mustelids are high, due to their thermo-energetically inefficient body form (Brown and Lasiewski, 1972), and elevated metabolic rate (Iversen, 1972; Moors, 1977; Casey and Casey, 1979; Powell, 1979a). Numerous attempts have been made to estimate maintenance requirements, in terms of ADE (Farrell and Wood, 1968b; Davison et al, 1978), and ME (Moors, 1977; Harper et al, 1978; Chwalibog et al, 1980, 1982).

Production energy incorporates energy stored in new body tissues arising from growth or reproduction, fat deposition (adipose tissue) or the synthesis of new pelage, and the work done in their production. The energetic cost of protein and lipid deposition, and the efficiency with which it is carried out, has been evaluated by Millward and Garlick (1976) and Pullar and Webster (1977). With a knowledge of these parameters and the gross composition of the new tissues, the partitionment of ingested energy may be completed.

## 3:3 The Present Study

From the fore-going discussion, it is apparent that the results obtained in feeding trials depend not only on the composition of the rations fed, but also on the nutritional physiology of the subject animal. Food intake and the efficiency with which ingested material is assimilated vary with changes in the digestive capacity of the animal in response to diet quality and nutritional requirements; these adaptations may be effected through changes in gut morphology and in nutrient metabolism.

Though extensively documented in the laboratory environment, the interaction of these factors in the context of the ecology of free-living animals has been little studied. In mustelids, the extreme sexual dimorphism in body size characteristic of most species is an additional consideration, though again it is one which few investigators have addressed.

Feeding trials with adults are usually conducted on groups of animals of the same sex, in order to reduce the effect of individual variation in subsequent analyses of the results obtained; of the nine studies carried out on adult mink quoted herein, five used all-male samples and four all-female. This practice of using single sex samples makes comparisons of male and female requirements difficult, though such comparisons are central to an understanding of this species' ecology.

In the present study, therefore, analyses of the nutritional value of various prey types have been combined with an investigation of the relationships between body size, energy requirements and nutrient metabolism, in both males and females. The results obtained are placed in the context of sex differences in the diets of free-living mink, which are believed to be size-related, in order to evaluate the significance of this particular aspect of sexual dimorphism.

## 3:4 Materials and Methods

### 3:4.1 Animal Housing and Management

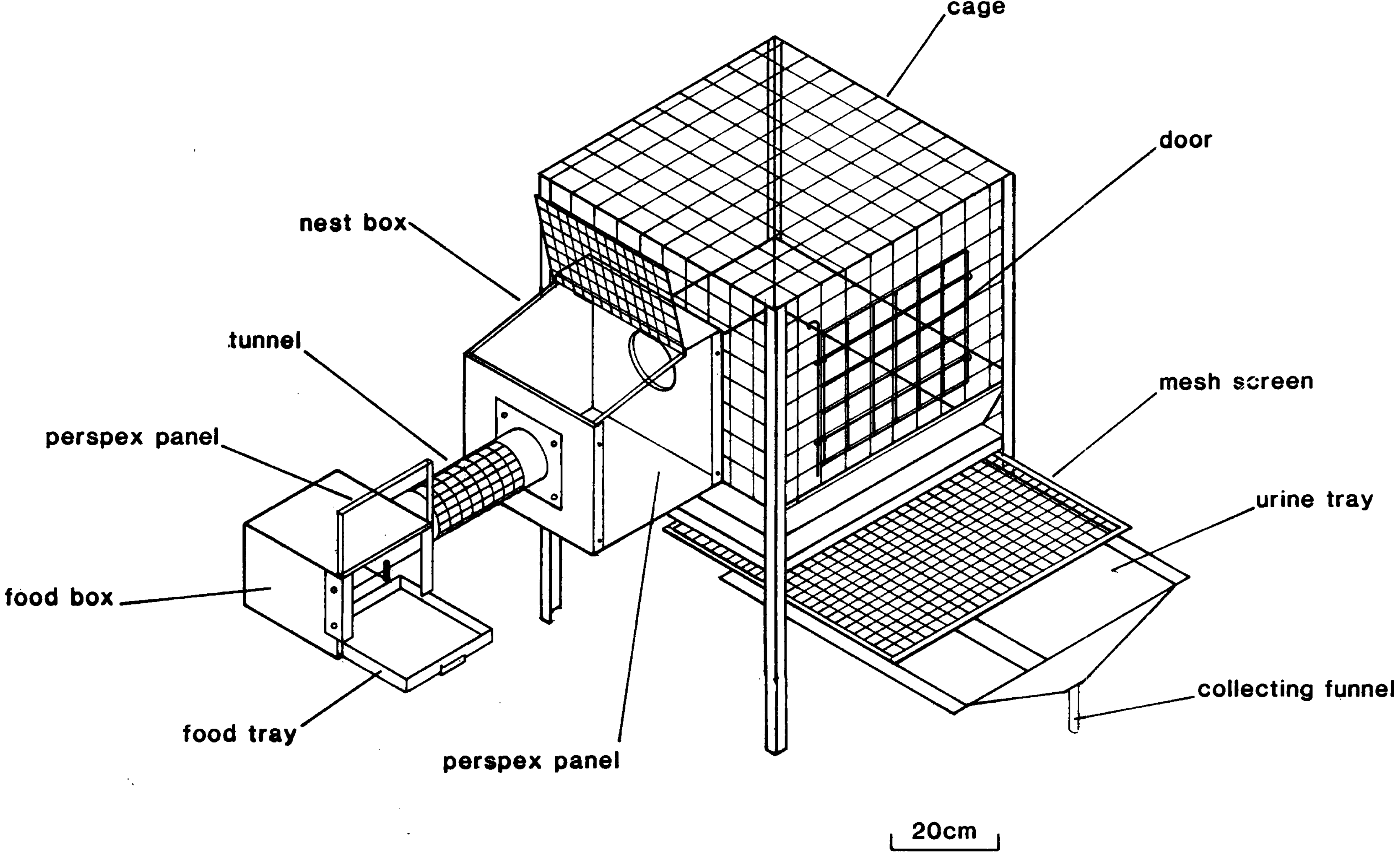
Animals were obtained from a local mink farmer as eight-week old weanling kits. All were of either the 'standard' or 'pastel' strains. The young mink were kept in mixed-sex peer groups of six to eight individuals, in a large, well-ventilated room permitting sufficient exercise for healthy growth and development. Wooden nest boxes containing straw bedding material and sawdust were provided, and water was available *ad libitum*.

The kits were fed once daily on a semi-liquid mixture of fish waste and slaughterhouse by-products identical with that used on the mink farm (the percentage composition of the rations presented is given in Appendix A). This procedure was continued until the kits were approximately three months old, when competition for food began to lead to unacceptable levels of aggression between individuals; they were then transferred to standard 'weldmesh' cages measuring 1.0 x 0.5 x 0.5 m, to which were attached wooden nest-boxes of dimensions 0.28 x 0.28 x 0.28 m, and subsequently maintained in the Department's animal-housing facilities. There, the once-daily feeding schedule was again followed, and water provided *ad libitum*. A 12:12 light regime obtained throughout.

### 3:4.2 Apparatus

During each feeding trial, animals were housed in 'metabolism' cages of the type illustrated in Fig. 3:3, which permitted the determination of food consumption and the separate collection of urine and faeces. The design of

# Fig. 3.3: Metabolism Cage



the cages, based on those published by Sinclair and Evans (1962) and Glem-Hansen and Jørgensen (1973), was intended to minimise contamination of excreta with undigested food, a problem particularly associated with studies of mink, in which waste and spillage of feed are commonplace due to the mink's habit of carrying mouthfuls of food from one area of the cage to another (Sinclair et al, 1962; Glem-Hansen and Jørgensen, 1973; Bleavins and Aulerich, 1981).

Observing that it is the natural habit of the mink to assume an arched-back posture during urination and defecation, Sinclair and Evans (1962) reasoned that these activities could be confined, by dimensional restrictions, to one part of the cage some distance from the feeding area and, by making the route between the two sufficiently indirect, the tendency to carry food from one to the other discouraged.

Accordingly, the metabolism cages used in the present study were designed to incorporate both of these features. Each cage consisted of an exercise pen of dimensions 0.5 x 0.5 x 0.5 m, constructed of 25.4 mm (1 in.) stainless steel mesh, to which was attached a wooden nest-box measuring 0.27 x 0.27 x 0.27 m. The nest-box was fitted with a perspex panel which permitted observation of the animal at all times, and a hinged wire-mesh lid to enable retrieval of orts and unconsumed feed.

Rations were presented on a removable steel tray contained in a food box, also equipped with perspex panel, to which the mink gained access via a narrow tunnel. The tunnel was constructed of 10 mm wire mesh for ease of cleaning, and a different size was used for each sex; 120 mm diameter for the males, 80 mm for the smaller females.

With these arrangements it was anticipated that defecation and urination could be restricted to the exercise pen, and consumption of feed to the food box. Faeces were collected on a 5 mm mesh screen placed beneath the pen, and urine channelled into plastic collecting bottles via the stainless steel tray below. Feeding behaviour was monitored by fitting each tunnel with a photo-electric trip by which the presence of the mink in the feed box could be recorded. The trip was activated by interruption of an infra-red beam passing across the threshold of the feed box to a detector (R. S. Components, Corby, Northants.) in the side of the tunnel. Entry of the mink into the box caused a break in the signal, which was registered as a deflection on a chart recorder. By examination of the traces from each tunnel, the relative frequency of feeding bouts and the proportion of time spent feeding by each individual could be calculated.

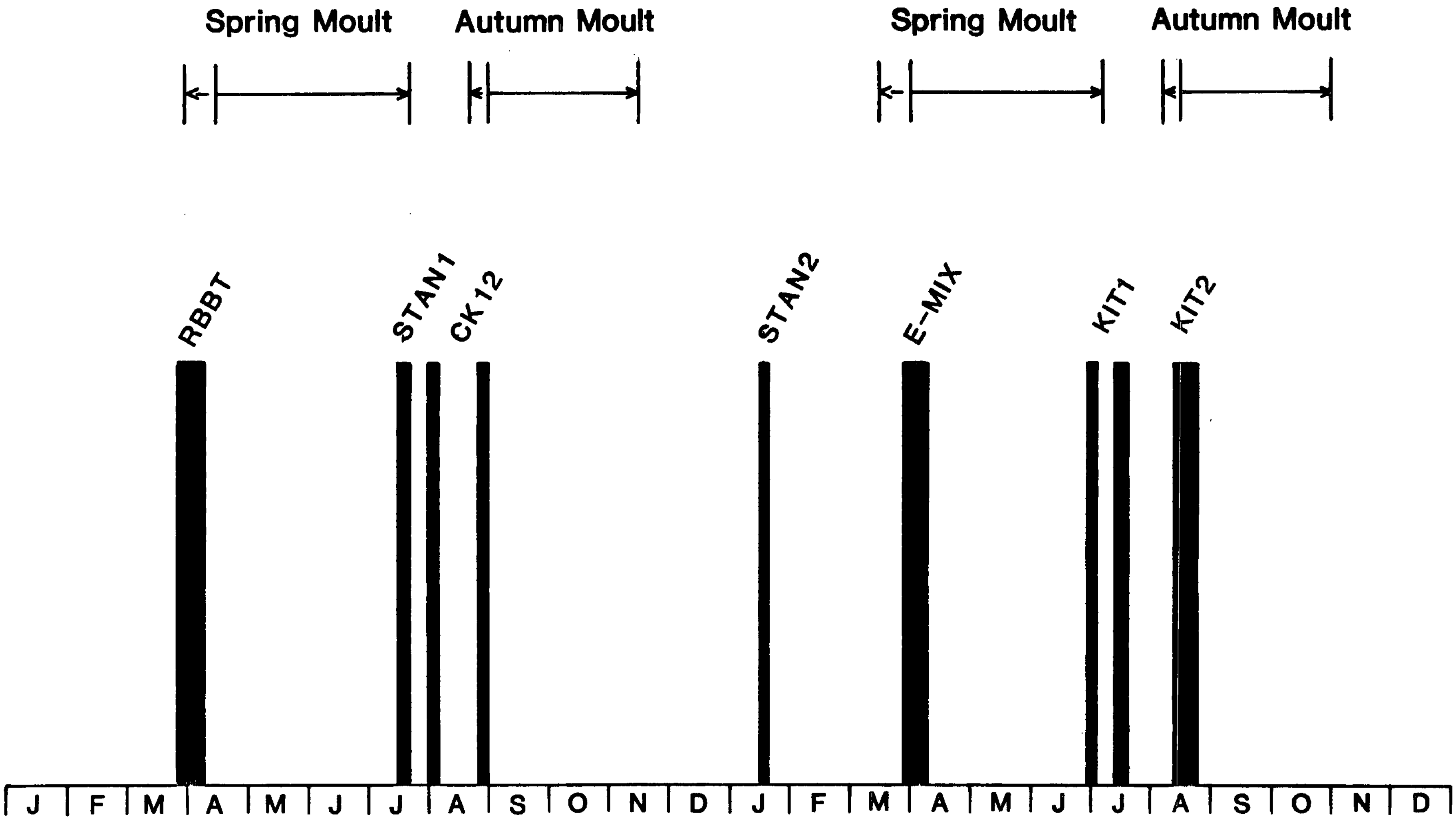
### 3:4.3 Experimental Procedures

Feeding trials were run according to the schedule depicted in Fig. 3:4, in which their relationship to the onset of spring and autumn moult is also indicated.

The spring moult is generally assumed to extend from April 15th to July 21st, and autumn moult from September 1st until November 15th (Bassett and Llewellyn, 1949; Rust, Shackelford and Meyer, 1965; Boissin-Agasse, Maurel and Boissin, 1980). These dates, however, relate to the pelage cycles of animals reared in North America and Canada; in Northern Britain the onset of the moult occurs slightly earlier in the year, with spring moult beginning in the first week of April, and the autumn moult in the last week of August (local mink farmer, pers. comm.). To facilitate comparisons with the work of other investigators, Fig. 3:4 includes both dates.

The extended duration of the moulting process, which in mink may last for seven months of the year, constitutes an obvious complication in the organisation of feeding trials, particularly studies of Nitrogen balance;

Fig. 3.4: Feeding Trials and the Moult



Glem-Hansen and Eggum (1974) stated that such trials could only be carried out during the months July to September, if the variations in nutrient metabolism attributable to the moult were to be avoided. Clearly, such an approach is impractical and, while comparative studies are made more difficult by the fact that the animal's metabolism is changing continuously for most of the year, a number of alternatives are available. For instance, Glem-Hansen and Eggum (1974) used laboratory rats as surrogates for the mink to estimate True Digestibility and Biological Value of protein diets, having first 'calibrated' the animals during the summer months.

In the present study, a reference diet, comprising minced rabbit, was fed to a group of standard animals prior to each feeding trial. This permitted comparisons between experimental diets, controlling for the effect of the moult, and also made possible a direct evaluation of the moult as a determinative factor in work of this kind.

Three diets were chosen for use in the feeding trials, RBBT, CK12 and EEL. These were selected as having both diversity of composition, and ecological relevance (Birks and Dunstone, 1985; Dunstone and Birks, 1987).

Eight adult mink were used in each trial with the experimental diets, four males and four females 1 - 3 years old. The standard group comprised two males and two females aged 1 - 2 years.

Each trial consisted of two phases, a pre-trial phase lasting four days, and a trial period of five to eight days duration. The pre-trial phase allowed time for the subject animals to grow accustomed to the new diet, and to their confinement in the metabolism cages. It also provided a period during which residues from the previous diet could be eliminated and the faecal matter equilibrate with the ingested material. The pre-trial phase was used to assess the palatability of the diet presented and to determine each individual's consumption; the rations subsequently presented during the trial period were thus calculated to be commensurate with the animal's level of intake.

All the diets proved to be highly palatable to the mink, though consumption of the EEL diet declined sharply after the second day and the faeces collected during this period were of a 'tarry' consistency and voided with difficulty. These were indications that the diet was of a very high digestibility, such that the minimal amounts of residual matter were insufficient to maintain normal passage of material along the gut (see section 2:2.2).

Attempts were therefore made to modify the composition of the ration, in order to provide a higher proportion of indigestible material. Minced rabbit was added as 10, 25 and 50% of wet weight, and each mix presented to the mink. No significant improvement in palatability was observed with the 10% and 25% rations, but intake increased markedly on the 50% mix. It was felt, however, that using a ration containing such a low proportion of the experimental diet would be of little theoretical value, and so a second mixture was prepared comprising 60% EEL and 30% RBBT by weight, to which was added approximately 10% powdered cellulose ('Solkafloc', Special Diet Services Ltd.) as a filler. This feed, named 'E-MIX', was taken readily. Its composition as fed is shown in Table 3:1.

Data collection commenced on the first day of the trial period. Portions of feed were weighed on the presentation trays to 0.01 g and inserted into the food boxes. An additional portion of similar size was placed beside the cages, as a control for evaporative losses in the determination of food consumption. The body weight of each mink was then determined to the nearest 5 g before release into the exercise pen, from which access to the food tray could be gained via the nest-box. Standard water bottles were attached to each cage and 1 - 2 ml toluene placed in the

Table 3:1 Proximate Composition of E-Mix Diet

| Percentage | Wet | Dry | Ash-free Dry |
|---|---|---|---|
| Protein | 18.65 | 42.73 | 45.0 |
| Lipid | 14.92 | 34.18 | 36.0 |
| Ash | 2.20 | 5.05 | - |
| Water | 56.35 | - | - |
| Other | 7.94 | 18.19 | 19.12 |
| Total | 100.06 | | |
| Dry-Matter | 43.65 | - | - |
| Gross Energy (KJ/g) | 11.54 | 26.44 | 27.84 |

urine bottles as a preservative (Greaves and Scott, 1960). Ambient temperature within the experimental room was also recorded, and the animals then left for a period of 24 hr.

The following morning, the food intake of each animal during the intervening period was calculated by weighing the feed remaining in the tray, together with orts and undigested food found in the tunnels, nestboxes and pens. Corrections for evaporative losses were made by comparison with the reference ration.

Faeces were collected and weighed to 0.01 g then put into screw-top plastic cartons and retained for later analysis. Urine volumes were measured to 1 ml, from which aliquots of approximately 2 ml were taken and placed in stoppered plastic vials. After these measurements had been made, the faeces screens and urine trays were cleaned and replaced. The food trays were also washed and dried, and the water bottles emptied and replenished, after which further rations of feed were presented. This marked the start of the next trial day.

At the end of each complete trial, the mink were returned to the Department animal-housing facility, and were there maintained on the regime detailed in section 3:4.1 until required for subsequent experiments. The metabolism cages were dismantled and thoroughly cleaned in hot water and disinfectant; urine trays and funnels were steeped in nitric acid (50% v/v) for 30 min. and rinsed in distilled water prior to reassembly, in order to remove accumulated mineral deposits.

### 3:4.4 Analytical Procedures

#### 3:4.4.1 Faecal Analysis

Faecal analyses were carried out according to the methods detailed in section 2:3.1. Determinations of Nitrogen and lipid content were made on the day of collection, and water content after the usual period of oven-drying (24 hrs.). Samples each comprised several stools, in whole or in part, to ensure consistency of composition and minimise individual variation.

Estimations of caloric value were usually made within 14 days of collection, on sub-samples of the dried material which had been stored at -20 °C in sealed plastic bags until required. After thawing, the procedure followed was as described in section 2:3.1.3.

#### 3:4.4.2 Urine Analysis

(i) Determination of urine Nitrogen

Assays of urine Nitrogen were performed using the method of Martinek (1964). The technique, like that of Mitchell (1972) employed in the determination of faecal Nitrogen (see section 2:3.1.4), made use of the phenol-hypochlorite reaction, following enzymatic break-down of urea to ammonia by the action of urease.

Urine samples of 0.5 ml were diluted to 50 ml with de-ionised water, and aliquots of 0.02 ml placed in tubes containing 0.2 ml of a urease solution, buffered to pH 6.6. The solution was composed of 0.5% (w/v) urease (urea amidohydrolase from Jack beans, type VI, Sigma Chemical

Company), 20-mM Potassium di-hydrogen orthophosphate and 27-mM EDTA. After gently shaking each tube to ensure adequate mixing, foil caps were fitted and the samples incubated in a water bath at 56 $^{O}$C for 10 min.

On completion of incubation, 5 ml of phenol reagent consisting of 0.11-M phenol and 0.17-μM Sodium nitroprusside was added to each tube, and colour developed by the addition of 5 ml of hypochlorite reagent comprising 0.126-M Sodium hydroxide and 10-mM Sodium hypochlorite solution (approximately normal in 0.1-N Sodium hydroxide), followed by incubation for a further 15 min. Absorbance was measured immediately at 640 nm.

Standard curves, prepared using a 36-mM solution of urea were found to be linear for concentrations 0.35 - 14-mM, and determinations of urinary Nitrogen made on the basis of direct proportionality. Three assumptions were made in doing so:-

1). Non-urea Nitrogen comprised a negligible proportion of total urinary Nitrogen (Keyes, Barron and Ross, 1971; Keiver _et_ _al_, 1984)

2). Urea Nitrogen was mainly of dietary origin

3). Non-urea Nitrogen was derived largely from endogenous sources (Varley _et_ _al_, 1980)

Readings obtained were consistently higher than the values given by Martinek (1964), but no explanation of the observed differences could be found. There was no evidence of any colour-promoting contaminant in the urea standards, as equimolar series of ammonium sulphate standards run in parallel gave identical results. Blanks with and without active urease were found to have the same absorbance, and standards run with de-natured urease (heated at 100 °C for 1 min.) gave readings similar to those of the blanks, demonstrating that no colour development took place independent of the action of urease on the urea present.

As a final check on the reliability of the procedure, the molar extinction coefficient 'E' was calculated for indophenol blue, using the Beer-Lambert equation,

$$\text{Absorbance} = E \times \text{concentration} \tag{Eqn. 3:6}$$

for molar solutions of urea and ammonium sulphate. A value of $2.187 \times 10^{+4}$ was derived with urea, and $2.265 \times 10^{+4}$ with ammonia, both of which were within the range recorded by Mann (1963). Since no fault could be found with the procedure, this assay was implemented without further modification.

All determinations were made in triplicate, with blanks and standards included in each series. Reagents were stored in the dark at 4 °C and replenished as necessary at intervals of not more than 14 days. Aliquots of urease solution were stored at -20 °C in stoppered plastic vials until required.

### (ii) Determination of urine energy

The energy content of urine is usually determined directly by combustion of freeze-dried residues in a bomb-calorimeter (Moors, 1977; Keiver et al, 1984; Ronald et al, 1984). Due to technical difficulties, however, freeze-drying of the samples collected during the present study was not practicable, and the alternatives tried, such as oven-drying under vacuum (Harri and Brockway, 1985; Dewey, 1985, pers. comm.), were considered too time-consuming in view of the schedules to which the feeding trials were run. Therefore, a procedure based on the methods described by Street et al (1964) and Paladines et al (1964) was employed, by which urine energy was estimated from assays of urinary Nitrogen.

Following determination of urea Nitrogen content by the assay described in (i) above, the energy value of each sample was estimated by substitution of a caloric equivalent of the urea present. The caloric value of urea was determined directly by combustion of pellets of powdered urea in a ballistic bomb calorimeter; benzoic acid was used as a promoter to ensure a complete burn and contributed approximately 20% of the total energy released. The value obtained, 10.87 KJ/g (S.E. = 0.49), was slightly higher than that of 10.57 KJ/g, quoted by Robbins (1983), but was used in preference to the latter in order to retain comparability with the other analyses carried out during the study. The caloric equivalent of urine derived therefrom was 23.29 KJ/g urea Nitrogen, which is in close agreement with the values of 24.36 - 28.5 KJ/g determined empirically by Litvaitis and Mautz (1980) for the urine of the coyote Canis latrans.

The substitutive method is to be contrasted with those of Paladines et al (1964) and Street et al (1964), in which regression techniques were used to estimate energy content for urines of varying composition; it's use required that several assumptions were made about the composition of the urines on which the estimates were based.

The first of these was that total urine energy was derived almost entirely from urea. The second was that the absolute amounts of the non-urea constituents remained constant with changes in diet, and correlated changes in Nitrogen retention associated with the metabolism of dietary protein, and also with the requirements of the moult (Varley et al, 1980)

With the use of diets having a very high protein content, these assumptions were taken to be valid, and subsequent calculations made on this basis.

### 3:4.4.3 Bowel Transit Time and Gut Morphology

(i) Estimation of bowel transit time

Bowel transit time (BTT) was estimated by faecal marker recovery. The markers used were variously coloured strips of polyethylene, measuring approximately 1 x 15 mm. Observations were made over a two-day post-trial period and entailed presentation of the experimental diet, supplemented with one of the several markers, with BTT being measured as the interval between ingestion and first appearance in the faeces.

To enable several determinations to be made on each animal, the usual ration was divided into portions, to each of which was added a different coloured marker. The portions were then presented in succession, each one being replaced by the next on first appearance of that marker in the faeces. On passage of the last coloured marker, the balance of the ration was returned to the food tray, and the trial concluded.

Implicit in the use of particulate markers in studies of transit time is the assumption that the rates of passage for marker and faecal matter are equivalent. Though not valid in certain applications (Kotb and Luckey, 1972; Robbins, 1983), in the present study, similar transit times were ascribed to marker and ingesta, in view of the large amounts of indigestible material (bones, hair, etc.) which the experimental diets contained.

(ii) Examination of gut morphology

Superficial examinations were carried out on the alimentary tracts of wild and farm-bred mink to investigate the effects of sex, body size and nutritional history on the gross morphology of the gut.

The sample comprised the carcasses of 28 farm-bred mink (15 males, 13 females) aged approximately nine months, together with 12 wild adult mink (6 males, 6 females) of undetermined age, caught during a trapping programme organised by the Ministry of Agriculture.

After weighing each carcass, the viscera were removed and the total length of the intestine measured from pylorus to anus. An incision was then made along the length of the organ and the tissue spread out to enable measurement of the circumference. Five measurements were taken at equal intervals along the length of the tract, and the mean of these incorporated into a formula by which the approximate capacity (volume) of the gut could be estimated, as shown below:-

$$\text{Luminal volume} = \frac{(\text{Circumference})^2}{(\ 4 \times \text{Pi}\ )} \times \text{length}$$

(Eqn. 3:7)

Serosal surface area, given by the product of length and circumference (Barry, 1976), was also calculated in order to determine the ratio of surface area to volume.

## 3:5 Results

### 3:5.1 Behaviour of Subject Animals

All animals adapted well to confinement in the metabolism cages, though most individuals displayed various forms of aberrant, stereotypical behaviour. Those most commonly seen included gnawing or scratching at the bars of the cage and the perspex panels of the food boxes and nest boxes. Males, in particular, developed a habit of repeatedly butting the water bottles and fittings, which occasionally led to leakage of water into the urine trays and subsequent dilution of the urine samples. More generalised motor activity was also frequently observed, often as a characteristic weaving motion of the body, or repeated leaping into and out of the nest box. These types of activity usually occurred in bouts, sustained for periods of 10 - 15 min. For much of the time, however, the animals were quiescent, or sleeping.

Feeding took place at intervals of 2 - 3 hrs. throughout the 24 hr. period but, contrary to experimental design, was not confined to the food boxes. The animals were observed to remove feed from the boxes, a mouthful at a time, and deposit it either in the nest boxes or on the floors of the cages. In general, feeding did not commence until a large proportion of the ration had first been removed from the feed tray. The practice of caching food in various parts of the apparatus was noted as being more prevalent among females, though with both sexes spillage of feed was considerable; estimation of consumption entailed retrieval of orts from the tunnels, nest boxes and also from the mesh screens beneath the cages. Though defecation and urination were confined to the exercise pens, as anticipated, the contamination of excreta with waste feed remained a possibility throughout, and constituted a serious flaw in the design of the apparatus as used in these trials.

It was further noted that, after emptying the feed trays, the animals often sat in the tunnels for extended periods, scratching at the perspex panels of the feed boxes, and the smaller females were observed to sleep there in preference to the nest boxes. This habit was discouraged by adding a metal partition to the feed tray, which effectively reduced the size of the box by half. Although this prevented the animals from sleeping in the food boxes, the tendency to sit in the tunnels connecting them to the cages remained and, since consumption of the rations usually took place outside the feed boxes, attempts to measure the frequency and duration of feeding bouts were abandoned, and the infra-red movement detectors subsequently removed.

### 3:5.2 Changes in the Body Weight of Subject Animals

(i) Changes in body weight between trials

Mean body weights of males and females on each trial were calculated from the means for individual subject animals over the trial period and are presented in Table 3:2, together with the standard errors of the means. Comparisons of mean body weights between trials were made for males and females separately, using 'Student's' t-test, and the results are tabulated in Tables 3:3 and 3:4. STAN1 and STAN2 refer to presentations of the reference diet to the Standard animals prior to the CK12 and E-MIX trials, respectively.

No significant differences were found in the body weights of males, although the Experimental animals weighed substantially more during the trials with the CK12 diet (1.929 Kg) than either RBBT (1.810 Kg) or E-MIX (1.805 Kg). Similarly, the mean body weight of the Standard animals was

Table 3:2 Mean Body Weight of Subject Animals During Feeding Trials

| Trial | Body Weight (Kg) | | | |
|---|---|---|---|---|
| | N | Males | N | Females |
| RBBT | 4 | 1.810 (0.167) | 4 | 0.895 (0.025) |
| STAN1 | 2 | 1.716 (0.046) | 2 | 0.739 (0.081) |
| CK12 | 3 | 1.929 (0.059) | 4 | 0.957 (0.023) |
| STAN2 | 2 | 1.825 (0.046) | 2 | 0.800 (0.032) |
| E-MIX | 3 | 1.805 (0.087) | 4 | 0.824 (0.026) |

Table 3:3 Comparison of Body Weights of Subject Animals Between Trials (Males)

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 4 | * | N.S. | N.S. | N.S. | N.S |
| STAN1 | 2 | | * | N.S. | N.S. | N.S |
| CK12 | 3 | | | * | N.S. | N.S |
| STAN2 | 2 | | | | * | N.S |
| E-MIX | 3 | | | | | * |

Table 3:4 Comparison of Body Weights of Subject Animals Between Trials (Females)

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 4 | * | N.S. | N.S. | N.S. | N.S |
| STAN1 | 2 | | * | 0.05 | N.S. | N.S |
| CK12 | 3 | | | * | N.S. | 0.05 |
| STAN2 | 2 | | | | * | N.S |
| E-MIX | 3 | | | | | * |

considerably greater during the STAN2 trial than STAN1 (1.825 Kg in comparison with 1.716 Kg) though, again, the increase was not significant.

The body weights of females also remained relatively constant between trials, and comparison of means for the Experimental animals revealed no significant differences. The slight variations observed paralleled those recorded in the males, with body weights being markedly greater during presentation of the CK12 ration (0.957 Kg) than on the trials with RBBT (0.895 Kg) and E-MIX (0.824 Kg). Standard animals gained weight between the STAN1 and STAN2 trials, but not significantly so, although in both instances the mean values (0.739 Kg and 0.800 Kg, respectively) were significantly lower than that recorded for the Experimental animals on the CK12 trial.

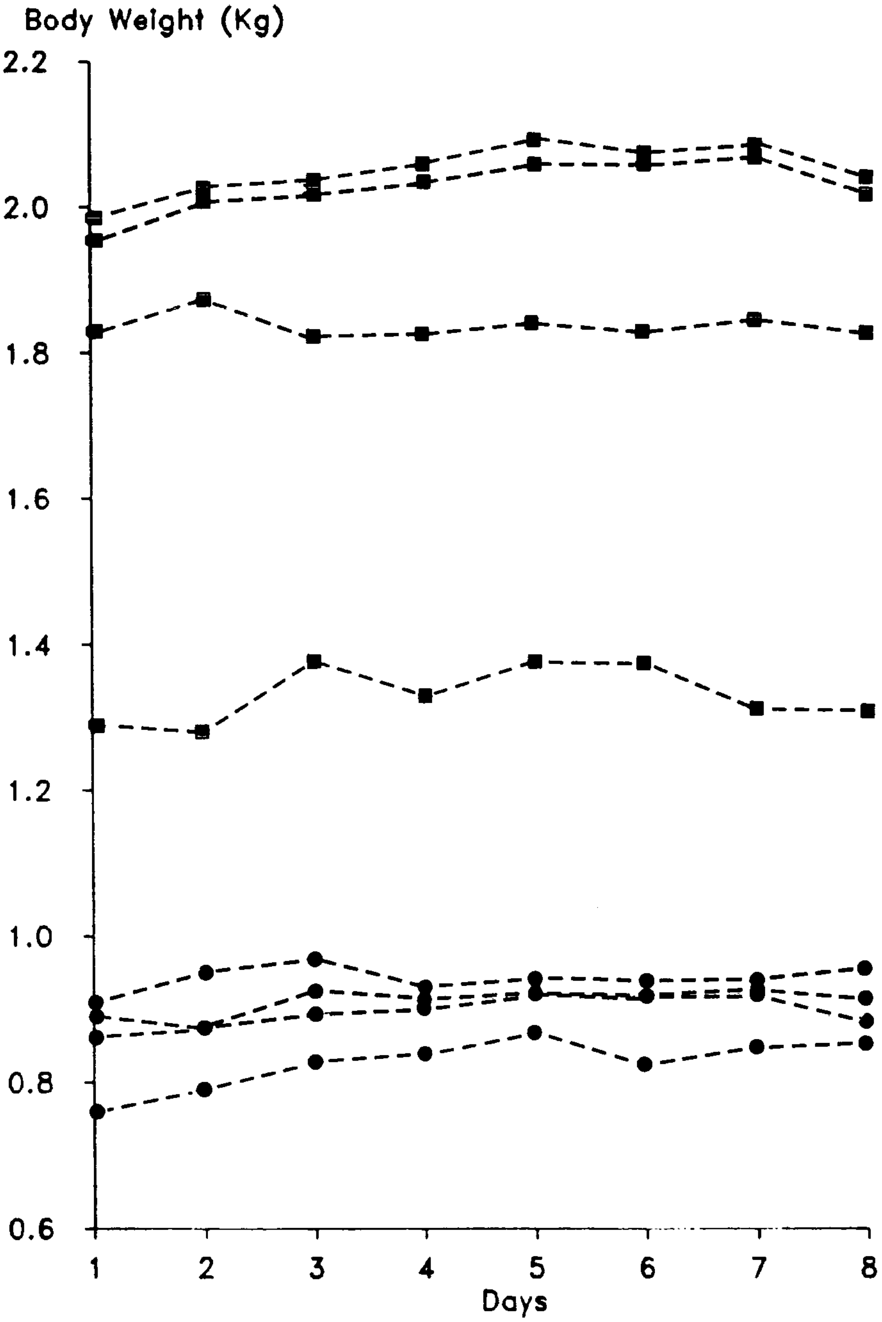
### (ii) Changes in body weight during feeding trials

The body weights of subject animals during the trials are depicted in Figs. 3:5 - 3:9, from which it may be seen that most individuals underwent a slight gain in weight over each trial period. In order to determine the rate of change in weight, the body weights of each animal were recalculated as a proportion of the initial weight, recorded on the first day of the trial, and analyses of covariance performed using this transformed value as the dependent variable and trial day as the covariate. Data from males and females were examined separately, using the daily mean values for each sex.

The slopes of the regressions so-obtained are tabulated in Table 3:5. Comparisons between males and females made using the variance-ratio test revealed no significant differences in the rate of weight gain on RBBT and STAN1. On CK12, however, males underwent a net loss in weight at appproximately 0.09% of initial body weight per day, while females gained weight at a rate of 1.08% per day. Sex differences were also apparent

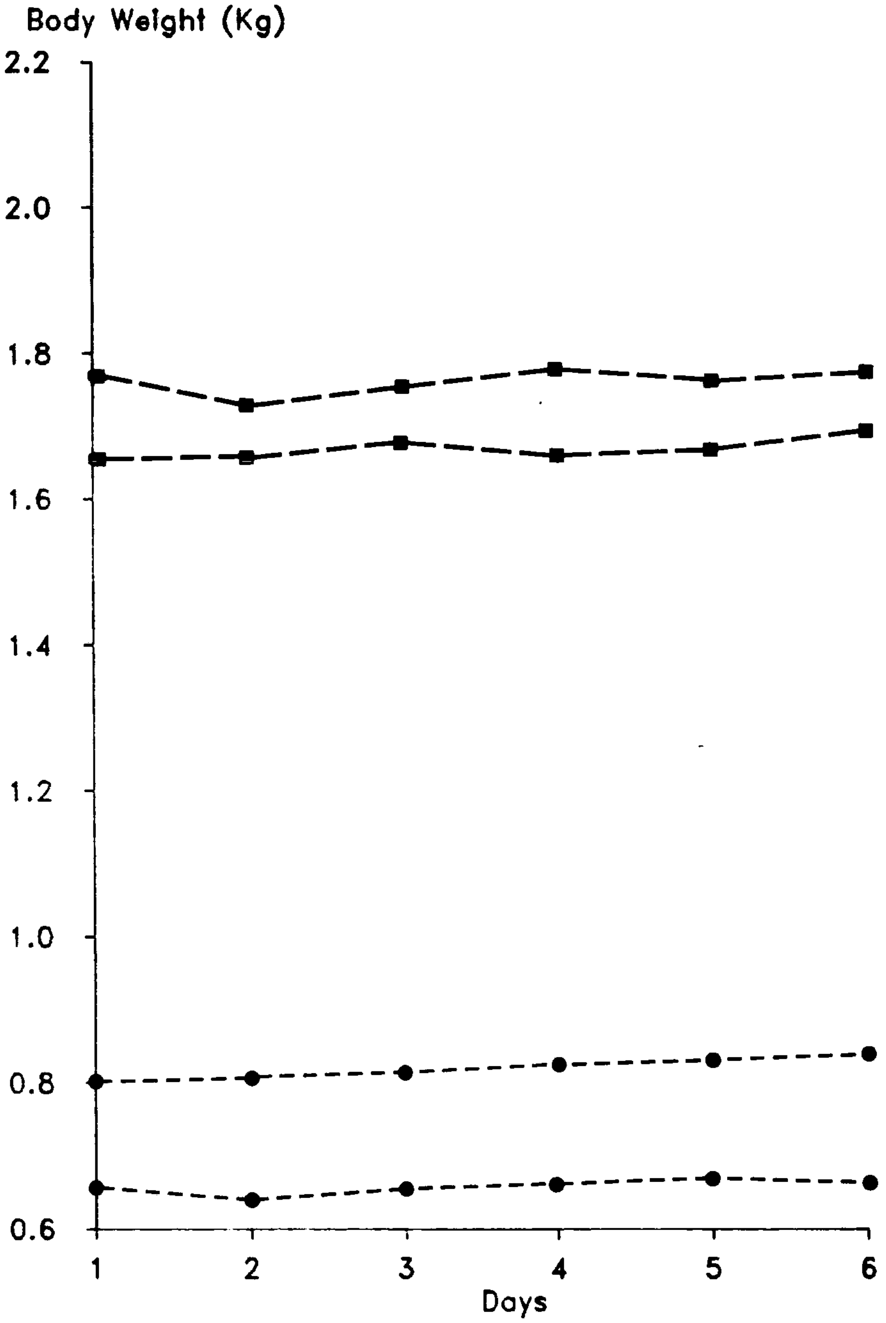
## Fig. 3:5 Variation in Body Weight of Animals Over Trial Period

(Diet = RBBT)



## Fig. 3:6 Variation in Body Weight of Animals Over Trial Period

Diet = STAN1



# Fig. 3:7 Variation in Body Weight of Animals Over Trial Period

(Diet = CK12)

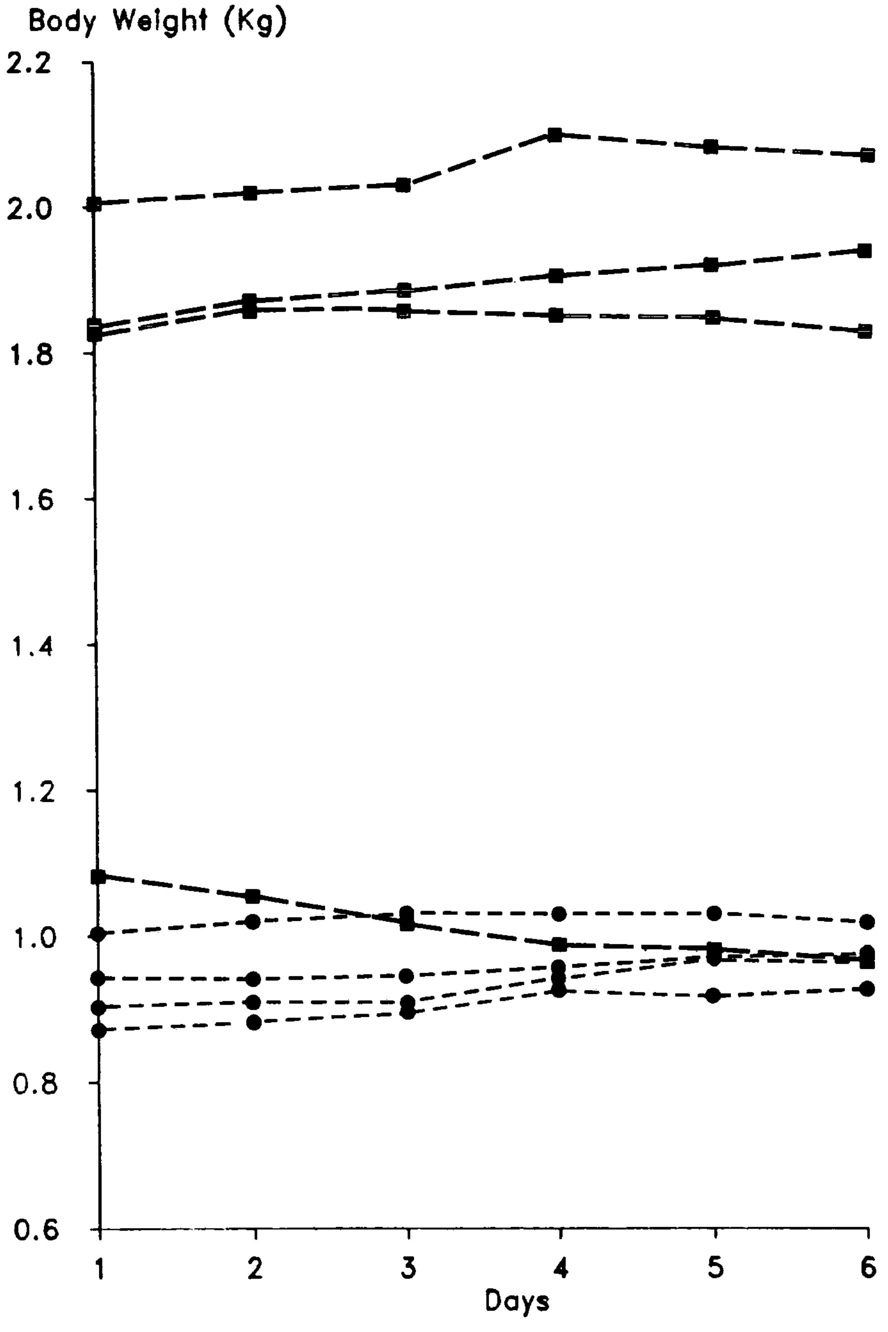
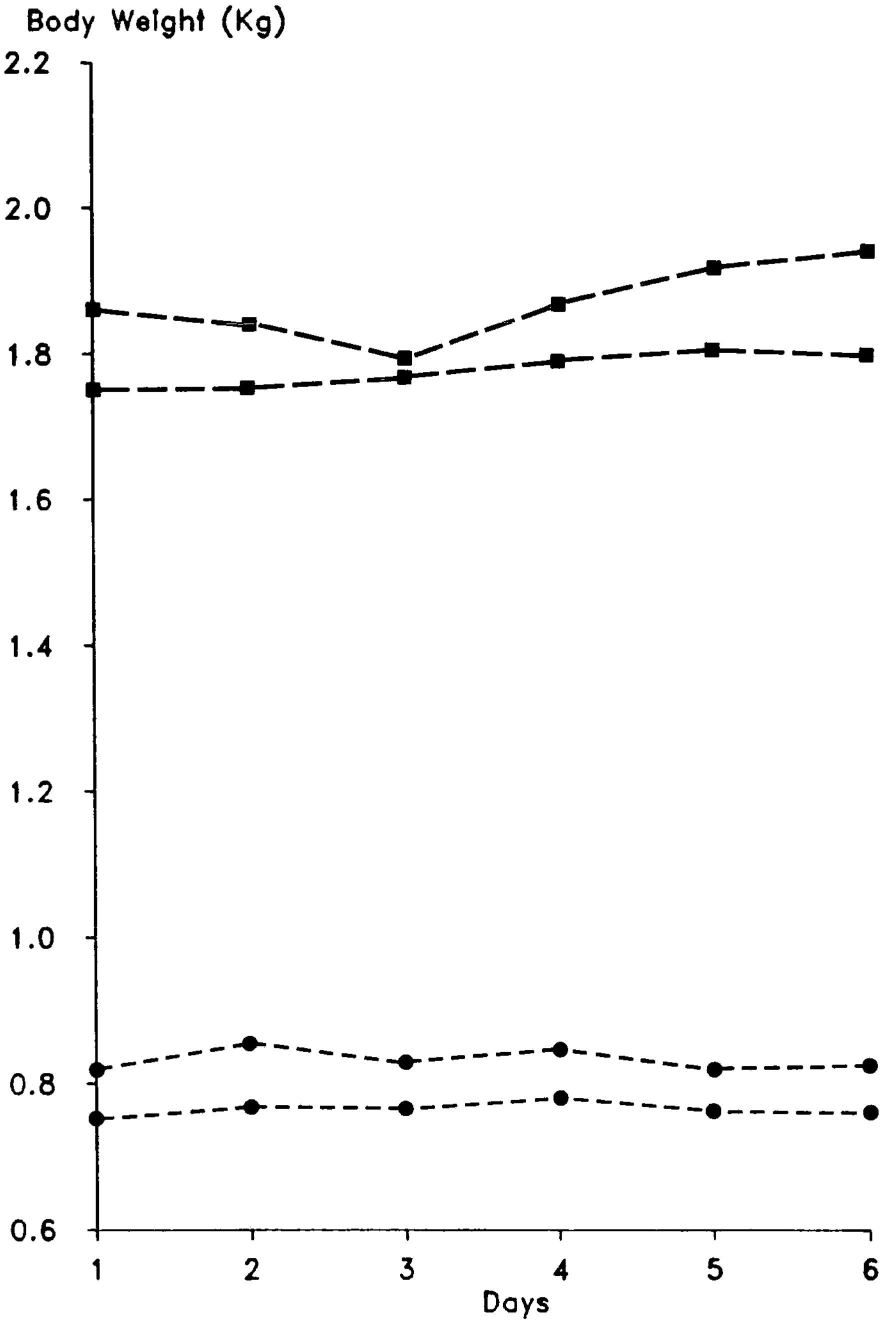


Fig. 3:8 Variation in Body Weight of Animals Over Trial Period (Diet = STAN2)



# Fig. 3:9 Variation in Body Weight of Animals Over Trial Period

(Diet = E-MIX)

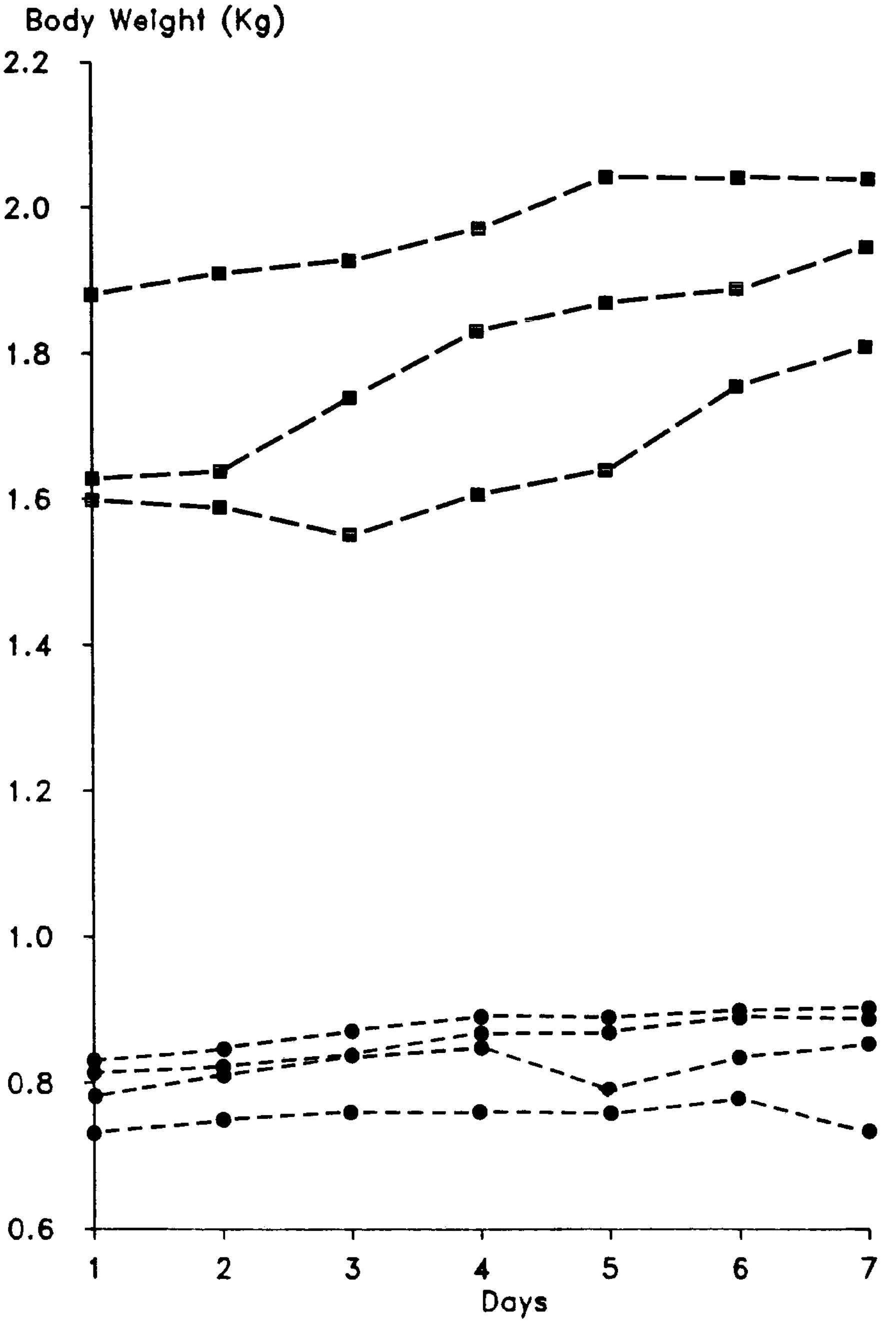


Table 3:5 Changes in the Body Weight of Subject Animals During Feeding Trials (I): Comparisons of Rate of Weight Change Between Sexes

| Trial | | N | Slope | S.E. | F-Value | d.f. | Sig. |
|---|---|---|---|---|---|---|---|
| RBBT | M | 8 | 0.0030 | (0.0021) | 2.0966 | 1, 12 | N.S. |
| | F | 8 | 0.0079 | (0.0027) | | | |
| STAN1 | M | 6 | 0.0029 | (0.0014) | 4.8055 | 1, 8 | N.S. |
| | F | 6 | 0.0078 | (0.0017) | | | |
| CK12 | M | 6 | -0.0009 | (0.0007) | 100.49 | 1, 8 | 0.001 |
| | F | 6 | 0.0108 | (0.0009) | | | |
| STAN2 | M | 6 | 0.0088 | (0.0030) | 7.6233 | 1, 8 | 0.05 |
| | F | 6 | -0.0028 | (0.0029) | | | |
| E-MIX | M | 7 | 0.0274 | (0.0026) | 16.0327 | 1, 10 | 0.005 |
| | F | 7 | 0.0092 | (0.0038) | | | |

Table 3:6 Changes in the Body Weight of Subject Animals During Feeding Trials (II): Rate of Weight Change Within Trials

| Trial | | N | Slope | S.E. | T-Value | d.f. | Sig. |
|---|---|---|---|---|---|---|---|
| RBBT | | 16 | 0.0054 | (0.0018) | 3.065 | 14 | 0.01 |
| STAN1 | | 12 | 0.0054 | (0.0$^{0}_{\wedge}$132) | 4.071 | 10 | 0.005 |
| CK12 | M | 6 | -0.0009 | (0.0007) | 1.278 | 4 | N.S. |
| | F | 6 | 0.0108 | (0.0009) | 11.400 | 4 | 0.001 |
| STAN2 | M | 6 | 0.0088 | (0.0030) | 2.950 | 4 | 0.05 |
| | F | 6 | -0.0028 | (0.0029) | -0.947 | 4 | N.S. |
| E-MIX | M | 7 | 0.0274 | (0.0026) | 10.740 | 5 | 0.001 |
| | F | 7 | 0.0092 | (0.0038) | 2.438 | 5 | N.S. |

during STAN2 and E-MIX, though most marked in the latter. The gain for males, at approximately 2.74% of initial body weight, was nearly three times higher than that for females (0.92% per day) and represented an average daily weight gain of nearly 49 g, in comparison with only 7.6 g per day in the females. On each of these diets, the sex differences were statistically significant ($p < 0.001$, $p < 0.05$ and $p < 0.005$ for CK12, STAN2 and E-MIX, respectively).

To evaluate the importance of the observed changes in body weight, 'Student's' t-test was applied to the regression coefficients for each diet as a test that the slopes differed significantly from zero. For the RBBT and STAN1 trials, data from males and females were pooled and the test applied to the common slope; for CK12, STAN2 and E-MIX, the regression coefficients were tested separately. The results are presented in Table 3:6.

Significant weight changes were recorded in both sexes on the RBBT and STAN1 trials, for which an average daily weight gain of 0.54% of initial body weight was recorded ($p < 0.005$). On CK12, females underwent a percentage weight gain of 1.08%, though no such changes were apparent on STAN2 or E-MIX. In contrast, the males underwent highly significant increases in body weight over the trial period on each of these diets (STAN2, $p < 0.05$; E-MIX, $p < 0.001$).

Since the rations presented differed in their capacity to promote gains in weight, the regression coefficients derived for each diet were compared by means of the variance-ratio test. Analyses of the data for males and females were carried out separately, and the results are given in Table 3:7 and 3:8. Reference to Table 3:7 indicates that, in males, weight gains were similar on all diets, though the very large increases recorded during presentation of E-MIX were significantly greater than those observed during

Table 3:7 Changes in the Body Weight of Subject Animals During Feeding Trials (IIIa): Comparisons of Rate of Weight Change Between Trials (Males)

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S.<br>(0.000) | N.S.<br>(1.649) | N.S.<br>(2.480) | 0.001<br>(56.13) |
| STAN1 | 6 |  | * | 0.05<br>(6.230) | N.S.<br>(3.161) | 0.001<br>(55.65) |
| CK12 | 6 |  |  | * | 0.05<br>(9.987) | 0.001<br>(82.08) |
| STAN2 | 6 |  |  |  | * | 0.01<br>(22.11) |
| E-MIX | 7 |  |  |  |  | * |

Table 3:8 Changes in the Body Weight of Subject Animals During Feeding Trials (IIIb): Comparisons of Rate of Weight Change Between Trials (Females)

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S.<br>(0.000) | N.S.<br>(0.582) | 0.05<br>(5.746) | N.S.<br>(0.087) |
| STAN1 | 6 |  | * | N.S<br>(2.370) | 0.05<br>(9.579) | N.S.<br>(0.087) |
| CK12 | 6 |  |  | * | 0.005<br>(19.401) | N.S.<br>(0.127) |
| STAN2 | 6 |  |  |  | * | 0.05<br>(5.356) |
| E-MIX | 7 |  |  |  |  | * |

presentation of the other rations. In females, a different, though less consistent, pattern emerged (see Table 3:8). Gains in weight were greatest on the CK12 ration, and were significantly higher than on STAN2 ($p < 0.005$), though comparison with RBBT, CK12 and STAN2 revealed no such difference. These anomalous results may be attributable to disparities in the variances of the data sets compared.

### 3:5.3 Relationships Between Body Size and Feed Intake

Food consumption during each trial, expressed per unit body weight (g/Kg/day), is presented in Table 3:9. On RBBT, the weight-specific intake of females (214.2 g/Kg/day) exceeded that of males (173.3 g/Kg/day) by 23.6%, and on STAN1, by 75.4% (mean values for males and females 148.6 and 260.6 g/Kg/day, respectively), and on all diets the food consumption of males was markedly lower than that of females, in proportion to their size.

To enable comparison of individuals independent of body size, a measure of metabolic weight was derived by calculation of the constant 'b' (equation 3:1) from a double-logarithmic regression of metabolisable energy intake (KJ) on body weight (Kg), as depicted in Fig. 3:10. Since each datum was the aggregate mean of several sample means, both dependent and independent variables had an attendant error and, consequently, the computation of a regression equation was inappropriate. The data were therefore characterised using the method of principal axes, as described by Sokal and Rohlf (1981), and the equation of the principal axis is included in Fig. 3:10. The slope 'b' so-derived (0.762) accords well with the value of 0.75 which is generally employed in comparative studies of this kind, and computation of the 95% confidence limits of 'b' (0.745 and 0.779) demonstrated that the latter value was not significantly different from this

Table 3:9 Weight-Specific Feed Consumption of Subject Animals: Comparisons Between Sexes

| Trial | | N | Consumption (g/Kg Body Wt.) | Sig. |
|---|---|---|---|---|
| RBBT | M | 4 | 173.3 (13.80) | 0.05 |
| | F | 4 | 214.2 (17.41) | |
| STAN1 | M | 2 | 148.6 ( 3.00) | 0.05 |
| | F | 2 | 260.6 (21.75) | |
| CK12 | M | 3 | 149.6 ( 7.96) | N.S. |
| | F | 4 | 186.2 (15.58) | |
| STAN2 | M | 2 | 166.1 ( 6.56) | N.S. |
| | F | 2 | 185.7 (22.19) | |
| E-MIX | M | 3 | 129.9 (12.72) | N.S. |
| | F | 4 | 154.7 ( 6.19) | |

Table 3:9 Weight-Specific Feed Consumption of Subject Animals: Comparisons Between Sexes

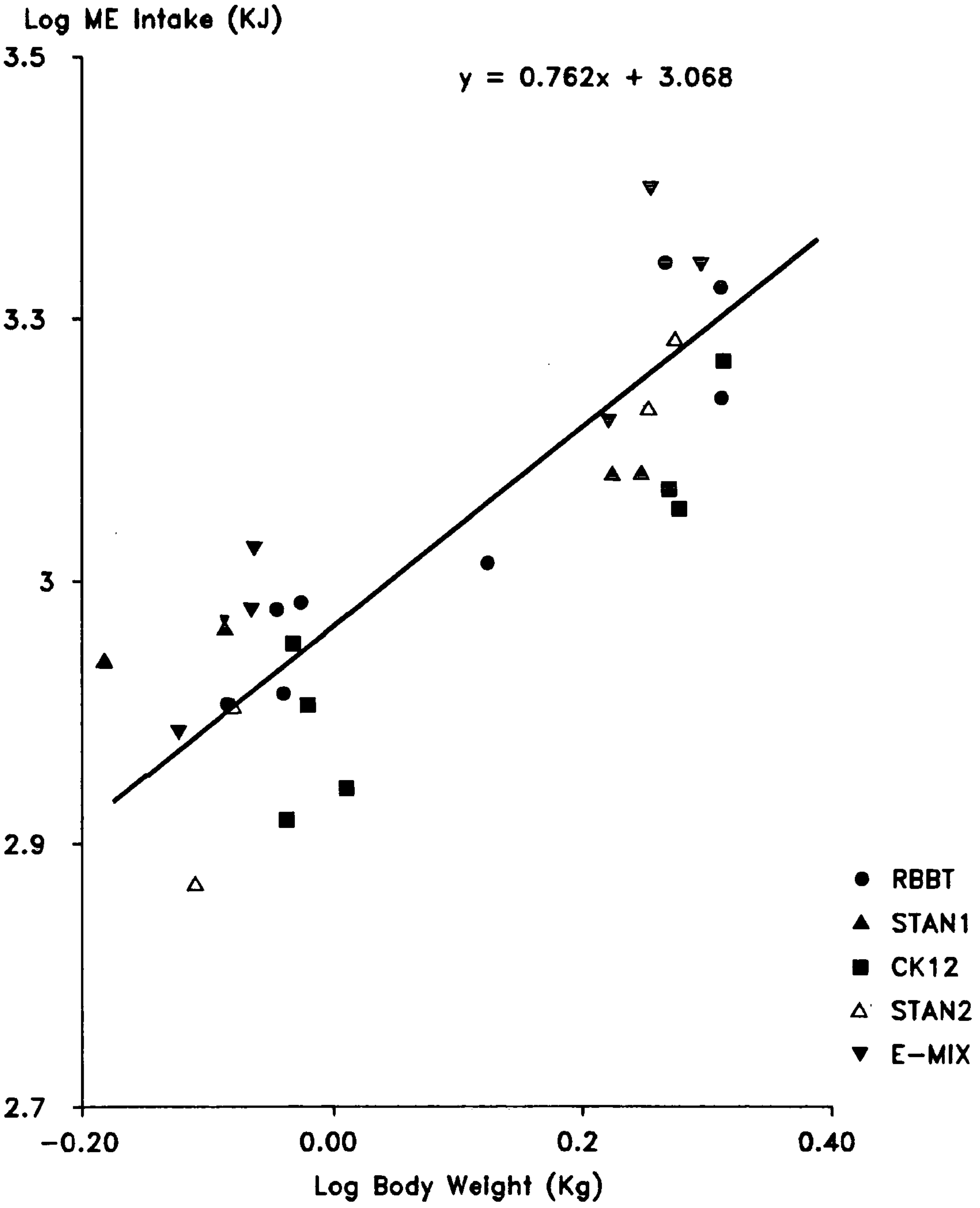
| Trial | | N | Consumption (g/Kg Body Wt.) | Sig. |
|---|---|---|---|---|
| RBBT | M | 4 | 173.3 (13.80) | 0.05 |
| | F | 4 | 214.2 (17.41) | |
| STAN1 | M | 2 | 148.6 ( 3.00) | 0.05 |
| | F | 2 | 260.6 (21.75) | |
| CK12 | M | 3 | 149.6 ( 7.96) | N.S. |
| | F | 4 | 186.2 (15.58) | |
| STAN2 | M | 2 | 166.1 ( 6.56) | N.S. |
| | F | 2 | 185.7 (22.19) | |
| E-MIX | M | 3 | 129.9 (12.72) | N.S. |
| | F | 4 | 154.7 ( 6.19) | |

Fig. 3:10 Relationship of Metabolisable Energy Intake to Body Weight (All Diets)

Double logarithmic regression of metabolisable energy intake against body weight. Figure includes equation of principal axis.

n.b: When males and females are treated separately, no relationship between metabolisable energy intake and body weight can be established.

Fig 3:10 Relationship of Metabolisable Energy Intake to Body Weight
(All Diets)



standard. Therefore, in order to facilitate comparison of the results obtained in the present study with those of other workers, metabolic weight was calculated as body weight $(Kg)^{0.75}$, and subsequent weight-specific measures of nutrient metabolism were made on this basis. (For a derivation of the principal axis equation and it's descriptors, see Appendix B).

Following recalculation of food consumption per unit metabolic weight $(g/Kg^{0.75}/day)$, comparisons between males and females on each diet were effected by means of 'Student's' t-test. Though the disparity in intake on STAN1 remained statistically significant $(p < 0.05)$, there were no significant sex differences on any of the other rations, and therefore, data for males and females were pooled. The means for each diet are presented in Table 3:10.

'Student's' t-test was again used to evaluate observed differences between trials, and the results are tabulated in Table 3:11, from which it is apparent that, with the exception of the very low value recorded on the E-MIX ration (149.1 $g/Kg^{0.75}/day$), weight-specific intake did not vary significantly between trials. Feed consumption on the latter diet was, however, significantly lower than on each of the others, particularly in comparison with RBBT, for which a mean intake of 204.7 $g/Kg^{0.75}/day$ was recorded $(p < 0.001)$.

Mean values for gross energy intake are included in Table 3:12, statistical comparisons in Table 3:13. The figure of 1720 $KJ/Kg^{0.75}/day$ calculated for E-MIX was significantly higher $(p < 0.05)$ than those for RBBT (1277 KJ) and STAN2 (1158 KJ), both rations of comparatively low energy content (see Table 2:4). No significant differences were observed between any of the other trials.

Disparities in the mass-specific intake of the various elements of the diet were, in part, correlated with the level of feed consumption, though

Table 3:10 Feed Consumption of Subject Animals per Unit Metabolic Weight

| Trial | N | Consumption ($g/Kg^{0.75}$) |
|---|---|---|
| RBBT | 8 | 204.7 ( 8.77) |
| STAN1 | 4 | 205.4 (21.13) |
| CK12 | 7 | 180.9 ( 9.12) |
| STAN2 | 4 | 184.5 (11.11) |
| E-MIX | 7 | 149.1 ( 6.83) |

Table 3:11 Comparison of Feed Consumption Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | N.S. | N.S. | 0.001 |
| STAN1 | 4 | | * | N.S. | N.S. | 0.05 |
| CK12 | 7 | | | * | N.S. | 0.05 |
| STAN2 | 4 | | | | * | 0.05 |
| E-MIX | 7 | | | | | * |

Table 3:12 Gross Energy Intake of Subject Animals per Unit Metabolic Weight

| Trial | N | Intake ($KJ/Kg^{0.75}$) |
|---|---|---|
| RBBT | 8 | 1277 ( 54.75) |
| STAN1 | 4 | 1282 (131.92) |
| CK12 | 7 | 1295 ( 65.31) |
| STAN2 | 4 | 1152 ( 69.34) |
| E-MIX | 7 | 1720 ( 78.82) |

Table 3:13 Comparison of Gross Energy Intake Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | N.S. | N.S. | 0.001 |
| STAN1 | 4 |  | * | N.S. | N.S. | 0.05 |
| CK12 | 7 |  |  | * | N.S. | 0.001 |
| STAN2 | 4 |  |  |  | * | 0.001 |
| E-MIX | 7 |  |  |  |  | * |

differences in the composition of the rations presented also contributed to the patterns observed, as analysis of Nitrogen and lipid intake during each trial clearly indicates.

Mean values for Nitrogen intake on each diet (g $N/Kg^{0.75}/day$) are tabulated in Table 3:14 and the results of statistical comparisons of the means in Table 3:15. Similarly, the figures for lipid intake (g $FFA/Kg^{0.75}/day$) are included in Table 3:16, and the results of t-tests between the means in Table 3:17.

Nitrogen intake was highest on the rabbit-based diets, RBBT, STAN1 and STAN2 (means of 8.11, 8.14 and 7.31 $g/Kg^{0.75}/day$, respectively), and no significant differences were found between these three though, in each case, intake was higher than on CK12 and E-MIX (5.66 and 4.45 $g/Kg^{0.75}/day$), both of which were relatively low in Nitrogen content (see Table 2:4). The mean value for E-MIX was 21.4% lower than that calculated for CK12, and represented only 54.9% of the mean daily intake recorded on RBBT.

In contrast with these results, the figures for lipid intake indicate that daily means were highest on E-MIX (21.09 $g/Kg^{0.75}/day$) and CK12 (7.70 $g/Kg^{0.75}/day$), and lowest on RBBT, STAN1 and STAN2. Again, no significant differences in intake were recorded between the latter three rations, though the mean values calculated (5.67, 5.69 and 5.11 $g/Kg^{0.75}/day$, respectively) were all significantly lower than the figure recorded for the CK12 diet, which was, in turn, exceeded by that computed for E-MIX ($p < 0.001$).

As with dietary Nitrogen, the range in lipid intake between the five trials was considerable, the 3.7-fold difference between the highest and lowest values recorded being due principally to the very high lipid content of the E-MIX ration (approximately 149 mg/g as fed).

Table 3:14 Nitrogen Intake of Subject Animals per Unit Metabolic Weight

| Trial | N | Intake ($g\ N/Kg^{0.75}$) |
|---|---|---|
| RBBT | 8 | 8.11 (0.35) |
| STAN1 | 4 | 8.14 (0.847 |
| CK12 | 7 | 5.66 (0.29) |
| STAN2 | 4 | 7.31 (0.44) |
| E-MIX | 7 | 4.45 (0.20) |

Table 3:15 Comparison of Nitrogen Intake Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | 0.001 | N.S. | 0.001 |
| STAN1 | 4 |  | * | 0.001 | N.S. | 0.001 |
| CK12 | 7 |  |  | * | 0.01 | 0.005 |
| STAN2 | 4 |  |  |  | * | 0.005 |
| E-MIX | 7 |  |  |  |  | * |

Table 3:16 Lipid Intake of Subject Animals per Unit Metabolic Weight

| Trial | N | Intake (g FFA/Kg$^{0.75}$) |
|---|---|---|
| RBBT | 8 | 5.673 (0.24) |
| STAN1 | 4 | 5.693 (0.59) |
| CK12 | 7 | 7.699 (0.39) |
| STAN2 | 4 | 5.114 (0.31) |
| E-MIX | 7 | 21.093 (0.97) |

Table 3:17 Comparison of Lipid Intake Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | 0.001 | N.S. | 0.001 |
| STAN1 | 4 | | * | 0.05 | N.S. | 0.001 |
| CK12 | 7 | | | * | 0.001 | 0.001 |
| STAN2 | 4 | | | | * | 0.001 |
| E-MIX | 7 | | | | | * |

### 3:5.4 Apparent Digestibility

Coefficients of Apparent Digestibility were calculated for dry-matter, gross energy, Nitrogen and lipid, using equation 3:2. To enable statistical analysis of the values obtained, data were first normalised by means of the angular, or arc sine, transformation, and the transformed means then compared using 'Student's' t-test. Though Apparent Digestibility was usually higher in males, no significant differences were found between the sexes for any of the variables measured and therefore, data for males and females were pooled; the figures quoted are the back-transformed aggregate means for each group. Since confidence limits about these means are asymmetrical, tables include the upper and lower bounds of a confidence interval equivalent to the arc sine mean, plus or minus it's standard error.

#### (i) Apparent Digestibility of dry-matter and gross energy

The Apparent Digestibility of dry-matter did not vary markedly between the rations presented, as may be seen from Table 3:18. The value recorded for CK12 (0.728) was somewhat low in comparison with the other diets fed, but differences were not significant ($p > 0.05$). The mean for all rations was 0.747. (Data were not collected during RBBT trials).

Coefficients of Apparent Digestibility for gross energy intake are presented in Table 3:19, and the results of statistical comparisons of the means in Table 3:20. With the exception of RBBT, comparisons between trials revealed no significant differences. Similar values were obtained for RBBT and STAN1, though Apparent Digestibility was significantly higher on RBBT than on all the other rations, with ADE constituting nearly 87% of the energy ingested.

Table 3:18 Apparent Digestibility of Dry-matter

| Trial | N | Mean | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| STAN1 | 4 | 0.769 | 0.740 | 0.797 |
| CK12 | 7 | 0.728 | 0.695 | 0.761 |
| STAN2 | 4 | 0.762 | 0.731 | 0.792 |
| E-MIX | 7 | 0.745 | 0.735 | 0.755 |

Table 3:19 Apparent Digestibility of Gross Energy

| Trial | N | Mean | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 0.869 | 0.859 | 0.879 |
| STAN1 | 4 | 0.830 | 0.808 | 0.851 |
| CK12 | 7 | 0.797 | 0.784 | 0.810 |
| STAN2 | 4 | 0.798 | 0.774 | 0.820 |
| E-MIX | 7 | 0.823 | 0.816 | 0.829 |

Table 3:20 Comparison of Apparent Digestibility of Gross Energy Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | 0.001 | 0.01 | 0.005 |
| STAN1 | 4 | | * | N.S. | N.S. | N.S. |
| CK12 | 7 | | | * | N.S. | N.S. |
| STAN2 | 4 | | | | * | N.S. |
| E-MIX | 7 | | | | | * |

(ii) Apparent Digestibility of Nitrogen

The Apparent Digestibility of Nitrogen varied considerably between trials, as shown in Table 3:21, with the highest values recorded on RBBT and E-MIX (0.940 and 0.918, respectively). A significant difference was found between these two ($p < 0.05$), and on both trials ADN was significantly higher than on the other three, between which no significant differences were apparent (see Table 3:22).

(iii) Apparent Digestibility of lipid

Reference to Table 3:23 shows that, on all rations, the Apparent Digestibility of lipid was very high, and absolute differences between trials were comparatively small. Comparison of the means (tabulated in Table 3:24) revealed significant differences between E-MIX (0.988) and each of the other trials, and between RBBT and CK12, for which the values recorded were 0.971 and 0.936, respectively. Observed differences between STAN1, CK12 and STAN2 were not statistically significant.

### 3:5.5 <u>Partitionment of Ingested Gross Energy</u>

Following the scheme delineated in Fig. 3:1, ADE was apportioned between urinary energy losses and Retained, or Metabolisable Energy (ME). The former was estimated from direct assay of urea Nitrogen, as described in section 3:4.4.2 above, the latter obtained by difference. Aggregate mean values for urinary energy and ME, expressed as percentages of ingested Gross Energy (GE), are presented in Tables 3:25 and 3:27. Confidence intervals about the means (+/- one standard error of the arc sine transformed means)

### Table 3:21 Apparent Digestibility of Nitrogen

| Trial | N | Mean | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 0.940 | 0.933 | 0.947 |
| STAN1 | 4 | 0.844 | 0.835 | 0.853 |
| CK12 | 7 | 0.817 | 0.796 | 0.837 |
| STAN2 | 4 | 0.841 | 0.823 | 0.858 |
| E-MIX | 7 | 0.918 | 0.914 | 0.922 |

### Table 3:22 Comparison of Apparent Digestibility of Nitrogen Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | 0.001 | 0.001 | 0.005 | 0.05 |
| STAN1 | 4 | | * | N.S. | N.S. | 0.001 |
| CK12 | 7 | | | * | N.S. | 0.001 |
| STAN2 | 4 | | | | * | 0.05 |
| E-MIX | 7 | | | | | * |

Table 3:23 Apparent Digestibility of Lipid

| Trial | N | Mean | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 0.971 | 0.968 | 0.973 |
| STAN1 | 4 | 0.954 | 0.941 | 0.964 |
| CK12 | 7 | 0.936 | 0.925 | 0.946 |
| STAN2 | 4 | 0.941 | 0.925 | 0.955 |
| E-MIX | 7 | 0.988 | 0.985 | 0.990 |

Table 3:24 Comparison of Apparent Digestibility of Lipid Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | 0.01 | N.S. | 0.001 |
| STAN1 | 4 | | * | N.S. | N.S. | 0.005 |
| CK12 | 7 | | | * | N.S. | 0.001 |
| STAN2 | 4 | | | | * | 0.001 |
| E-MIX | 7 | | | | | * |

Table 3:25 Estimated Urine Energy as a Proportion of Ingested Gross Energy

| Trial | N | Mean (%) | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 6.29 | 5.77 | 6.82 |
| STAN1 | 4 | 7.67 | 7.51 | 7.83 |
| CK12 | 7 | 4.92 | 4.71 | 5.14 |
| STAN2 | 4 | 7.10 | 6.54 | 7.69 |
| E-MIX | 7 | 2.57 | 2.40 | 2.76 |

Table 3:26 Comparison of Urine Energy Losses Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | 0.05 | N.S. | 0.001 |
| STAN1 | 4 | | * | 0.001 | N.S. | 0.001 |
| CK12 | 7 | | | * | 0.005 | 0.001 |
| STAN2 | 4 | | | | * | 0.001 |
| E-MIX | 7 | | | | | * |

Table 3:27 Metabolisable Energy as a Proportion of Ingested Gross Energy

| Trial | N | Mean (%) | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 80.47 | 79.04 | 81.85 |
| STAN1 | 4 | 76.03 | 78.37 | 75.42 |
| CK12 | 7 | 74.70 | 73.33 | 76.04 |
| STAN2 | 4 | 72.53 | 70.01 | 74.97 |
| E-MIX | 7 | 79.64 | 78.79 | 80.48 |

Table 3:28 Comparison of Metabolisable Energy Retention Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | 0.05 | 0.05 | N.S. |
| STAN1 | 4 | | * | N.S. | N.S. | N.S. |
| CK12 | 7 | | | * | N.S. | 0.01 |
| STAN2 | 4 | | | | * | 0.01 |
| E-MIX | 7 | | | | | * |

are also included.

Urinary losses were greatest on the rabbit-based diets, RBBT, STAN1 and STAN2. Comparison of the means using 'Student's t-test revealed no significant differences between these three (see Table 3:26), though the value of 6.29% of GE recorded for Experimental animals on RBBT was somewhat lower than the figures obtained for Standard animals on the same ration (7.67% and 7.10% for STAN1 and STAN2, respectively). On the CK12 trial, urinary losses constituted 4.92% of GE, and on E-MIX, only 2.99%, both values significantly lower than those derived for the other three trials, and significantly different from each other ($p < 0.001$).

Reference to Table 3:27 indicates that ME remained a relatively constant proportion of GE on all trials, and no significant differences were observed between STAN1, CK12 and STAN2 (see Table 3:28). The figure obtained for RBBT (80.47% GE) and E-MIX (79.64% GE) were, however, markedly higher, and differed significantly from the values recorded on each of the other trials except STAN1, at 76.03% GE.

Metabolisable Energy was resolved into two components; the heat of nutrient metabolism or SDA, and Net Energy (NE). The calculation of SDA was based on estimates of the daily intake of apparent digestible protein and lipid, according to the formula

$$SDA = Consumption.((P_p.D_p.C_p) + (P_l.D_l.C_l)) \qquad \text{(Eqn. 3:8)}$$

in which the constant 'P' is the percentage of protein or lipid in the ration, 'D' is the Coefficient of Apparent Digestibility (see Table 3:4), and 'C' a caloric equivalent. Hill (1976) reported that the SDA of proteins approximated to 30% of total energy content, and that of lipids, 10%. Therefore, using Kleiber's (1961) figures of 23.83 KJ/g and 39.71 KJ/g for the total energy content of protein and lipid, respectively, a value of 7.15 KJ/g was derived as the caloric equivalent of protein, and 3.97 KJ/g for lipid.

Net Energy was obtained by difference, and the aggregate means for each component, expressed as a percentage of Gross Energy, are given in Tables 3:29 and 3:31. Statistical comparisons between the means of different trials are tabulated in Tables 3:30 and 3:32, for SDA and Net Energy respectively.

SDA constituted a highly variable proportion of GE, as may be seen from Table 3:29, with aggregate mean values ranging from 15.38% GE on E-MIX to 28.25% on RBBT. The observed differences were correlated with variations in both the composition of the rations presented, and their Apparent Digestibility. Disparities between the values obtained for the three rabbit-based diets were, accordingly, relatively small. The difference between STAN1 (25.46% GE) and STAN2 (25.37%) was not statistically significant (see Table 3:30), though the figure obtained for RBBT was significantly higher ($p < 0.001$). On the other two rations SDA constituted a significantly smaller proportion of GE intake (18.07% and 15.38% for CK12 and E-MIX, respectively), due to the lower protein content of these diets.

Mean values for Net Energy (NE) are given in Table 3:31, in which a similar, though reversed, pattern may be observed. Again, there were no significant differences between RBBT, STAN1 and STAN2, in all of which Net Energy formed a significantly smaller proportion of GE intake than in trials with the CK12 and E-MIX rations (see Table 3:32).

As shown in Fig. 3:1, Apparent Net Energy is conventionally assigned either to Maintenance or Production energy. Maintenance requirements,

Table 3:29 SDA as a Proportion of Ingested Gross Energy

| Trial | N | Mean (%) | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 28.25 | 28.04 | 28.45 |
| STAN1 | 4 | 25.46 | 25.20 | 25.71 |
| CK12 | 7 | 18.07 | 17.62 | 18.52 |
| STAN2 | 4 | 25.37 | 24.86 | 25.89 |
| E-MIX | 7 | 15.38 | 115.34 | 15.43 |

Table 3:30 Comparison of SDA Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | 0.001 | 0.001 | 0.001 | 0.001 |
| STAN1 | 4 | | * | 0.001 | N.S. | 0.001 |
| CK12 | 7 | | | * | 0.001 | 0.001 |
| STAN2 | 4 | | | | * | 0.001 |
| E-MIX | 7 | | | | | * |

Table 3:31 Net Energy as a Proportion of Ingested Gross Energy

| Trial | N | Mean (%) | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 51.89 | 50.63 | 53.16 |
| STAN1 | 4 | 50.16 | 48.23 | 52.09 |
| CK12 | 7 | 56.46 | 55.39 | 57.53 |
| STAN2 | 4 | 46.93 | 44.92 | 48.94 |
| E-MIX | 7 | 64.15 | 63.26 | 65.03 |

Table 3:32 Comparison of Net Energy Retention Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | 0.05 | N.S. | 0.001 |
| STAN1 | 4 | | * | 0.05 | N.S. | 0.001 |
| CK12 | 7 | | | * | 0.001 | 0.001 |
| STAN2 | 4 | | | | * | 0.001 |
| E-MIX | 7 | | | | | * |

comprising the energy expended in sustaining BMR, thermoregulation and activity at maintenance, were assumed to be constant between trials. Production costs, which include the energy invested in reproduction and growth, and the synthesis of new tissues such as pelage and depot fat, were expected to vary with physiological changes associated with the moult and the deposition of adipose tissue.

Since all subject animals were non-reproductive adults, the costs of reproduction and growth were discounted, and the relative proportions of Production and Maintenance energy on different trials evaluated using estimates of the energy expended in the synthesis and storage of adipose tissue.

These estimates were based on the assumption that the weight gains recorded in subject animals during the feeding trials represented deposition of body fat. Mitchell (1945) found that human adipose tissue comprised 42.44% lipid and 7.06% protein, and determined the energy content to be 17.47 KJ/g.[1] Pullar and Webster (1977) showed that the energy costs of protein and lipid deposition were approximately 52.9 KJ/g and 53.4 KJ/g, respectively. Thus, caloric equivalents of the observed weight gains were calculated by substitution of these figures into the following formula:-

$$\text{Production energy (Adipose tissue)} = \text{Weight Gain.}(W_p.P_p + W_l.P_l) \qquad \text{(Eqn. 3:9)}$$

in which '$P_p$' and '$P_l$' are the percentages of protein and lipid in the tissue, and '$W_p$' and '$W_l$' the Pullar-Webster constants. The aggregate means of the values so-derived, expressed as percentages of GE intake, are presented in Table 3:33, from which it is clear that the energy expended in

[1] Footnote: No data on the composition of mink adipose tissue are known to have been published.

Table 3:33 Energy Invested in Deposition of Adipose Tissue as a Proportion of Gross Energy Intake

| Trial | N | Mean (%) | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 3.096 | 0.216 | 5.976 |
| STAN1 | 4 | 5.574 | 1.180 | 9.968 |
| CK12 | 7 | 15.818 | 10.265 | 21.371 |
| STAN2 | 4 | 4.783 | -3.181 | 12.747 |
| E-MIX | 7 | 31.457 | 23.335 | 39.579 |

deposition of body fat was greatly increased on the lipid-rich rations CK12 and E-MIX, in comparison with the other rations having a lower lipid content. Standard errors were, however, very large, owing to the variability in the direction and magnitude of the daily weight changes of subject animals, from which these estimates were derived. The value of 31.46% GE obtained for E-MIX was demonstrably higher than each of the other trials ($p < 0.05$), but no significant differences were apparent between these four.

Deduction of this component of Production Energy from total NE yielded a value here defined as 'Maintenance' Energy, which included the requirements for maintenance and the additional costs associated with the moult. Reference to Fig. 3:4 indicates that the presentations of RBBT and E-MIX took place at the onset of the vernal moult, each trial commencing in the last week of March, while those of CK12 were carried out in August, immediately prior to the autumn moult. In contrast, both trials with the Standard animals were undertaken during the inter-moult period. It was anticipated, therefore, that comparisons of the energy metabolism of subject animals at different stages in the moult cycle would provide a means of evaluating the magnitude of the costs of moulting, thereby enabling a more accurate estimate of true maintenance requirements to be made.

The partitionment of ingested Gross Energy during the feeding trials was based on the scheme delineated in Fig. 3:1, and the means for each element are tabulated in Table 3:34a, which also includes estimates of the Apparent Net Energy Coefficient (NEC). Estimates of Production Energy (adipose tissue) and Maintenance Energy, the two components into which NE was resolved, are given in Table 3:34b. Data are expressed on a mass-specific basis, per unit metabolic weight ($KJ/Kg^{0.75}/day$), to facilitate direct comparison between trials.

Table 3:34a Apparent Mass-Specific Partitionment of Ingested Gross Energy During Feeding Trials

| Trial | N | Ingested | Faeces | Urine | SDA | Net Energy | Net Energy Coefficient |
|---|---|---|---|---|---|---|---|
| RBBT | 8 | 1277 ( 54.7) | 169.5 (16.9) | 83.8 (8.9) | 358 (12.8) | 658 ( 21.6) | 0.648 |
| STAN1 | 4 | 1282 (131.9) | 217.6 (36.1) | 89.6 (5.7) | 327 (31.4) | 636 ( 67.0) | 0.660 |
| CK12 | 7 | 1295 ( 65.3) | 267.5 (26.2) | 63.7 (3.6) | 234 (12.6) | 739 ( 41.3) | 0.760 |
| STAN2 | 4 | 1152 ( 69.4) | 236.7 (37.6) | 82.3 (9.8) | 292 (14.0) | 545 ( 27.5) | 0.652 |
| E-MIX | 7 | 1720 ( 78.8) | 302.8 ( 9.9) | 44.0 (3.4) | 263 (12.8) | 1110 (119.2) | 0.808 |
| | | | | | | | |
| MOULT | 15 | 1484 ( 74.4) | 231.7 (20.3) | 65.3 (7.1) | 314 (15.4) | 869 ( 67.4) | 0.732 |
| NON-MOULT | 15 | 1253 ( 48.9) | 246.0 (18.0) | 75.6 (4.4) | 274 (14.6) | 660 ( 33.4) | 0.706 |

Table 3:34b Apparent Mass-specific Partitionment of Net Energy During Feeding Trials

| Trial | N | Net | Production (Adip. tissue) | Maintenance |
|---|---|---|---|---|
| RBBT | 8 | 658 (21.6) | 145.7 (47.9) | 494 ( 51.5) |
| STAN1 | 4 | 636 (67.0) | 113.5 (42.4) | 529 ( 80.8) |
| CK12 | 7 | 739 (41.3) | 239.8 (79.1) | 500 ( 58.3) |
| STAN2 | 4 | 545 (27.5) | 91.7 (77.1) | 453 ( 50.4) |
| E-MIX | 7 | 1110 (63.7) | 593.6 (119.2) | 586 (119.6) |

Table 3:35 Comparison of Mass-Specific Energy Retention Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | N.S. | 0.005 | 0.001 |
| STAN1 | 4 |  | * | N.S. | N.S. | 0.001 |
| CK12 | 7 |  |  | * | 0.005 | 0.001 |
| STAN2 | 4 |  |  |  | * | 0.001 |
| E-MIX | 7 |  |  |  |  | * |

It is evident from these results that absolute differences in maintenance-energy requirements were attributable primarily to the variations in the relative magnitude of SDA already described, and to the energy expended in the deposition of adipose tissue, particularly for E-MIX. The elevated NEC's recorded for CK12 and E-MIX (0.76 and 0.81, respectively) indicated that SDA constituted a much lower proportion of ME on these rations than on the rabbit-based diets, in consequence of their relatively low ratios of protein to lipid. Since both CK12 and E-MIX rations were also characterised by a comparatively high lipid content (see section 2:4), total Net Energy retained per unit metabolic weight was considerably higher during these trials in comparison with the other three, as may be seen from Table 3:34b; values of 1110 and 740 KJ/$Kg^{0.75}$/day were obtained for Experimental animals feeding on the E-MIX and CK12 rations, in contrast with the 658 KJ calculated for the same individuals during trials with RBBT. No significant differences were apparent between trials with the Standard animals, nor between RBBT, STAN1 and CK12 (See Table 3:35). Net Energy retention was, however, significantly lower on STAN2, with an estimated mean of 545 KJ/$Kg^{0.75}$/day, than on each of the other trials ($p < 0.005$).

Comparisons between trials revealed no evidence of a change in energy metabolism associated with the moult; observed differences were primarily a consequence of variations in the composition of the rations presented. For instance, estimated Net Energy retention during the E-MIX trials exceeded that on RBBT by 69%, while data obtained from animals on the RBBT and STAN1 trials did not differ significantly for any of the parameters measured. The amount of energy invested in the deposition of adipose tissue paralleled the variations in NE intake, and ranged from 3.1% GE on RBBT to 31.5% GE on E-MIX; the residual component of NE, designated Maintenance Energy, therefore remained relatively constant at about 513 KJ/$Kg^{0.75}$/day. There were no significant differences between trials ($p > 0.05$) and consequently, attempts to evaluate the energetic costs of the moult were not taken further.

### 3:5.6 Nitrogen Metabolism

Nitrogen metabolism was characterised with reference to three components; ingested or dietary Nitrogen, faecal losses and urinary losses. Apparent Digestible Nitrogen (ADN) was calculated by difference from comparison of dietary intake and faecal Nitrogen, and apportioned between urine losses, measured by direct assay of urine urea, and a residual element here defined as 'retained' or metabolisable Nitrogen. The aggregate means of the three components tabulated in Table 3:36. Data are expressed on a mass-specific basis, per unit metabolic weight (g N/ $Kg^{0.75}$/day) to facilitate direct comparisons between trials.

It is clear from these data that differences in dietary Nitrogen content and the Apparent Digestibility of the rations presented were the major factors in determining absolute Nitrogen retention. The mean values for retained Nitrogen per unit metabolic weight, were consistently higher on the rabbit-based diets, RBBT, STAN1 and STAN2, than on the rations of lower protein content, CK12 and E-MIX; statistical comparisons between trials indicated that the figure of 3.99 g N /$Kg^{0.75}$/day obtained for the Experimental animals during trials with RBBT differed significantly from those recorded on each of the other trials except STAN1 (3.00 g N /$Kg^{0.75}$/day ). Nitrogen retention was lowest on the CK12 diet, at only 1.87 g N /$Kg^{0.75}$/day, though this value did not differ significantly from those estimated for STAN2 and E-MIX (2.57 and 2.17 g N /$Kg^{0.75}$/day, respectively).

Table 3:36 Apparent Mass-Specific Partitionment of Ingested Nitrogen During Feeding Trials

| TRIAL | N | Ingested | Faeces | Urine | Retained |
|---|---|---|---|---|---|
| RBBT | 8 | 8.11 (0.35) | 0.52 (0.07) | 3.60 (0.38) | 3.99 (0.24) |
| STAN1 | 4 | 8.14 (0.84) | 1.29 (0.09) | 3.85 (0.25) | 3.00 (0.14) |
| CK12 | 7 | 5.66 (0.29) | 1.06 (0.03) | 2.73 (0.15) | 1.87 (0.23) |
| STAN2 | 4 | 7.31 (0.44) | 1.20 (0.01) | 3.53 (0.42) | 2.57 (0.16) |
| E-MIX2 | 7 | 4.45 (0.20) | 0.37 (0.04) | 1.89 (0.15) | 2.17 (0.17) |
| MOULT | 15 | 6.40 (0.53) | 0.45 (0.04) | 2.80 (0.31) | 3.14 (0.28) |
| NON-MOULT | 15 | 6.76 (0.39) | 0.16 (0.01) | 3.24 (0.19) | 2.36 (0.20) |

The parity in Nitrogen retention between E-MIX and the other trials, during which intake was substantially greater, was attributable, in part, to the greatly elevated coefficient of Apparent Digestibility calculated for this ration (see Table 3:22). Similarly, the differences in the Apparent Digestibility of RBBT and STAN2 contributed to the disparity in Nitrogen retention recorded for these diets, for which the estimates of mass-specific intake were not significantly different (Table 3:15).

Urine losses constituted a relatively constant proportion of intake, and were closely correlated with Apparent Digestible Nitrogen, as shown in Fig. 3:11. Indices of Nitrogen utility, however, varied markedly between trials; mean values, expressed as the proportion of ADN retained, are presented in Table 3:37. Statistical comparisons of the means were made using 'Student's' t-test, and the results are tabulated in Table 3:38.

A marked disparity is evident between the values derived for RBBT and E-MIX (53.28 and 53.06% ADN), and the remaining three trials, in which Nitrogen utility was considerably lower (mean values of 41.81, 39.15 and 41.74% ADN for STAN1, CK12 and STAN2, respectively). Statistical analyses of the differences observed were, however, inconclusive; a significant difference was recorded between RBBT and CK12, but comparison with STAN1 and STAN2 revealed no such difference. In contrast, the means of both CK12 and STAN1 were found to be significantly lower than the figure given for E-MIX. These anomalies may be due to inequalities in the variances of the samples compared.

Estimates of the percentage of ingested Nitrogen retained are presented in Table 3:39. Again, the highest values recorded were for RBBT and E-MIX (49.87 and 48.54%, respectively), and statistical comparison revealed no significant difference between these two (see Table 3:40). On both these trials, however, Nitrogen retention was significantly higher than on each of

Fig 3:11 Relationship of Urine Nitrogen to Apparent Digestible Nitrogen

(Per Unit Metabolic Weight)

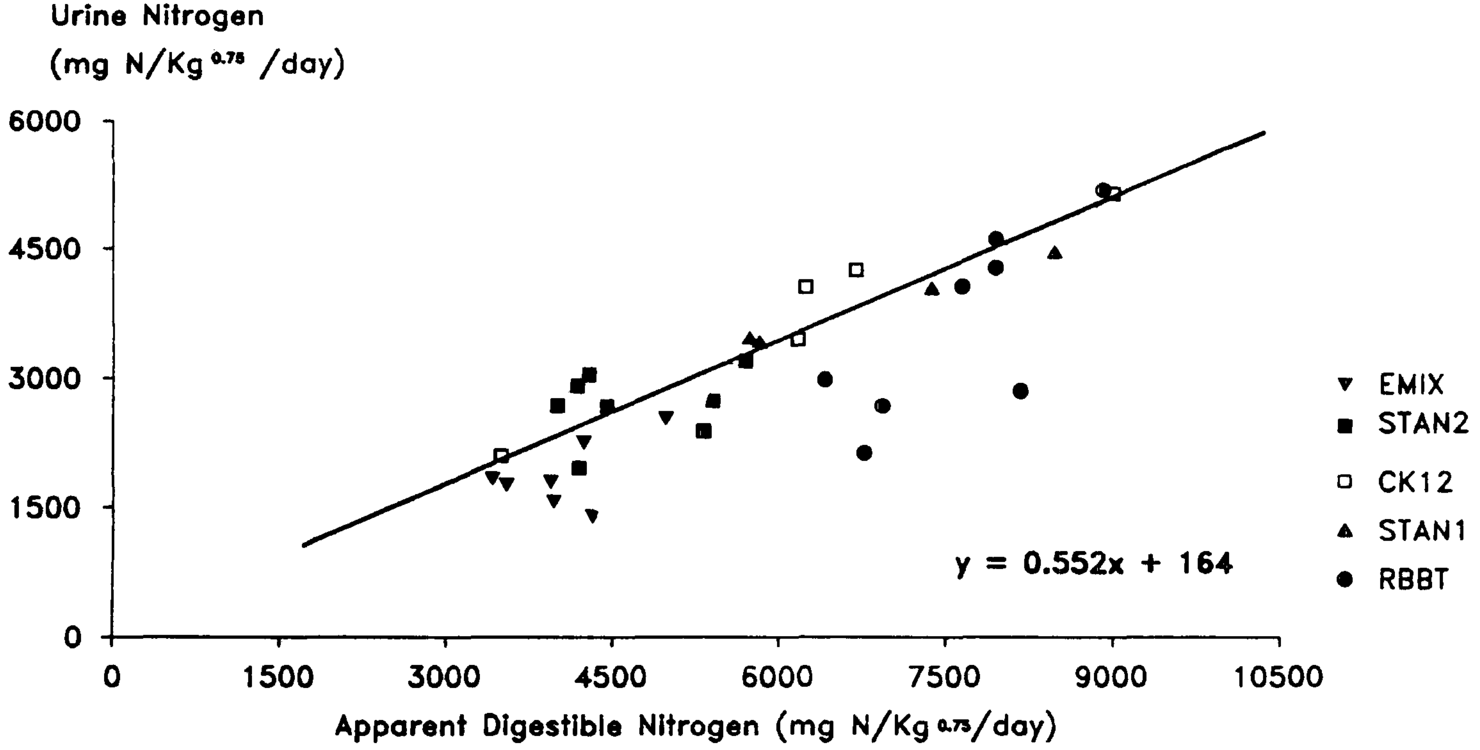


Table 3:37 Retained Nitrogen as a Proportion of Apparent Digestible Nitrogen

| Trial | N | Mean (%) | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 53.28 | 49.23 | 57.30 |
| STAN1 | 4 | 41.81 | 39.64 | 43.99 |
| CK12 | 7 | 39.15 | 35.60 | 42.75 |
| STAN2 | 4 | 41.74 | 37.06 | 46.50 |
| E-MIX | 7 | 53.06 | 50.05 | 56.06 |

Table 3:38 Comparison of Nitrogen Utility Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | N.S. | 0.05 | N.S. | N.S. |
| STAN1 | 4 | | * | N.S. | N.S. | 0.05 |
| CK12 | 7 | | | * | N.S. | 0.05 |
| STAN2 | 4 | | | | * | N.S. |
| E-MIX | 7 | | | | | * |

Table 3:39 Retained Nitrogen as a Proportion of Ingested Nitrogen

| Trial | N | Mean (%) | Confidence Interval | |
|---|---|---|---|---|
| | | | Lower lim. | Upper lim. |
| RBBT | 8 | 49.87 | 45.81 | 53.94 |
| STAN1 | 4 | 35.26 | 33.71 | 36.83 |
| CK12 | 7 | 32.01 | 28.54 | 35.58 |
| STAN2 | 4 | 34.92 | 30.70 | 39.26 |
| E-MIX | 7 | 48.54 | 46.05 | 51.05 |

Table 3:40 Comparison of Nitrogen Retention Between Trials

| Trial | N | RBBT | STAN1 | CK12 | STAN2 | E-MIX |
|---|---|---|---|---|---|---|
| RBBT | 8 | * | 0.05 | 0.01 | 0.05 | N.S. |
| STAN1 | 4 | | * | N.S. | N.S. | 0.005 |
| CK12 | 7 | | | * | N.S. | 0.005 |
| STAN2 | 4 | | | | * | 0.05 |
| E-MIX | 7 | | | | | * |

the other three, between which no significant differences were apparent (mean values of 35.26, 32.01 and 34.91% for STAN1, CK12 and STAN2, respectively).

The emergent pattern was thus one of increased Nitrogen retention on those trials carried out during the moult, effected through changes in the digestibility and metabolism of ingested Nitrogen. In order to test this hypothesis, the data collected from each trial were assigned to one of two groups, either Moult (for RBBT and E-MIX) or Non-Moult (for STAN1, STAN2 and CK12); the combined means so-derived have been included in Table 3:34, to permit comparison with the values obtained from individual trials. Hotelling's multiple t-test was then applied to the three principal descriptors of Nitrogen metabolism; the coefficients of Apparent Digestibility and utility, and the percentage of ingested Nitrogen retained. The results of separate and collective analyses of these parameters are tabulated in Table 3:41, from which it may be seen that the differences between the two groups were highly significant in each case. The value for Hotelling's $T^2$ (81.89) provides a measure of the difference in group means across all variables and, similarly, indicated a significant difference in the Nitrogen metabolism of Moulting and Non-moulting animals ($p < 0.001$).

### 3:5.7 Bowel Transit Time

Determinations of Bowel transit times were made for each of the three experimental diets, RBBT, CK12 and E-MIX, and statistical comparisons effected using 'Student's' t-test. There were no significant differences between the sexes and therefore data for males and females were pooled; mean values for each diet are presented in Table 3:42.

Table 3:41 Variations in Nitrogen Metabolism Associated with the Moult

| VARIABLE | MOULT (n = 15) | NON-MOULT (n = 15) | SIG. |
|---|---|---|---|
| Apparent Digestibility | 0.930 (0.925, 0.935) | 0.831 (0.820, 0.841) | 0.001 |
| Pct. intake Retained | 49.25 (46.87, 51.63) | 33.64 (31.69, 36.65) | 0.001 |
| Pct. ADN Retained | 53.17 (50.69, 55.65) | 40.54 (38.49, 42.61) | 0.001 |

| Hotelling-T | F-value | d.f. | SIG. |
|---|---|---|---|
| 81.8899 | 25.347 | 3, 26 | 0.001 |

Table 3:42 Bowel Transit Times

| Diet | N | Transit Time (hr.) |
|---|---|---|
| RBBT | 13 | 2.88 (0.14) |
| CK12 | 13 | 3.25 (0.15) |
| E-MIX | 16 | 3.87 (0.19) |

Rates of passage were highest on the RBBT rations, with a mean transit time of 2.88 hr.; on CK12, transit times increased slightly (mean = 3.25 hr.), but the difference between the two diets was not statistically significant. The lowest rates of passage were recorded on E-MIX, for which the mean transit time was 3.87 hr. Though not significantly different from the figure obtained for CK12, this value was significantly greater than that given for the RBBT diet ($p < 0.001$), the difference representing a decrease of over 25% in the passage rate of this ration.

### 3:5.8 Gut Morphology

The means of the measurements taken are tabulated in Tables 3:43a and 3:43b for males and females, respectively. For purposes of comparison, derived variables based on these measures are also included.

In both sexes, the body weight of farm-bred animals exceeded that that of the wild-caught individuals, but the relative difference was much greater in males than in females; commercially raised animals averaging more than twice the weight of their wild counterparts (means of 1.95 Kg and 0.85 Kg, respectively). In contrast, the mean body weights of farm-bred females (1.0 Kg) exceeded that of the wild-caught specimens by only 22%.

Gut length was found to be linearly correlated with body weight, as illustrated in Fig. 3:12. Data obtained from wild males accorded well with the general trend, and this was interpreted as an indication that the low body weights of these individuals represented a generally smaller frame size, rather than simply poor condition. Comparisons between the two samples revealed that, in both sexes, the ratio of gut length to body weight was significantly higher in wild-caught animals, although the low body weights of the wild males undoubtedly contributed to the differences

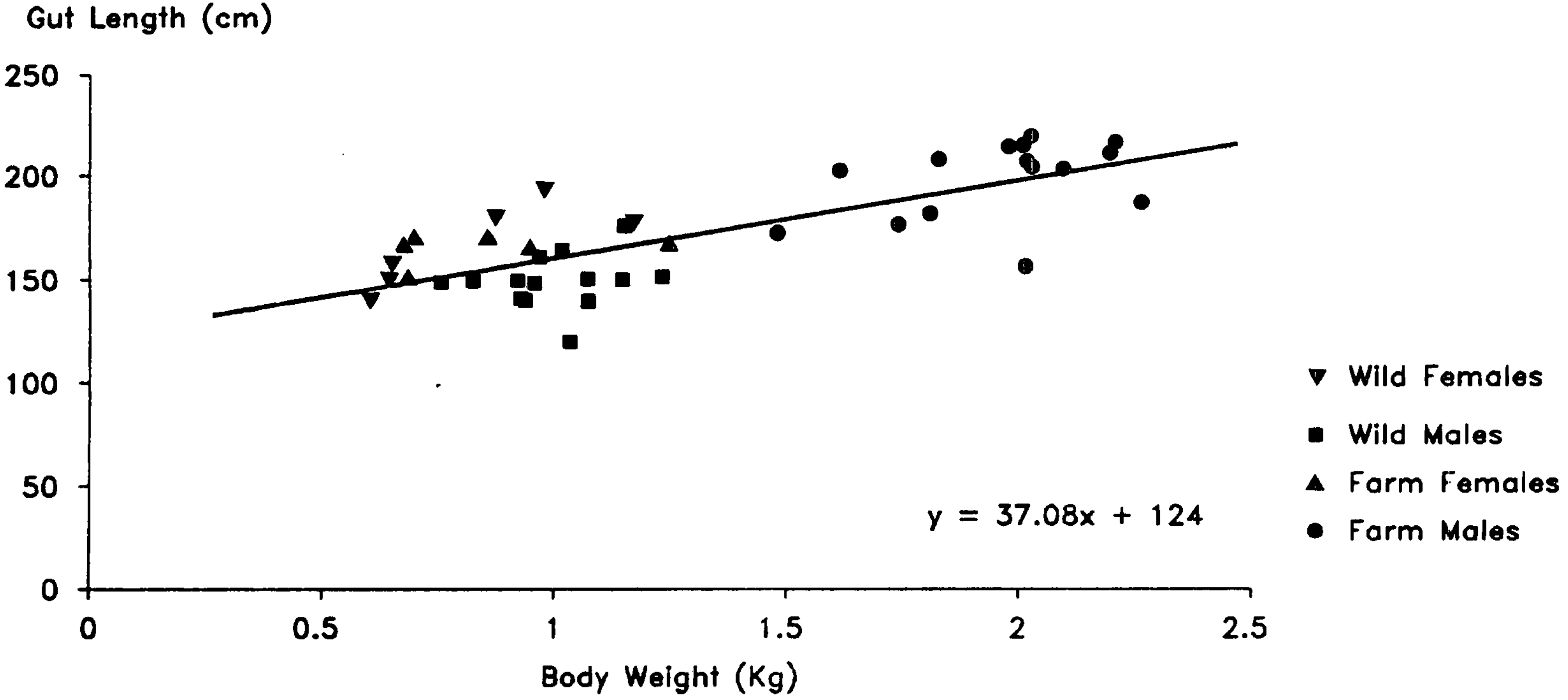
Table 3:43a Gut Morphology: Comparisons between Wild and Farm-bred Animals (Males)

| | WILD (n = 6) | FARM-BRED (n = 15) | SIG. |
|---|---|---|---|
| Body Weight (Kg) | 0.850 (0.091) | 1.950 (0.057) | 0.001 |
| Gut Length (cm) | 164.9 (2.92) | 198.3 (4.88) | 0.001 |
| Circumference (cm) | 1.530 (0.07) | 1.890 (0.03) | 0.001 |
| Ratio of Length to Body Weight | 202.46 (17.7) | 102.34 ( 3.1) | 0.001 |
| Volume (cm3) | 31.19 (3.13) | 56.38 (2.25) | 0.001 |
| Weight-specific Capacity (cm3/Kg) | 37.62 (4.14) | 28.86 (0.80) | N.S. |
| Ratio of Surface Area to Volume | 8.30 (0.39) | 6.68 (0.12) | 0.01 |

Table 3:43b Gut Morphology: Comparisons between Wild and Farm-bred Animals (Females)

| | WILD (n = 6) | FARM-BRED (n = 14) | SIG. |
|---|---|---|---|
| Body Weight (Kg) | 0.82 (0.093) | 1.00 (0.035) | N.S. |
| Gut Length (cm) | 167.8 (8.40) | 149.3 (3.44) | 0.05 |
| Circumference (cm) | 1.60 (0.06) | 1.67 (0.03) | N.S. |
| Ratio of Length to Body Weight | 212.3 (13.8) | 151.3 ( 6.0) | 0.001 |
| Volume (cm3) | 34.83 (3.83) | 33.53 (1.60) | N.S. |
| Weight-specific Capacity (cm3/Kg) | 43.08 (3.44) | 33.73 (5.90) | 0.01 |
| Ratio of Surface Area to Volume | 7.92 (0.32) | 7.55 (0.15) | N.S. |

Fig 3:12 Relationship of Gut Length to Body Weight in Wild and Farm-bred Mink

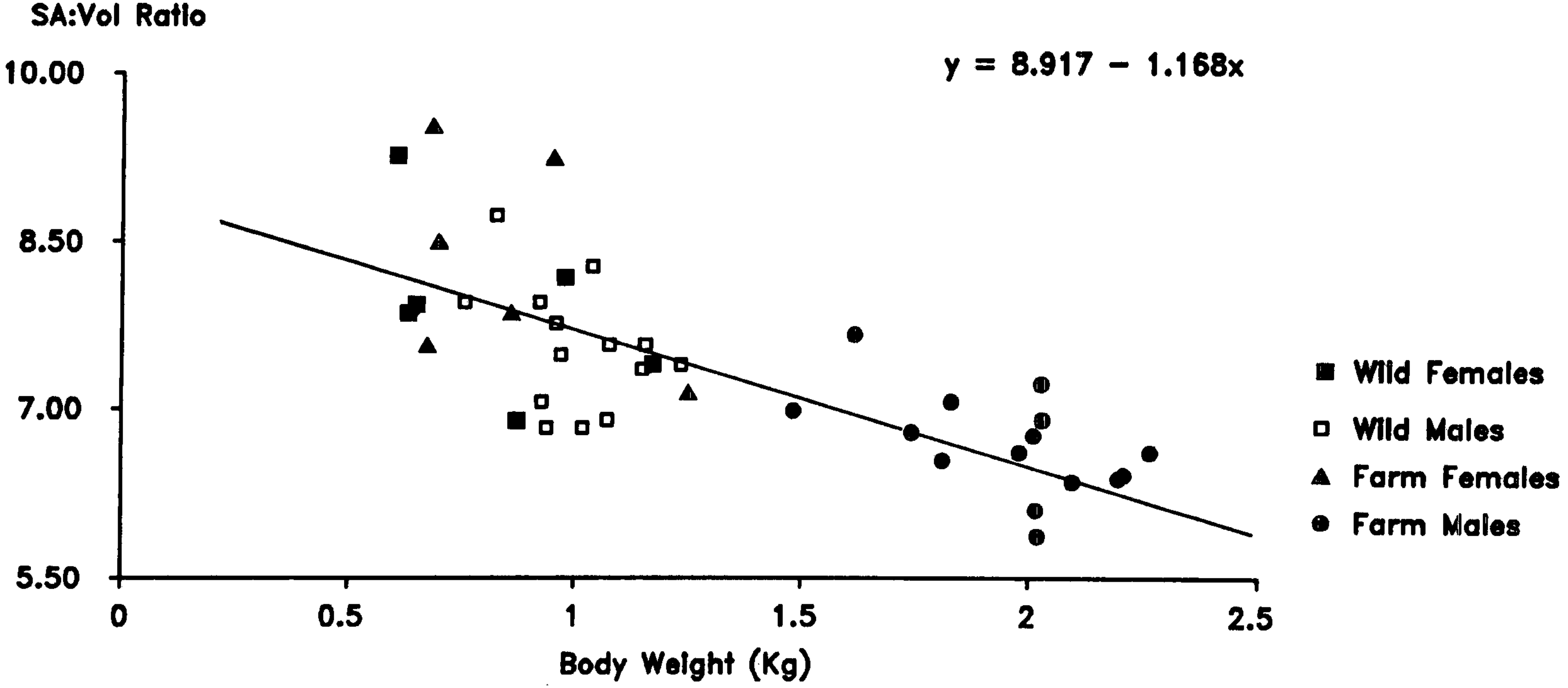


observed. It may be noted, however, that in females, in which the difference in mean body weights was much less marked, the gut lengths of wild-caught specimens were, on average, 40% longer in proportion to body weight than those of commercially-raised animals (212.28 cm/Kg and 151.27 cm/Kg, respectively).

The circumference of the intestine, also a correlate of body weight, was found to be significantly greater in the farm-bred than in the wild males (18.9 mm compared with 15.3 mm, $p < 0.005$), though no such difference was apparent between females. In both sexes, however, the intestine was relatively wider, in proportion to length, in the wild-caught specimens. Consequently, total gut capacity per unit body weight was found to be substantially greater in these individuals than in the farm animals. For the males, the figures obtained were 37.62 and 28.56 $cm^3$/Kg for wild and commercially-raised animals, respectively; the equivalent values for females were 43.08 and 33.73 $cm^3$/Kg, a difference which was statistically significant ($p < 0.01$). Comparisons between sexes revealed that, in farm-bred animals, the weight-specific gut capacity of females exceeded that of males by 16.86% ($p < 0.01$); for the wild-caught specimens the figure was 14.86%, though this difference was not statistically significant.

The ratio of serosal surface area to luminal volume can be shown to reduce to 4.Pi/circumference and, therefore, varied inversely with body weight, as illustrated in Fig. 3:13. The regression equation describing the relationship is also included.

Fig 3:13 Relationship of Intestinal Surface Area:Volume Ratio to Body Weight in Wild and Farm-bred Mink



## 3.6 Discussion

### 3.6.1 The Effect of Body Size on Nutritional Requirements

The aims of the work as detailed in the preceding sections were three-fold; to evaluate the relationship between body-size and energy requirements, to compare and contrast the digestion and metabolism of the diets presented, and to investigate the possibility that males and females differ in their capacity to assimilate ingested material.

Regarding the first of these, it is apparent that the relationship between body-size and energy requirements in mink does not differ markedly from that described for other species, with Metabolisable Energy intake being a function of body weight raised to a fractional power approximating to 0.75. In consequence, the weight-specific energy requirements of the males were proportionately lower than those of the smaller females; the mean daily intake on all diets was estimated to be 832 KJ/Kg/day for males (mean body weight 1.817 Kg), compared with approximately 1475 KJ/Kg/day for females (body weight 0.843 Kg). Since subject animals underwent only minor weight change in the course of the feeding trials, these values have been taken to represent the energy requirement for maintenance, and are in close agreement with the estimate of 1141 KJ/Kg/day for mink, provided by the U.S. National Academy of Sciences (1968).

Of wider application is the index of intake per unit metabolic weight, the use of which permits comparison with the results obtained in more recent investigations. Expressed on this basis, it may be shown that the daily GE intake of the animals used in this study averaged 1299 $KJ/Kg^{0.75}$/day. This value is almost twice the figure of 659 $KJ/Kg^{0.75}$/day given by Chwalibog *et al* (1980), who used respirometric techniques in combination with feeding trials, to estimate maintenance requirements from measurements of retained and metabolisable energy.

That the two approaches are directly comparable has, however, been brought into question by the work of Moors (1977) on weasels. Using natural diets comprising small mammals, birds and rabbits, Moors found large disparities between the estimates of energy requirements derived from feeding trials and those obtained using respirometry. He attributed these differences to variations in the amount of activity of subject animals during each trial, although differences in the digestibility and nutritional value of the diets used may provide an alternative explanation.

The Gross Energy intake of females, for instance, ranged from 721 $KJ/Kg^{0.75}$/day when feeding on an Apodemus diet, to 1304 $KJ/Kg^{0.75}$/day on Microtus, though estimates of metabolic rate during the same period were almost identical at 1054 and 1084 $KJ/Kg^{0.75}$/day, respectively. Natural diets were also used by Davison et al (1978), in their studies of the fisher, and again there were wide variations in GE intake between diets; from 549 $KJ/Kg^{0.75}$/day on Snowshoe hare, to 789 $KJ/Kg^{0.75}$/day on Coturnix quail.

It is evident from these findings that GE intake is not a reliable predictor of maintenance energy requirements. Moreover, differences in the digestibility and nutritional value of the rations used, and in the capacity of subject animals to metabolise them, make inter- and intra-specific comparisons almost meaningless. The evaluation of maintenance energy requirement must, of necessity, incorporate estimates of ADE, ME and SDA, if valid comparisons are to be made.

### 3:6.2 Energy Metabolism

The Apparent Digestibility of dietary energy did not vary greatly between the rations presented, the overall mean being 81.8% of GE intake. Exclusion of the value obtained for RBBT (86.9% GE), which differed significantly from the other four, yields a mean of 80.5% for STAN1, CK12, STAN2 and E-MIX. These values are somewhat lower than those obtained by other workers for trials in which natural diets were used.

Moors (1977) estimated the digestive efficiency of weasels feeding on a small mammal (Microtus) diet to be 81.6%, increasing to 96.05% on whole rabbit carcasses, and similar values were given by Davison et al (1978) for fishers consuming rations comprising small mammals, snowshoe hare and white-tailed deer (80.9, 90.0 and 92.6% GE, respectively). Comparable results have been obtained in feeding trials with other carnivores; for polar bear, 91.7% (Best, 1985); red fox, 85.4 - 97% (Litvaitis and Mautz, 1976) and bobcat, 89.5 - 91% (Golley et al, 1965).

In each of these studies, however, the experimental diets comprised either whole carcasses or, in the case of larger prey items such as white-tailed deer (Golley et al, 1965; Litvaitis and Mautz, 1976; Davison et al, 1978) or Ringed seal (Best, 1985), those portions of the carcass adjudged by the experimenters to be palatable to the subject animals. These generally included the muscle tissue and viscera only; the skin, skeletal structure and adipose tissue being discarded. The higher coefficients of digestibility obtained for these types of ration may, therefore, be attributable to the removal of such components of low digestibility prior to the start of the trial. In feeding trials with polar bears, inclusion of the skeletal material in rations based on selected components of seal carcasses reduced the Apparent Digestibility of dry-matter from 87% to 54%

(Best, 1985). Similarly, Davison et al (1978) estimated the dry-matter digestibility of small mammal diets fed to fishers to be 72.3%, which is in close agreement with the mean of 75.1% obtained for the diets used in the present study, but found that for a ration of deer meat the value was 92%.

The use of whole carcasses in feeding trials may also have an attendant potential for error, as discussed in Chapter Two, in that the composition of the ration presented may differ substantially from that of the material actually consumed. The disparity would be most significant in trials in which subject animals displayed a degree of selectivity in their utilisation of the carcass, as might occur with the use of prey items of comparatively large size in relation to themselves. This too, would result in the derivation of higher coefficients of digestibility, such as those recorded by Moors (1977) for weasels feeding on rabbit.

The lower digestibility of the rations used in the present study may also be attributable to increased rates of passage of ingesta along the gut, in consequence of the higher proportion of indigestible matter which these diets contained. Rates of passage in carnivores have not been widely reported, but several studies of BTT in mink feeding on commercial rations are available for comparison.

Bleavins and Aulerich (1982), using ferric oxide as the faecal marker, estimated transit time to be approximately 3.11 hrs., which accords well with the values of 2.88 and 3.25 hrs. obtained for the RBBT and CK12 diets, though it is somewhat lower than the time of 3.87 hrs. recorded for E-MIX. Sibbald et al (1962) measured the transit times of carmine-dyed feedstuffs to be between 1.03 and 2.37 hrs., while Wood (1957), employing a variety of markers, presented values from 1.48 - 2.05 hrs.

The dicrepancies in these estimates of transit time are due, in part, to the choice of marker in each study, with the highest passage rates being recorded for soluble markers and dyes, which move in association with the gastrointestinal fluids (Robbins, 1983). For trials with feeds containing a high proportion of indigestible material, however, the use of particulate markers is more appropriate. By presenting rations containing fuchsin-stained wheat chaff, Slawinski, Slawon and Bednarz (1962) demonstrated that transit time may be considerably longer, ranging from 2 - 7 hrs. Their findings suggest that the passage rates recorded for the diets used in the present study are not dissimilar from those determined for other types of feed and, therefore, are unlikely to explain their comparatively low digestibility.

Although ADE constituted a lower proportion of ingested Gross Energy in these diets than in the rations used in other studies, Gross Energy intake greatly exceeded the level which might be explained by compensatory increases in food consumption. The mean daily intake of ADE was 1106 $KJ/Kg^{0.75}$/day. Similar values, ranging from 848 - 1078 $KJ/Kg^{0.75}$/day were obtained by Farrell and Wood (1968b) for mink feeding on commercial rations, also of low digestibility (76.1% for ADE) but, in general, the ADE intake of carnivores is considerably lower.

Cowan, Wood and Kitts (1957) presented a value of 635 $KJ/Kg^{0.75}$/day for mink, while Davison _et_ _al_ (1978) estimated the maintenance requirements of fishers to be between 501 and 727 $KJ/Kg^{0.75}$/day. In studies with large felids (puma, leopard, lion and tiger), Barbiers _et_ _al_ (1982) found that the daily ADE intake ranged from 694 - 760 $KJ/Kg^{0.75}$/day.

There is, however, some evidence to suggest that energy intake is not always related to metabolic requirements; several species of carnivore having been reported to consume more food than is apparently required for subsistence (Harlow, 1981). This has been interpreted as an adaptive trait, conferring an advantage upon opportunist predators which exploit food resources of varying abundance and availability. The habit of caching food, a behaviour pattern characteristic of many carnivores and one which is widely documented in mink (Yeager, 1943; Sinclair et al, 1962; Bleavins and Aulerich, 1982; this study) is similarly believed to have arisen in response to extended periods of prey scarcity (Errington, 1967).

The high levels of food consumption recorded in the present study could, therefore, be a consequence of the ad libitum feeding regime implemented. There remain, however, substantial differences between the estimates of ADE intake during these trials and those obtained by other workers, of a magnitude not adequately explained by aberrations in feeding behaviour.

Errington (1967) records that during periods of prey abundance, mink gorged on the plentiful supplies of food, but that their faeces were subsequently found to contain large quantities of undigested material. Similarly, Lampe (cited in Harlow, 1981) has shown that digestive efficiency is negatively correlated with biomass consumed. In contrast, the digestive efficiencies calculated for these feeding trials remained relatively high throughout, so giving rise to the elevated mass-specific intake recorded in these animals.

Particularly notable in this regard were the trials with E-MIX, a ration comprising a high proportion of lipid and, consequently, having a very high energy content (11.54 KJ/g wet weight). Although feed consumption during these trials averaged only 123 g/Kg$^{0.75}$/day, the mass-specific intake of ADE was 1417 KJ/Kg$^{0.75}$/day, due principally to the high digestibility of the lipid moiety (98.8%). The Apparent Digestibility of energy was somewhat lower (82.3%), owing to the inclusion of the cellulose filler. Very high coefficients of Apparent Digestibility for lipid have been recorded in several studies of carnivore feeding energetics: in mink, 97.2% (Leoschke,

1959) and 97.8% (Sinclair et al, 1962); in fisher, 99.4% (Davison et al, 1978); puma, 99.0% (Barbiers et al, 1982) and Polar bear, 99.3% (Best, 1985). The last author also noted that BTT was positively correlated with the lipid content of the diet, a finding which he attributed to the longer period required for the digestion and assimilation of fatty substances. This trend was also apparent in the diets used in the present study, and it is probable that the slower passage rate of E-MIX further contributed to the high ADE intake of animals consuming this ration.

Such high levels of ADE intake may indicate an unusually high energy requirement for maintenance or, alternatively, signify a low assimilation efficiency in feeding on the rations presented. The former hypothesis was proposed by Farrell and Wood (1968b) in explanation of the elevated levels of ADE intake which they recorded in mink consuming a typical commercial feed. These authors noted that BMR, determined in a previous study to be 323 $KJ/Kg^{0.75}$/day (Farrell and Wood, 1968a), represented only one third of the daily energy requirement, in contrast with the finding in most other species that the energy expenditure at maintenance is approximately twice the basal level (Kleiber, 1961).

They attributed the differences in ADE requirements to an increment in maintenance energy expenditure associated with activity. As supporting evidence for this hypothesis they cited significant decreases in ADE intake following the confinement of subject animals in metabolism cages, which permitted only a limited degree of activity. Similar findings have been reported in cats (Miller and Allison, 1958). That the measurements of BMR in mink made by Farrell and Wood (1968a) lie within the range established for other species of mustelid, which extends from 302 $KJ/Kg^{0.75}$/day in the fisher (Davison et al, 1978) to approximately 400 $KJ/Kg^{0.75}$/day in smaller species (Iversen, 1972), lends further support to this hypothesis.

Conversely, there was no evidence to suggest that the estimates of assimilation efficiency derived for these animals differed significantly from those obtained by other workers for carnivores consuming this type of diet. In weasels, assimilation efficiency has been found to range from 71.3 - 83.1% (Moors, 1977), and in fishers from 73.9 - 86.9% (Davison, 1978); these results accord well with the values obtained in the present study, which varied between 72.5% for STAN2 and 80.5% for RBBT.

Urinary energy losses were, however, proportionately lower during these trials than have been found in other investigations of this kind. On STAN1, urine energy constituted only 7.67% of GE, and on E-MIX less than 3%. In comparison, Moors (1977) derived values of 6.6 - 10.9% for weasels feeding on small mammals (Apodemus, Microtus), increasing to between 12% and 15.5% on a rabbit diet. In the latter case, however, the ingested material may have contained a significantly higher proportion of protein than the rations used in the present study (see section 3:6.1) and therefore, the elevated urine energy losses on this diet may have derived from increases in Nitrogen excretion.

In consequence of the comparatively high assimilation efficiency of subject animals on each of the diets presented, the levels of ME intake were also considerably higher than would be predicted from estimations of maintenance requirements; averaging 838 KJ/Kg$^{0.75}$/day when on STAN1, and 1369 KJ/Kg$^{0.75}$/day on E-MIX. The calculated SDA of these rations accounted for approximately 30% of ME, so leaving a residual value for Net Energy of between 545 and 1110 KJ/Kg$^{0.75}$/day to sustain BMR, body temperature and activity.

Variation in the magnitude of these components was expected to be small. All feeding trials were carried out at temperatures within the minks' thermoneutral zone, established by Farrell and Wood (1968a) to extend from 16 - 29 °C, and the amount of energy expended in thermoregulation was, therefore, assumed to be minimal.

Increments in the maintenance requirement arising from increased activity were also assumed to be small, and constant between trials. The suggestion that inequalities in ME intake might be explained largely by differences in the activity of the subject animals (Farrell and Wood, 1968b; Moors, 1977) was therefore rejected, particularly in view of the evident correlation between ME and ADE intake, which was directly related to the composition of the ration presented.

In resolving NE into the two components 'Production' and 'Maintenance', it was apparent that such increases in energy intake were paralleled by a similar increment in the amount of energy stored as depot fat. This relationship was most marked during the trials with E-MIX; from the substantial gains in body weight recorded, it was estimated that the energy expended in deposition of adipose tissue represented 31% of GE, in comparison with only 3.1% for RBBT. The value for Maintenance Energy remained constant between trials and averaged 513 KJ/Kg$^{0.75}$/day, or about 1.6 times BMR. These results suggest that energy expended in activity constituted a smaller proportion of the total energy requirement than Farrell and Wood (1968b) had postulated, even though the metabolism cages in which subject animals were confined were slightly larger than those used by these authors. That the animals underwent a gain in weight during each trial does, however, further substantiate the hypothesis that the *ad libitum* feed intake of carnivores is not commensurate with metabolic capacity; ingested GE may exceed the maintenance requirement, particularly under conditions affording only a limited degree of activity.

### 3:6.3 Nitrogen Metabolism

The Coefficients of Apparent Digestibility for Nitrogen ranged from 0.817 on CK12 to 0.94 on RBBT and are not dissimilar from those recorded in other investigations of carnivore nutrition. Roberts and Kirk (1964) obtained values of 0.87 - 0.91 in mink; in fishers, ADN has been estimated at between 78.9% and 83.5% of intake (Davison et al, 1978), and in Polar bears, from 72.1 - 94.9% (Best, 1985). In each of these studies the lowest values were obtained with rations containing relatively large amounts of indigestible proteinaceous matter such as fur, feather, etc., and the presence of this highly keratinised material may have depressed estimates of ADN still further, by increasing the losses of endogenous faecal Nitrogen through desquamation of the intestinal epithelium (Best, 1985).

Particularly notable are the changes in Nitrogen digestibility believed to be associated with the moult. As the composition of E-MIX differed markedly from that of the other diets, direct comparisons are difficult, but it is evident from the results of the trials with rabbit-based diets that changes in Nitrogen digestibility did occur between trials; from a value of 0.94 on the RBBT trials, carried out in early spring, the Apparent Digestibility of Nitrogen declined by more than 10% to an average of 0.84 during presentation of the Standard rations later in the year. These results may be contrasted with the data presented by Seier et al (1971) in a study of the mink's autumn moult, which indicated that the elevated requirements for protein Nitrogen during the moult were met by increases in food consumption, rather than a change in Nitrogen digestibility. Urine losses were not evaluated, but since Nitrogen retention declined after the moult while feed intake continued to increase, it is probable that the change in tissue balance was effected through an increase in the amount of

Nitrogen excreted as urea.

In the present study, urinary losses were closely correlated with ADN intake, and constituted approximately 43.4% of ingested Nitrogen during the moult, increasing to 47.9% in the inter-moult period. By comparison, Roberts and Kirk (1964) gave values of 70.7 - 73.9% for mink consuming a commercial ration, while Keiver et al (1984) presented an estimate of 90% for Harp seals feeding on a herring diet. The disparities between these results indicate that, in consequence of their elevated feed intake, the Nitrogen retention of the animals used in the current trials was also very high, ranging from 1.87 g/Kg$^{0.75}$/day on CK12, up to 3.99 g/Kg$^{0.75}$/day during presentations of RBBT. These latter values are equivalent to approximately 3.51 and 6.25 g N/animal/day. As Seier et al (1971) estimated the Nitrogen retention of adult male mink to be only 0.89 g N/animal/day during the autumn moult, it seems probable that an error was incurred in the determination of urine Nitrogen.

Underestimates of urine Nitrogen could have been due to decomposition of urea to ammonia prior to analysis. Although the use of toluene as a preservative (Greaves and Scott, 1960) served to minimise Nitrogen losses through bacterial degradation, the addition of strong acid such as sulphuric (Glem-Hansen and Jørgensen, 1973; Chwalibog et al, 1982; Harri and Brockway, 1985) or hydrochloric acid (Roberts and Kirk, 1964; Seier et al, 1971) may have yielded more satisfactory results by inhibiting the decomposition of urea to ammonia during storage (See Appendix C).

It is also possible that certain of the assumptions made about the composition of the urine were invalid, in that total urine Nitrogen may have comprised a much larger proportion of exogenous non-urea Nitrogen than had originally been assumed. In animals feeding on natural diets of the type used in the present study, an important source of such Nitrogen would be creatinine, derived principally from the muscle tissue of the carcasses consumed. At very high levels of feed intake, therefore, failure to include this component in the total would have contributed substantially to the low values obtained.

### 3:6.4 Gut Morphology

Comparisons of gross gut morphology revealed significant size-related sex differences in the dimensions of the alimentary tract. The weight-specific gut capacity of females was considerably higher than that of males, possibly reflecting their proportionately higher metabolic requirements (Myrcha, 1964; Fell, 1972; Gross et al, 1985); conversely, the surface area:volume ratio decreased with increasing size. This latter relationship may explain the observation (Allen, 1962; Allen et al, 1964; this study) that coefficients of Apparent Digestibility are generally higher in males since, as noted by Robbins (1983), endogenous losses of energy, protein and lipid derived from the intestinal epithelium would be proportionately lower in animals of larger size.

In both sexes, the alimentary tracts of wild-caught individuals were longer and wider, in proportion to body weight, than were those of animals raised on farms. These findings are in accordance with the theory that intestinal hypertrophy also develops in response to diets of low nutritional value (Miller, 1975; Savory and Gentle, 1976; Al-Joborae, 1980), the increased gut capacity permitting rapid consumption of large quantities of material without a loss in digestive efficiency (Fell, 1972; Harlow, 1981).

## 3:6.5 Conclusions: Dimorphism, Nutrition and Feeding Ecology

From the fore-going discussion, it is apparent that females differ from males primarily in their proportionately higher energy demands. Consideration of the relationship between feeding energetics and the ratio of ADE:ADN in the diet (Sibbald et al, 1957; Sinclair et al, 1962; Allen et al , 1964) suggests that, in maintaining a greater biomass, the higher absolute nutritional requirements of males entail an increased need for protein Nitrogen. In contrast, the requirements of females necessarily include a demand for material of high caloric value in respect of their elevated metabolic rate.

As shown in Chapter Two, determinations of gross composition and energy content revealed significant differences between the diets used in the present study. The smaller prey types, including rodents, birds and fish, have a higher ratio of ADE:ADN than larger items such as rabbits, although comparisons with data presented by other workers (c.f. section 2:5) demonstrate that the variations in protein and energy content within these different groups are as great as, or greater than, those between the diets themselves. It is also apparent, from the results of the feeding trials described in the present chapter, that such diets do not differ significantly, either in digestibility or biological value; attempts to classify particular prey types in terms of their nutritional value are, therefore, of limited application in analyses of the feeding ecology of a generalist predator.

No evidence of metabolic or physiological differences associated with nutritional requirements was found in either sex, but comparisons of gut morphology indicate that, in females, hypertrophy of the alimentary tract may develop in response to increased energy demands. Since a similar adaptation was evident in both males and females from wild populations, it may be inferred that the natural diets of free-living mink are generally of a much lower quality than the rations fed to commercially raised animals, even though the constituent prey items are often of a comparatively high nutritional value and easily digested, as shown in the present study. Furthermore, the maintenance requirements of active predators are considerably greater than those of captive individuals, as Powell and Leonard (1983) were able to demonstrate in an analysis of the foraging energetics of female fishers; a dependence on such diets could, in consequence, place considerable constraints upon the nutrition and metabolism of both young and adults, particularly during extended periods of prey scarcity.

In Chapter Four, therefore, an attempt is made to evaluate the extent to which the development of growing kits is influenced by diet quality and, in particular, to assess the significance of nutritional factors in determining the magnitude of sexual dimorphism in adults.

# Chapter Four

# FEEDING TRIALS WITH KITS

## 4:1 Introduction

The American mink is now a species of considerable economic importance and, as such, has been the subject of a great deal of research directed towards determining its requirements for optimal growth and development. These have included numerous investigations into the nutrition and metabolism of growing kits, and their performance on different types of diet and dietary regime (c.f. section 1:3).

Such studies have shown that differentials in the growth rates of males and females are dependent not only on the plane of nutrition (Glem-Hansen, 1980a, 1980b; Tauson, 1985b), but also on the source and quality of dietary protein (Seier et al, 1970; Skrede, 1978a, 1978c), and the ratio of protein to energy in the ration presented (Sinclair et al, 1962; Allen et al, 1964). The importance of these various factors in determining adult body size is greatest during the first five months after weaning when growth rates, and hence metabolic requirements, are highest (Tauson and Alden, 1985).

In the light of these findings, the objectives formulated for the work described in the present chapter were three-fold; to compare and contrast the growth and development of kits feeding on 'natural' diets with those of animals raised on commercial feeds; to identify possible differences in the assimilation efficiency of male and female kits during the critical initial period of growth and, by comparison with adults feeding on rations of a similar type, to evaluate the extent to which nutrient metabolism varies with age and metabolic demands.

## 4:2 Methods

Attainment of the objectives outlined in 4:1 above required that individual variation between subject animals be reduced to a minimum. Ideally, the experimental groups would comprise kits of the same age and of a similar genetic constitution, preferably siblings. In addition, the animals used should all be from litters of the same size, in order to eliminate possible differences in their nutritional history related to maternal milk yield, prior to weaning.

To ensure that each of these requirements was met, it was necessary to establish two such litters by artificial means.

### 4:2.1 Establishment of Experimental Litters

Two pregnant 'pastel' females, mated at similar times, were obtained from a local mink farm. They were brought to the Department two weeks prior to the expected date of parturition and placed in metabolism cages, where they were subsequently maintained on a diet of minced rabbit, fed *ad libitum*. Straw bedding material was also provided and replenished as necessary. A 12:12 subdued light regime (appproximately 21.5 Lux) obtained throughout.

Three kits were born to the first female (designated Female 'A') on May 3rd 1986, of which two survived; a male weighing 9 g, and a female of 10.1 g. On May 4th, a further four kits were born; one male weighing 8.7 g, and three females, with birth weights of 7.0, 7.7 and 8.2 g.

The second female (Female 'B') also whelped on May 4th, delivering ten kits, of which five were male and five female; all survived. The mean body weights recorded at birth were 8.2 and 7.3 g, for males and females,

Table 4:1 Body Weights of Kits: Experimental Litters

| | Litter A | Litter B |
|---|---|---|
| Male | 10.1, 9.5 | 10.5, 9.5 |
| Female | 10.5, 8.8 | 9.8, 9.3 |

respectively.

The kits were separated from the dams at two days old, and the experimental litters established by division of Litter 'B' into two groups of four kits, each comprising two males and two females, of similar mean body weights (see Table 4:1). These animals were replaced in the nest boxes and the females were again allowed access; both litters were accepted immediately. The remaining eight kits were returned to the mink farm and similarly fostered with surrogate mothers.

Lactation lasted six weeks. Each day, small quantities of the dam's ration were placed in the nest boxes, to accustom the kits to solid food. The young animals began taking the diet when 22 days old, and were weaned at 42 days old, at which point the dams were removed from the cages and each litter was divided into single-sex pairs. Rations containing a vitamin supplement ('MA2', Farm Feed Formulators) were subsequently presented to each pair on an <u>ad libitum</u> basis for a further two weeks, until the commencement of the first feeding trial.

### 4:2.2 <u>Experimental Procedures</u>

Two feeding trials were conducted on the growing kits, beginning at 56 and 99 days <u>post partum</u>, and designated KIT1 and KIT2, respectively. The experimental procedures adopted during the trials were identical to those followed with adults (c.f. section 3:4.3), each animal being housed individually in a metabolism cage. At the end of the trials, the kits were transferred to the Department's animal-housing facility, and were there maintained on the same diet according to the regime detailed in section 3:4.1, until required for subsequent experiments.

All analytical procedures employed were as described in sections 2:3.1 and 3:4.4.

### 4:2.3 Mortality

Two animals were lost during the course of the study, both males. The first died at the age of 56 days, weighing only 290 g; considerably less than the other males, which then averaged 620 g. A replacement, of the same age and of a similar body weight (680 g) to the three remaining male siblings, was brought from the mink farm. This animal also died when 88 days old and, again, a replacement was found. In neither case was any attempt made to establish the cause of death.

## 4:3 Results

### 4:3.1 Growth of Kits

The rate of weight gain was calculated according to the formula for Instantaneous Percentage Growth Rate, derived by Brody (1945):-

$$\text{Growth Rate 'R'} = \frac{(\log W2 - \log W1) \times 2.303 \times 100}{(t2 - t1)} \qquad \text{(Eqn. 4:1)}$$

in which W1 and W2 are the body weights at the beginning and end of a given period of (t2 - t1) days. Determinations of body weight were made at intervals of 2 - 9 days (see Table 4:2) and the values for 'R' derived therefrom are tabulated in Table 4:3.

For the first six weeks of life, the weight gains of females paralleled those of males (see Fig. 4:1); at five days old the mean body weight of

Table 4:2 Body Weights of Kits During Study Period

Mean body weights for each sex, recorded at intervals of 2 - 9 days. Standard errors of the means in parentheses.

n.b: Values are based on those individuals which survived for the whole of the study period.

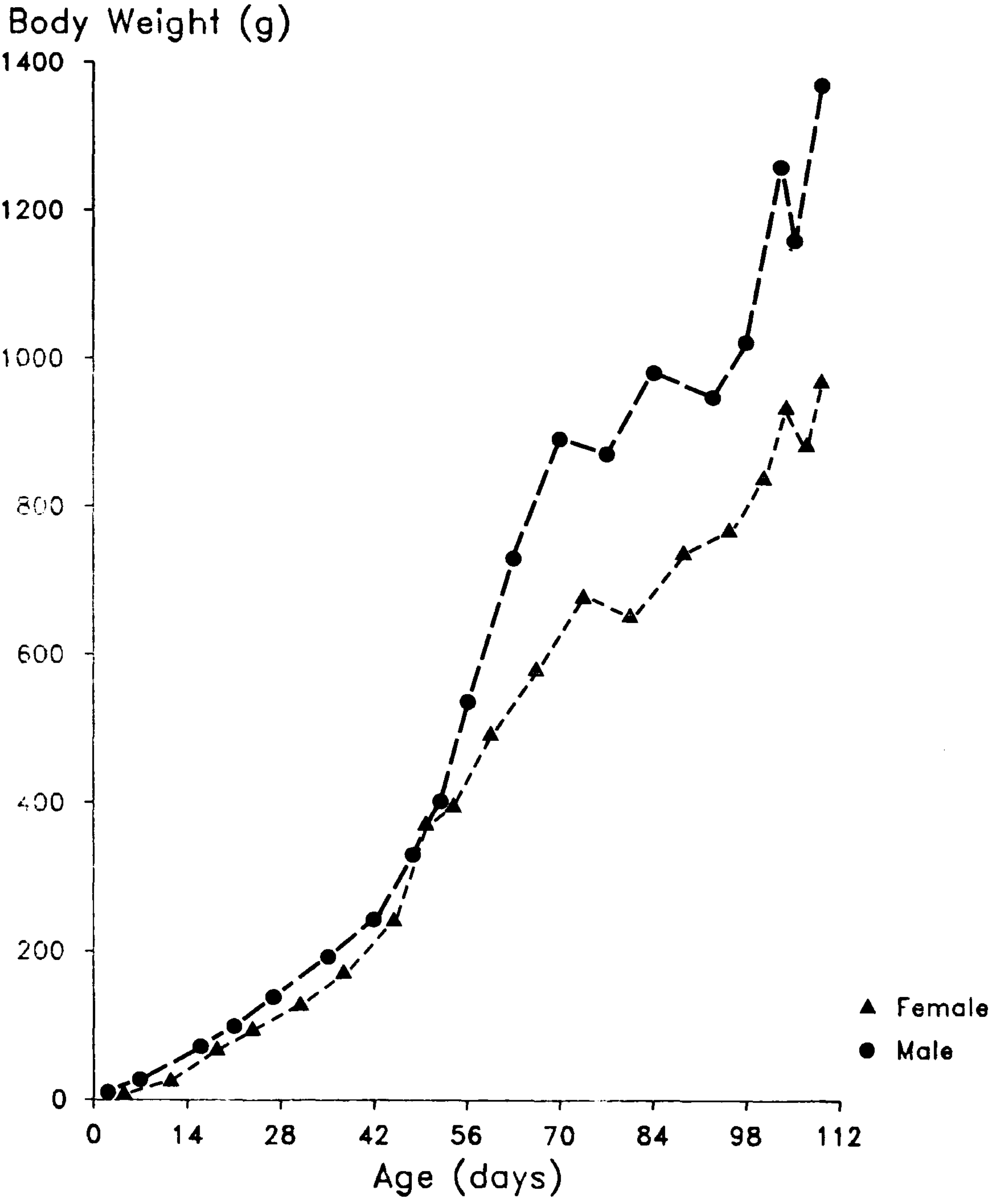
## Table 4:2 Body Weights of Kits During Study Period

| Age (days) | Weight (g) Males | | Weight (g) Females | |
|---|---|---|---|---|
| 2 | 9.75 | ( 0.25) | 9.5 | ( 0.50) |
| 7 | 27.00 | ( 0.71) | 26.75 | ( 0.48) |
| 16 | 70.75 | ( 2.53) | 68.50 | ( 1.19) |
| 21 | 98.00 | ( 5.83) | 94.25 | ( 2.25) |
| 27 | 139.00 | (11.66) | 128.75 | ( 4.73) |
| 35 | 193.00 | (25.53) | 172.50 | (11.81) |
| 42 | 242.25 | (34.57) | 243.25 | (10.10) |
| 48 | 329.50 | (57.22) | 372.00 | ( 9.89) |
| 52 | 402.50 | (60.60) | 396.25 | (11.43) |
| 56 | 537.50 | (89.78) | 493.75 | (12.48) |
| 63 | 760.00 | (34.88) | 581.25 | (13.44) |
| 70 | 926.25 | (52.89) | 677.50 | (11.99) |
| 77 | 917.50 | (64.73) | 653.75 | (26.64) |
| 84 | 1020.00 | (62.48) | 738.75 | (23.13) |
| 93 | 948.33 | (65.21) | 767.50 | (28.32) |
| 98 | 1021.67 | (94.68) | 838.75 | (50.51) |
| 103 | 1260.00 | (91.92) | 935.00 | (10.61) |
| 105 | 1160.00 | (92.41) | 885.00 | (10.61) |
| 109 | 1370.00 | (96.25) | 970.00 | (35.36) |

Table 4:3 Growth Rates of Kits During Study Period

| Period (days) | Growth Rate (%) | |
|---|---|---|
| | Males | Females |
| 2-7 | 20.37 (0.91) | 20.78 (0.72) |
| 7-16 | 10.70 (0.16) | 10.45 (0.06) |
| 16-21 | 6.42 (0.49) | 6.38 (0.64) |
| 21-27 | 5.75 (0.41) | 5.17 (0.27) |
| 27-35 | 3.89 (0.64) | 3.60 (0.40) |
| 35-42 | 3.20 (0.18) | 4.98 (0.50) |
| 42-48 | 4.80 (1.60) | 7.11 (0.68) |
| 48-52 | 5.27 (1.10) | 1.57 (0.24) |
| 52-56 | 6.92 (0.74) | 5.51 (0.63) |
| 56-63 | 3.04 (0.63) | 2.72 (0.32) |
| 63-70 | 2.81 (0.75) | 2.19 (0.19) |
| 70-77 | -.19 (0.79) | -.53 (0.84) |
| 77-84 | 1.56 (0.16) | 1.76 (0.16) |
| 84-93 | -.40 (0.17) | .42 (0.38) |
| 93-98 | 1.38 (0.98) | 1.70 (0.56) |
| 98-03 | 5.85 (0.59) | 4.24 (0.65) |
| 105-109 | 4.20 (0.47) | 2.30 (0.61) |

# Fig 4:1 Weight Gain of Kits During Trial Period



both sexes was 10 g, increasing to 243 g at 43 days. Growth rates during this period declined steadily from a mean value 20.6% in the first week to 6.0% in the sixth (Fig. 4:2).

From the age of seven weeks onwards, however, the rate of weight gain in males was consistently higher than that of females. At 52 days old, the mean daily percentage increase in body weight was calculated to be 5.3% for males, in comparison with only 1.6% for females. Thereafter, the disparity in the mean body weights of each sex increased progressively; by the ninth week, males weighed 25.6% more than females (mean body weights of 730 g and 581 g for males and females, respectively), and in the twelfth, 32.6% (980 g and 739 g). When the study was terminated at 109 days *post partum*, males were, on average, 41.2% heavier than females (mean body weights of 1370 g and 970 g, respectively).

### 4:3.2 *Feed Consumption and Body Weight*

The mean body weights of the kits during feeding trials are presented in Table 4:4, and the weight-specific nutrient intake for each sex tabulated in Tables 4:5a and 4:5b, for KIT1 and KIT2, respectively.

On both trials, the weight-specific feed consumption of males exceeded that of females. For KIT1, the values were 433.9 and 396.8 g/Kg body weight, for males and females, respectively; on KIT2, 429 and 408 g/Kg. The aggregate means for each sex were compared using 'Student's' t-test, though in neither case were the differences statistically significant. Comparisons between trials were carried out on the pooled data for males and females but, again, no significant differences were apparent. Subsequent analyses were, therefore, performed on the data from KIT1 and KIT2, combined.

Fig 4:2 Growth Rates of Kits During Trial Period

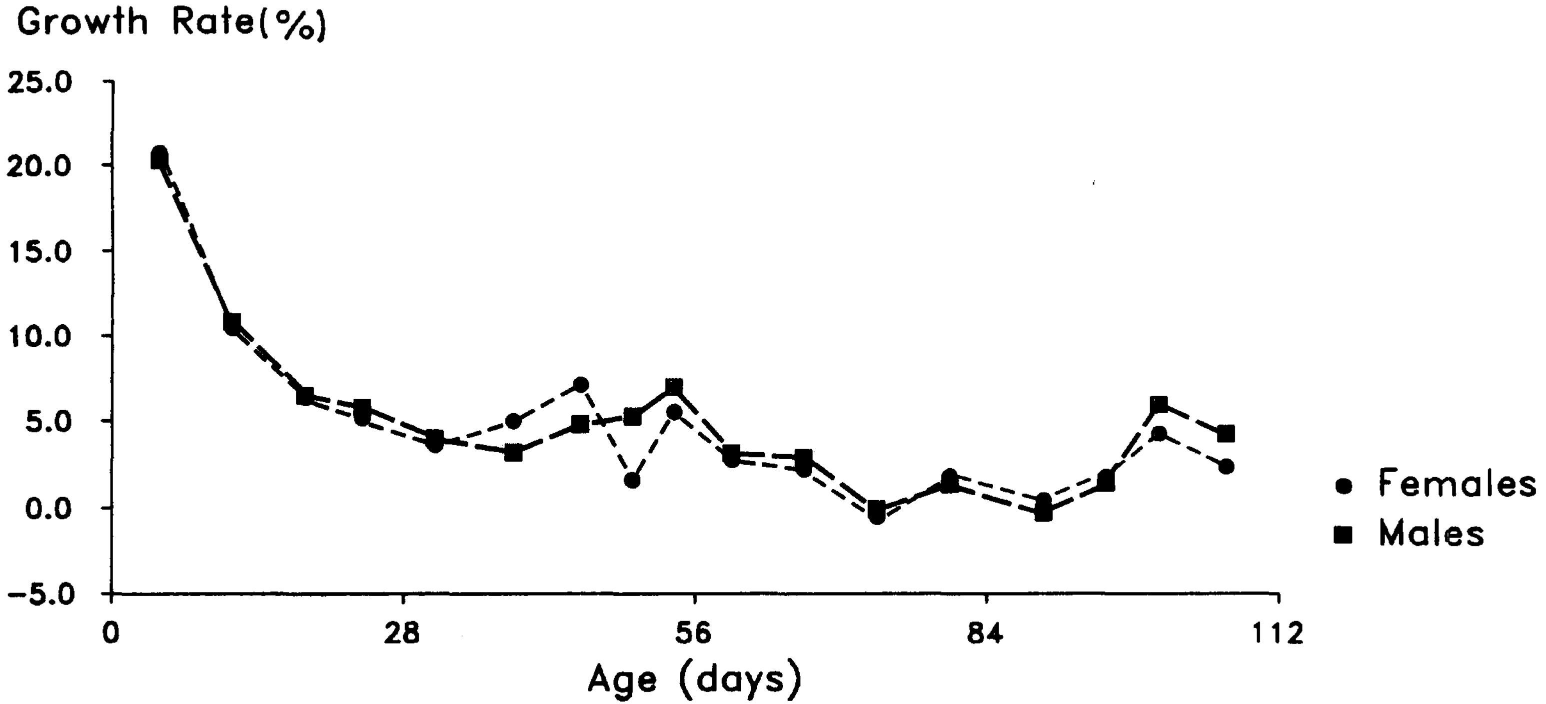


Table 4:4 Mean Body Weight of Subject Animals During Feeding Trials

| | KIT1 | KIT2 |
|---|---|---|
| Males | 0.787 (0.063) | 1.307 (0.116) |
| Females | 0.594 (0.020) | 0.891 (0.030) |

Table 4:5a Weight-Specific Nutrient Intake of Subject Animals: KIT1

| | Males | Females |
|---|---|---|
| Feed Intake (g/Kg) | 433.9 (14.88) | 396.8 ( 6.74) |
| Energy (KJ/Kg) | 2893 (99.26) | 2646 (44.96) |
| Nitrogen (g N/ Kg) | 13.64 (0.47) | 12.47 (0.21) |
| Lipid (g FFA/Kg) | 18.40 (0.63) | 16.82 (0.29) |

Table 4:5b Weight-Specific Nutrient intake of Subject Animals: KIT2

| | MALES | FEMALES |
|---|---|---|
| Feed Intake (g/Kg) | 429 (32.17) | 408 ( 6.74) |
| Energy (KJ/Kg) | 2858 ( 215) | 2720 (56.54) |
| Nitrogen (g N/ Kg) | 13.47 (0.10) | 12.82 (0.27) |
| Lipid (g FFA/Kg) | 18.40 (0.14) | 17.29 (0.36) |

To enable comparisons with adults, independent of the effect of body size, a measure of metabolic weight was obtained by calculation of the constant 'b' (Equation 3:1, c.f. section 3:5.3) from a double-logarithmic regression of Metabolisable Energy intake against body weight (Fig. 4:3). The principal axis equation derived therefrom (included in Fig. 4:3) yielded a mass-exponent of 1.0, thereby demonstrating that, in contrast with adults, the metabolic requirements of growing kits are directly proportional to body weight. The data for kits presented in the following analyses are, in consequence, expressed on the basis of unit body weight; those of adults are given in units of metabolic weight.

Comparisons of mass-specific Metabolisable Energy intake were effected by means of 'Student's' t-test. By virtue of the linear relationship between energy requirements and body weight in the growing animals, the ME intake of kits (2756 KJ/Kg) was considerably higher than the values recorded for adults during trials with RBBT and the Standard diets (means of 1277 and 1217 $KJ/Kg^{0.75}$. respectively). The greater than two-fold difference between these results was found to be highly significant ($p < 0.001$).

### 4:3.3 Apparent Digestibility

Coefficients of Apparent Digestibility were calculated for dry-matter, Gross Energy, Nitrogen and lipid, using equation 3:2. For the purpose of statistical analyses, the resulting values were normalised by means of the arc sine transformation. Table 4:6a contains the back-transformed aggregate means, together with the upper and lower bounds of a confidence interval equivalent to the arc sine mean plus or minus its standard error. For comparison, the values recorded for adults consuming diets of the same type (c.f. section 3:4.3) are presented in Table 4:6b; 'STAN' denotes the

# Fig 4:3 Relationship of Metabolisable Energy Intake to Body Weight

(Kits)

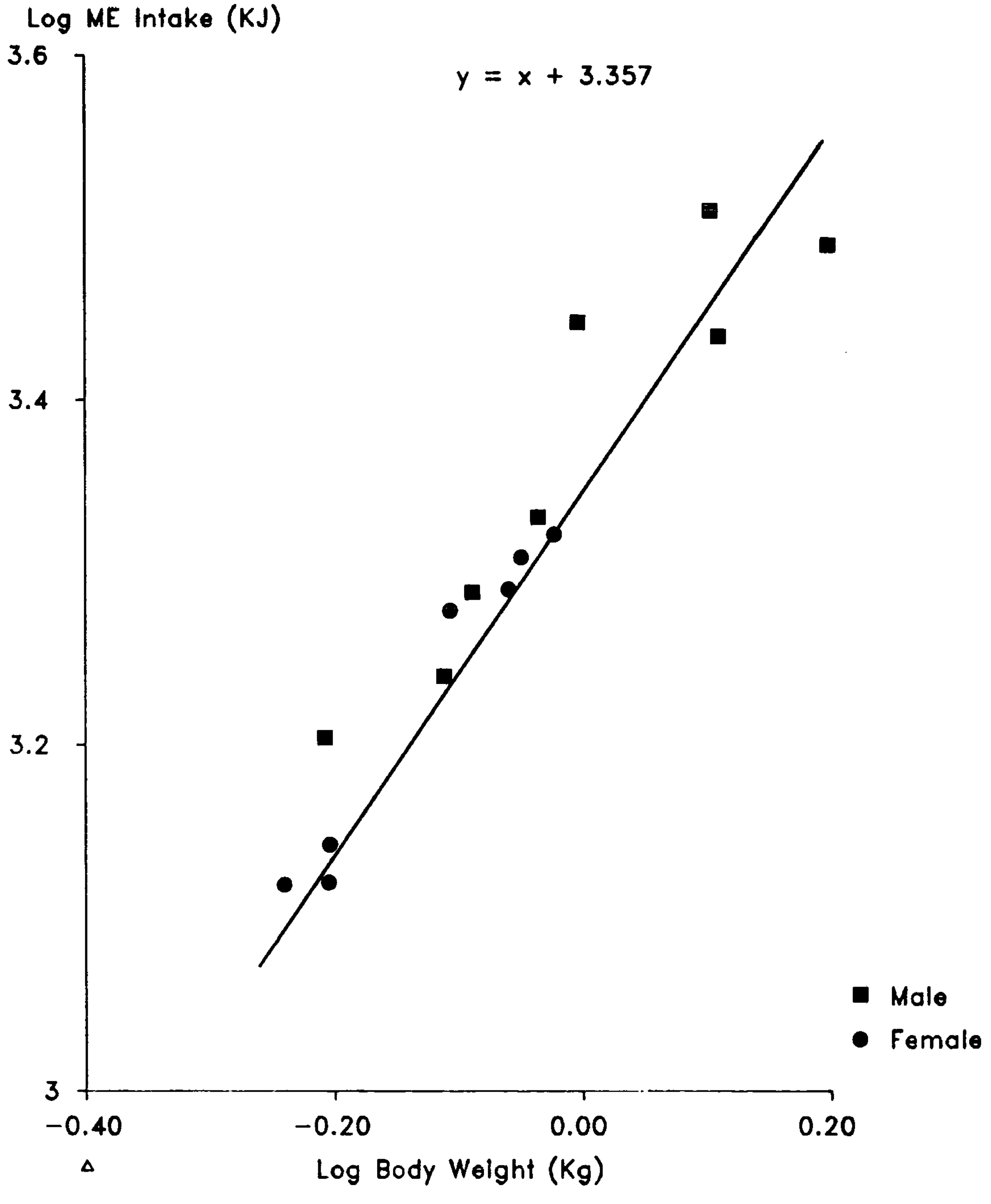


Table 4:6a Apparent Digestibility: Kits

| | Mean | Confidence Interval |
|---|---|---|
| Dry-matter | 0.809 | (0.799, 0.819) |
| Gross Energy | 0.852 | (0.844, 0.860) |
| Nitrogen | 0.765 | (0.749, 0.781) |
| Lipid | 0.972 | (0.969, 0.975) |

Table 4:6b Apparent Digestibility: Adults

| | RBBT | | STAN | |
|---|---|---|---|---|
| | Mean | Confidence Interval | | Confidence Interval |
| Dry-matter | – | ( – , – ) | 0.766 | (0.736, 0.794) |
| Gross Energy | 0.869 | (0.859, 0.879) | 0.814 | (0.791, 0.835) |
| Nitrogen | 0.940 | (0.933, 0.947) | 0.842 | (0.830, 0.854) |
| Lipid | 0.971 | (0.968, 0.973) | 0.947 | (0.934, 0.960) |

results from STAN1 and STAN2, combined. Comparisons between groups were made using 'Student's' t-test, and the results are tabulated in Table 4:7.

Excepting the values recorded for ADN, the digestive efficiency of kits was generally higher than that of adults, but not significantly so; statistical comparisons between kits and Standard animals revealed a significant difference in the coefficients of Apparent Digestibility for lipid (0.972 for kits, 0.947 for STAN; $p < 0.05$), but no such differences were apparent between the kits and adults consuming the RBBT ration.

Kits were, however, considerably less efficient than adults in digesting dietary Nitrogen. The Apparent Digestibility coefficient for Nitrogen derived for the Standard animals (0.842) was 10% higher than that obtained for the kits (0.765). Comparison with the results of trials carried out with adults during the spring moult revealed an even greater disparity, the value of 0.940 recorded for RBBT representing an increase in digestive efficiency of 22.9%. As shown in Table 4:7, these differences were found to be highly significant.

### 4:3.4 Energy Metabolism

Energy metabolism was characterised according to the parameters described in section 3:5.5. The apparent mass-specific partitionment of ingested Gross Energy in kits and adults is tabulated in Table 4:8. Statistical comparisons were effected by means of 'Student's' t-test and the results are given in Table 4:9, from which it may be seen that kits differed significantly from adults for each of the parameters measured.

The very high level of feed consumption in the young animals represented a 2.7-fold greater ADE intake relative to adults (2344 KJ/Kg for kits, in comparison with 985 - 1108 KJ/Kg $^{0.75}$ for STAN and RBBT,

Table 4:7 Apparent Digestibility: Comparisons with Adults

| | RBBT | STAN |
|---|---|---|
| Dry-matter | N.S. | N.S. |
| Gross Energy | N.S. | N.S. |
| Nitrogen | 0.001 | 0.01 |
| Lipid | N.S. | 0.05 |

Table 4:8 Apparent Mass-Specific Partitionment of Ingested Gross Energy During Feeding Trials

| | Kits (KJ/Kg) | Adults (KJ/Kg$^{0.75}$) | |
|---|---|---|---|
| | | RBBT | STAN |
| Ingested | 2756 (83.0) | 1277 (54.8) | 1217 (50.2) |
| Faeces | 412 (26.1) | 170 (16.9) | 227 (33.2) |
| Assimilated | 2344 (77.7) | 1108 (40.4) | 985 (38.7) |
| Urine | 153 ( 5.8) | 84 ( 8.9) | 86 ( 2.2) |
| Metabolisable | 2191 (73.4) | 1024 (34.3) | 899 (40.7) |
| SDA | 510 (18.8) | 358 (12.8) | 310 (10.0) |
| Net | 1682 (57.2) | 658 (19.6) | 591 (32.2) |

Table 4:9 Apparent Mass-Specific Partitionment of Ingested Gross Energy During Feeding Trials: Comparisons with Adults

| | RBBT | STAN |
|---|---|---|
| Ingested | 0.001 | 0.001 |
| Faeces | 0.001 | 0.05 |
| Assimilated | 0.001 | 0.001 |
| Urine | 0.001 | 0.001 |
| Metabolisable | 0.001 | 0.001 |
| SDA | 0.001 | 0.001 |
| Net | 0.001 | 0.001 |

respectively), and the values for Net Energy retention indicated that the estimated maintenance requirement of adults was only 35 - 40% of that calculated for the growing kits, the means for STAN and RBBT being 591 and 658 KJ/Kg $^{0.75}$ compared with 1682 KJ/Kg for the kits.

A percentage breakdown of the kits' energy metabolism, in relation to ingested Gross Energy, is presented in Table 4:10a and, for comparison, that of adults in Table 4:10b. The results of statistical comparisons between groups are tabulated in Table 4:11.

There were no significant differences in the apportionment of ingested Gross Energy between faecal and Apparent Digestible Energy, these results reflecting the parity in digestive efficiency noted in section 4:3.3 above. Urine losses were proportionately lower in kits than in adults, constituting only 5.55% of GE intake, compared with 6.29% GE on RBBT and 7.12% on STAN. These differences were, however, relatively small, and significant only in the latter case ($p < 0.001$).

By virtue of the very low coefficients of digestibility for Nitrogen calculated for the kits, SDA accounted for a significantly smaller proportion of ingested Gross Energy in the young animals (18.41%) than was found in adults, for which the values were 28.25 and 25.41% GE on RBBT and STAN, respectively. Retained Net energy constituted 60.88% of GE intake and was, therefore, **rather higher than** the estimates of 48.51% GE obtained for STAN trials and 52.22% GE for RBBT; statistical comparisons between group means indicated that these differences were highly significant ($p < 0.001$).

### 4:3.5 Nitrogen Metabolism

The apparent mass-specific partitionment of ingested Nitrogen in kits and adults is tabulated in Table 4:12, and the results of statistical

Table 4:10a Breakdown of Energy Metabolism in Relation to Ingested Gross Energy (Kits)

| | MEAN (%) | Confidence Interval |
|---|---|---|
| Faeces | 14.80 | (14.00, 15.62) |
| Assimilated | 85.20 | (84.38, 85.99) |
| Urine | 5.55 | ( 5.39, 5.71) |
| Metabolisable | 79.48 | (78.68, 80.27) |
| SDA | 18.41 | (18.09, 18.74) |
| Net | 60.88 | (60.11, 61.66) |

Table 4:10b Breakdown of Energy Metabolism in Relation to Ingested Gross Energy (Adults)

| | RBBT | | STAN | |
|---|---|---|---|---|
| | MEAN (%) | Confidence Interval | MEAN (%) | Confidence Interval |
| Faeces | 13.07 | (12.09, 14.08) | 18.64 | (16.47, 20.91) |
| Assimilated | 86.93 | (85.92, 87.91) | 81.36 | (79.09, 83.53) |
| Urine | 6.29 | ( 5.77, 6.82) | 7.12 | ( 6.70, 7.55) |
| Metabolisable | 80.47 | (79.04, 81.85) | 74.11 | (71.78, 76.37) |
| SDA | 28.25 | (28.04, 28.45) | 25.41 | (25.06, 25.77) |
| Net | 52.22 | (50.63, 53.16) | 48.51 | (46.53, 50.49) |

Table 4:11 Breakdown of Energy Metabolism in Relation to Ingested Gross Energy: Comparisons with Adults

| | RBBT | STAN |
|---|---|---|
| Faeces | N.S. | N.S. |
| Assimilated | N.S. | N.S. |
| Urine | N.S. | 0.001 |
| Metabolisable | N.S. | 0.05 |
| SDA | 0.001 | 0.001 |
| Net | 0.001 | 0.001 |

Table 4:12 Apparent Mass-Specific Partitionment of Ingested Nitrogen During Feeding Trials

| | Kits (g N/Kg) | Adults (g N/Kg$^{0.75}$) | |
|---|---|---|---|
| | | RBBT | STAN |
| Ingested | 12.99 (0.39) | 8.11 (0.35) | 7.72 (0.32) |
| Faeces | 3.10 (0.21) | 0.52 (0.70) | 1.25 (0.14) |
| Assimilated | 9.89 (0.38) | 7.59 (0.29) | 6.48 (0.21) |
| Urine | 6.56 (0.25) | 3.60 (0.38) | 3.69 (0.10) |
| Retained | 3.34 (0.20) | 3.99 (0.24) | 2.79 (0.29) |

Table 4:13 Apparent Mass-Specific Partitionment of Ingested Nitrogen During Feeding Trials: Comparisons with Adults

| | RBBT | STAN |
|---|---|---|
| Ingested | 0.001 | 0.001 |
| Faeces | 0.001 | 0.001 |
| Assimilated | 0.001 | 0.001 |
| Urine | 0.001 | 0.001 |
| Retained | N.S. | N.S. |

Table 4:12 Apparent Mass-Specific Partitionment of Ingested Nitrogen During Feeding Trials

| | Kits (g N/Kg) | Adults (g N/Kg$^{0.75}$) | |
|---|---|---|---|
| | | RBBT | STAN |
| Ingested | 12.99 (0.39) | 8.11 (0.35) | 7.72 (0.32) |
| Faeces | 3.10 (0.21) | 0.52 (0.70) | 1.25 (0.14) |
| Assimilated | 9.89 (0.38) | 7.59 (0.29) | 6.48 (0.21) |
| Urine | 6.56 (0.25) | 3.60 (0.38) | 3.69 (0.10) |
| Retained | 3.34 (0.20) | 3.99 (0.24) | 2.79 (0.29) |

Table 4:13 Apparent Mass-Specific Partitionment of Ingested Nitrogen During Feeding Trials: Comparisons with Adults

| | RBBT | STAN |
|---|---|---|
| Ingested | 0.001 | 0.001 |
| Faeces | 0.001 | 0.001 |
| Assimilated | 0.001 | 0.001 |
| Urine | 0.001 | 0.001 |
| Retained | N.S. | N.S. |

comparisons between aggregate group means are presented in Table 4:13.

As was found in the analysis of energy metabolism, the principal difference between adults and kits was one of magnitude. Owing to the elevated feed consumption of the young animals, their daily ADN intake (9.89 g N/Kg) was 30% higher than that of moulting adults during presentation of RBBT (7.59 g N/Kg $^{0.75}$), and 52.7% higher than the value recorded for the Standard animals (6.48 g N/Kg $^{0.75}$).

Urine losses, however, were also very high, approximately 6.56 g N/Kg, compared with only 3.60 g N/Kg$^{0.75}$ on RBBT and 3.69 g N/Kg $^{0.75}$ on STAN. In consequence, mass-specific Nitrogen retention did not differ significantly between the three groups (means of 3.99, 2.79 and 3.34 g N for RBBT, STAN and kits, respectively).

Table 4:14a comprises a breakdown of Nitrogen metabolism in kits, relative to total Nitrogen intake, and also includes the index of Nitrogen utility (c.f. section 3:5.6). The equivalent values for adults are given in Table 4:14b. Reference has already been made to the proportionately higher losses of faecal Nitrogen recorded in kits, in comparison with adults, Apparent Digestible Nitrogen representing only 76.5% of that ingested.

Urine losses constituted approximately 51% of dietary Nitrogen intake and were higher than had been recorded in either RBBT or STAN trials (43.44 and 48.39%, respectively), though statistical analysis revealed no significant differences between groups (see Table 4:15). In consequence, percentage Nitrogen retention in kits (23.71%) was less than 68% of that found in the Standard animals (35.09%) and only 47.5% of the value recorded during presentation of RBBT (49.57%). Nitrogen utility approximated to 31.14% of ADN, in comparison with 41.28% on STAN and 53.28% for RBBT, so indicating a marked difference in the Nitrogen metabolism of the growing

Table 4:14a Breakdown of Nitrogen Metabolism in Relation to Ingested Nitrogen

| | Mean (%) | Confidence Interval |
|---|---|---|
| Faeces | 23.49 | (21.94, 25.08) |
| Assimilated | 76.51 | (74.92, 78.06) |
| Urine | 51.05 | (49.60, 52.50) |
| Retained | 23.71 | (22.89, 24.53) |
| Nitrogen Utility | 31.14 | (30.05, 32.24) |

Table 4:14b Breakdown of Nitrogen Metabolism in Relation to Ingested Nitrogen (Adults)

| | RBBT | | STAN | |
|---|---|---|---|---|
| | Mean (%) | Confidence Interval | Mean (%) | Confidence Interval |
| Faeces | 5.99 | ( 5.29, 6.75) | 15.79 | (21.94, 25.08) |
| Assimilated | 94.00 | (93.25, 94.71) | 84.21 | (74.92, 78.06) |
| Urine | 43.44 | (39.90, 47.02) | 48.39 | (49.60, 52.50) |
| Retained | 49.87 | (45.81, 53.94) | 35.09 | (22.89, 24.53) |
| Nitrogen Utility | 53.28 | (49.23, 57.30) | 41.78 | (30.05, 32.24) |

able 4:15 Breakdown of Nitrogen Metabolism in Relation to Ingested Nitrogen: Comparisons with Adults

| | RBBT | STAN |
|---|---|---|
| aeces | 0.001 | 0.01 |
| ssimilated | 0.001 | 0.01 |
| rine | N.S. | N.S. |
| etained | 0.001 | 0.001 |
| itrogen Utility | 0.001 | 0.005 |

animals in comparison with that of the adults.

## 4:4 Discussion

### 4:4.1 Growth Rates

The principal aim of the work, as detailed in the preceding sections was to compare and contrast the growth response of male and female kits consuming 'natural' diets, with that of animals raised on commercial rations.

Comparisons between sexes revealed that, while the relative growth rates of male and female kits were similar during the first two months of life, marked sex differences developed thereafter, with males having a consistently higher rate of weight gain than females. Similar findings have been reported by Skrede (1978b), Glem-Hansen (1979) and Tauson and Alden (1985), for kits maintained on commercial feeds, although these authors noted that the development of sexual dimorphism in body size began at approximately 21 days post partum, provided that the rations presented met the nutritional requirements of the mothers during lactation. Skrede (1978b) found that maximal pre-weaning growth rates were obtained with diets containing 8.5 - 11 g of Apparent Digestible Protein/100 Kcal (418 KJ) ME, equivalent to 41 - 53% of ME from ADP; Glem-Hansen (1979, 1980b) obtained satisfactory results with levels from 34 - 42% ME, while Tauson and Alden (1985) recommended a protein-energy content of 41% ME. These values are all considerably lower than that of 15.94g ADP/100 Kcal calculated for the RBBT diet, in which ADP constituted approximately 81.4% of the total ME content (6.09 KJ/g), due to the comparatively high ratio of protein to lipid in this ration. Comparisons made according to these criteria suggest, therefore, that the composition of the 'natural' diet is superoptimal for promoting high pre-weaning growth rates. From the data presented by other workers, however, it is evident that this was not the case.

Tauson and Alden (1985) recorded mean body weights of 106 and 115 g for 21 day old male and female kits, increasing to 345 and 301 g at 42 days old. These latter values are in close agreement with the figures given by Glem-Hansen (1980c) for six-week old kits (367 and 284 g, for males and females, respectively), and provide an indication of typical mean body weights at weaning. Throughout the early growth period, however, the body weights of the kits used in the present investigation were markedly lower than the values recorded by these authors. At 21 days post partum males averaged only 98 g, and females 94.25 g; at 42 days, the mean body weight of both sexes was 243 g.

In the post-weaning growth period, the differences between the body weights of animals feeding on 'natural' diets and those consuming commercial feeds become more pronounced, owing to the higher rates of weight gain observed in the latter group. Between 6 and 11 weeks post partum, Sinclair et al (1962) recorded growth rates of up to 9.81% in males, and 6.93% in females; from 11 to 17 weeks, the values were 5.55 and 3.8% for males and females, respectively. Allen et al (1964) estimated the rate of weight gain between 6 and 16 weeks post partum to be 14.05% in males, and 11.13% in females. In comparison, the growth rates calculated for the kits feeding on minced rabbit during the same period were approximately 3.2 and 2.6% in males and females, respectively. When the study was terminated at 109 days post partum, the mean body weight of males (1370 g) was 14.4% lower than the value of approximately 1600 g presented by Skrede (1978a). In contrast, final body weights of female kits were somewhat higher (970 g compared with

900 g). These findings suggest that the nutritional quality of the 'natural' diet is high enough to support normal growth and development in females, but may be insufficient in meeting the elevated metabolic demands of males, so resulting in diminished growth rates and lower adult body weights. To evaluate the extent to which the growth rates of each sex are differentially constrained by dietary regime it is appropriate, therefore, to compare the nutrient metabolism of males and females during this critical initial period.

### 4:4.2 Nutrient Metabolism

In contrast with the findings reported for adults, the mass-specific nutrient requirements of growing males are higher than those of females; the mean ME intake of male kits was estimated at 2628 KJ/Kg, in comparison with 2451 KJ/Kg for females (equivalent to 2450 and 2286 KJ ADE/Kg for males and females, respectively). These differences, though not statistically significant, provide some indication of the disparities in nutrient metabolism between sexes. For both males and females, however, the recorded values for energy intake greatly exceed those reported by other workers for kits raised on commercial feeds though, in terms of gross composition, the RBBT diet compared favourably with the types of ration used in these studies, as noted in section 4:4.1 above.

Between the ages of 10 and 15 weeks, Glem-Hansen (1980a) estimated ME intake to be 1116 KJ/Kg for males, and 1195 KJ/Kg for females; Glem-Hansen and Engaard Hansen gave values of 884 and 1038 KJ/Kg. These sex differences in intake have been found to diminish with age, however. From the results of feeding trials conducted throughout the period from weaning to pelting

(8 - 29 weeks post partum), Skrede (1978a) concluded that the daily energy requirements of males and females were virtually the same, at 1317 KJ/Kg. A similar finding was reported by Chwalibog et al, (1982), who derived a value of 12 - 1300 KJ/Kg$^{0.75}$.

The high levels of energy intake recorded in the present study might be attributable either to a relatively lower digestibility or, alternatively, to a deficiency in the RBBT diet of one or more essential nutrients, thereby leading to compensatory increases in feed consumption. The ration was of a comparatively high digestibility; the coefficient for ADM being 0.809 and for ADE, 0.852. The latter value is comparable with the results presented by other investigators, which range from 0.76 (Allen, 1964) to 0.855 (Chwalibog et al, (1982). The digestibility of dietary Nitrogen was, however, somewhat low (0.765), particularly when compared with the values of 0.842 and 0.940 derived for adults consuming a ration of identical composition, although the ADN intake of kits exceeded that of adults by a considerable margin (9.892 g/Kg in comparison with 7.589 and 6.477 g/Kg for RBBT and STAN, respectively) and was much higher than the estimates of Nitrogen intake obtained in other studies of growing kits. Glem-Hansen (1980a) calculated the ADN requirement between 10 and 15 weeks post partum to be 4.90 - 5.33 g N/Kg, while Sinclair et al (1962) and Chwalibog et al (1982) each gave a value of approximately 2.7 g/Kg.

Of particular interest, however, are the very high urinary losses reported for the animals used in the present study. Urine urea Nitrogen constituted 66.28% of ADN intake and, in consequence, total Nitrogen retention (3.34 g/Kg) did not differ significantly from the values obtained for adults (3.99 and 2.79 g/Kg for RBBT and STAN, respectively). The low coefficient of utility calculated for kits indicates a comparatively high turn-over of ingested dietary Nitrogen and suggests that ADN intake exceeded metabolic requirements. In a similar investigation of the Nitrogen metabolism of growing kits, Glem-Hansen (1976) found that low levels of protein utilisation were attributable to deficiencies in sulphur-containing amino acids (SAA) in the diet, and several studies have shown that the level of SAA intake is the first limiting factor in determining post-weaning growth rate (Leoschke and Elvehjem, 1959; Perel' dik, 1970; Glem-Hansen, 1979, 1980c; Jørgensen, 1985). The supposition that the elevated rates of protein catabolism recorded in the present study are related to dietary deficiencies in SAA content may, however, be invalid. Estimates of the SAA requirement of growing kits presented by these authors suggest that, at high levels of protein intake, amino acid restriction would be minimal; Glem-Hansen (1980c) reported a satisfactory growth response with rations containing as little as 20% ME from ADP, considerably lower than the value of 62.3% calculated for the RBBT diet. Furthermore the demand for these potentially limiting amino acids is greatest during the latter part of the growth period, from 20 - 24 weeks <u>post partum</u>, when the kits begin to develop the winter pelage. At this time, approximately 61% of the Nitrogen metabolised is retained as fur, compared with only 10 - 12% between the ages of 10 and 17 weeks, when the present trials were carried out (Glem-Hansen and Enggaard Hansen, 1981). No analyses of amino acid composition were performed on the rations presented in these trials, and therefore the hypothesis that Nitrogen balance was affected by dietary deficiencies in SAA cannot be discounted. Such results may, however, be attributable to other factors.

The high urine Nitrogen losses observed in the kits may, in part, have been due to an elevated rate of excretion of endogenous urinary Nitrogen (EUN). The excretion of EUN is known to be a function of metabolic weight (Robbins, 1983) and in placental mammals approximates to 140 mg N/Kg/day.

In growing animals, EUN losses would be considerably greater, owing to their higher metabolic rate (Poczopko, 1979; this study).

Anomalous results for Nitrogen balance determinations have been reported by several investigators. Glem-Hansen (1980a) calculated that Nitrogen retention by growing kits between 10 and 21 weeks old declined from 4.8 to 2.6 g N/Kg/day, results which are in close agreement with the value of 3.3 g N/Kg/day obtained in the present study. Similar data have been presented by Skrede (1978a) and Chwalibog et al (1982). Each of these authors concluded, however, that such findings were attributable to underestimates of urinary Nitrogen arising from faults in the experimental procedures followed. Possible sources of error in the determination of urine Nitrogen content have been discussed in section 3:6.3 (see also Appendix C), and are probably systematic in nature but, as the magnitude of such errors remains to be evaluated, the conclusions drawn from analyses of this type can be no more than tentative.

### 4:4.3 Conclusions

Although certain of the results obtained in the course of the trials with kits were inconclusive, it is evident from the data presented that the sex differences in nutrient metabolism were comparatively small. Estimates of digestive efficiency were similar in males and females for each of the parameters measured, though the mass-specific requirements of male kits were slightly higher than those of females. In both sexes, however, feed intake and retention of ingested macro-nutrients were directly proportional to body weight and, in consequence, considerably higher than had been found in adults maintained on a similar diet.

Comparison with the findings of other workers suggest that, even at the very high levels of consumption recorded in the present study, the 'natural' diet was inferior to commercial rations in promoting a fast rate of weight gain and high adult body weights. The apparent restriction in growth rate was most marked in male kits, for which the mean body weights recorded were below average throughout the early growth period. For these kits, therefore, the sex differences in final adult body size were expected to be considerably smaller than those generally found in captive animals maintained on an _ad libitum_ feeding regime.

Though necessarily limited in scope, these observations support the hypothesis that the degree of sexual dimorphism in this species is dependent upon the extent to which the growth potential of males is constrained by dietary regime during the early phases of development.

# Chapter Five

# GENERAL DISCUSSION

## 5:1 Nutrition and Sexual Dimorphism: Causes and Effects

Sexual dimorphism in body size is a characteristic feature of the Mustelidae and a number of theories have been advanced in explanation of this phenomenon. The sex differences in diet and feeding ecology evidence differential niche utility, but arguments favouring the evolution of size dimorphism as a means of reducing niche overlap (Brown and Lasiewski, 1972) are confounded both by inconsistencies in the relationship between predator and prey sizes, and the theoretical difficulties in justifying dimorphism as the most effective means of reducing intersexual competition, particularly for the smaller of the dimorphic pair (Moors, 1980). Recent studies of mustelid biology have, therefore, sought to interpret the development of dimorphism in terms of separate selective pressures acting upon each sex, promoting large body size in males and small size in females. Gittleman (1984) has suggested that large size in males may have arisen through intrasexual competition for females. Conversely, Powell (1979a) and Moors (1980) have identified a selective advantage in small size for females in that, with lower maintenance requirements, the high costs of reproduction might be more easily met.

Central to each of these hypotheses is an understanding of the inter-relationships between body size, energetics and feeding ecology. From the data obtained in the present study it is apparent that the relationship between body size and energy requirement in mink does not differ markedly from that described for other species. Metabolisable Energy intake is a function of body weight raised to a fractional power approximating to 0.75 (c.f. section 3:5.3) and though females have proportionately higher weight-specific energy demands, their absolute maintenance requirements are considerably lower, and represent only 58% of the estimate derived for males.

The magnitude of the dimorphism in body size found in mustelids is known to be dependent upon the extent to which the growth potential of males is constrained by dietary regime during the early phases of development. Laboratory investigations have shown that differentials in the growth rates of males and females are related not only to the plane of nutrition, but also to the source and quality of dietary protein, and the ratio of protein to energy in the diet consumed (Glem-Hansen, 1980a, 1980b; Tauson, 1985b).

In the light of these findings, it has been postulated that the observed variations in dimorphism found in wild populations may be attributable to regional differences in diet and prey availability, particularly during the period when the young are growing (Ralls and Harvey, 1985). The results of the feeding trials carried out on kits in the course of the present study lend support to this hypothesis; the 'natural' diets presented did not support maximal growth and development, even at high levels of consumption, but led to low male body weights and reduced dimorphism in adult body size. The hypertrophy of the alimentary tract found in free-living adults is also indicative of the relatively low nutritional quality of the diets taken in the wild, though comparisons of gross composition with various commercial rations revealed no basis for these observations since each of the diets analysed was found to be of comparatively high biological value.

The finding that potential prey types do not differ markedly in terms of nutritional value has important implications in evaluating the significance of sex differences in feeding ecology. It is evident that attempts to identify the possible nutritional consequences of feeding on particular groups are of limited utility, particularly in studies of generalist predators such as the American mink. The disparities in the composition of the diets taken should, perhaps, be examined in the context of the different foraging strategies adopted by males and females, contingent upon the relationship between body size and hunting efficiency. Central to any such analysis is a detailed knowledge of the relative differences in maintenance requirements, together with reliable estimates of the energy expenditure associated with particular foraging strategies.

To date, investigations into the ecological energetics of mustelids have been based largely on determinations of maintenance requirements made under laboratory conditions, either in feeding trials (Stromberg, Rayburn and Clark, 1983), or through the use of respirometric techniques (Moors, 1977). There are, however, limitations inherent in these procedures which militate against application of the data so-obtained to investigations of free-living animals.

It is evident from the present study that the feed consumption of captive carnivores is not a reliable indicator of nutritional requirements, and whilst respirometry enables the direct determination of metabolic rate, its utility in evaluating the daily energy expenditure of active predators is necessarily confined to studies of the smaller species. For the larger members of the group, these objectives can only be achieved in combination with observations carried out in the field.

## 5:2 Suggestions for Further Work

The models proposed by Powell (1979a) and Powell and Leonard (1983) to describe the energy budget of fishers are founded on the premise that the energy expended in any given activity is a linear function of the animal's body weight raised to a specified power, and its running speed. From data on activity patterns collected from radio-tracked individuals these workers on activity patterns collected from radio-tracked individuals these workers were able to compile estimates of daily energy expenditure, based on measurements of running costs made in the laboratory.

Through implementing similar techniques in future studies of the mink, it may be possible to establish an empirical basis for the postulate that the extreme size dimorphism in this species developed because of differentials in feeding energetics between males and females. Williams (1983a, 1983b) has presented data on the costs of locomotion, and sex differences in activity and habitat usage have been investigated by Dunstone and Birks (1983). In a short-term study of a coastal-living population, the latter authors found a higher level of activity in females than in males, which may reflect a lower foraging efficiency or, alternatively, a greater requirement for food.

Attempts to evaluate the relative costs of these differing activity patterns have yet to be made. Of central importance is a detailed examination of breeding biology and its bearing on the nutrition of free-living predators. The costs of pregnancy are well documented in the mink and several other species, but after weaning the demand for energy, protein and minerals, etc., increases still further as the females attempt to find sufficient food to meet their own needs and those of their young, whose combined requirements may be several times greater. This, in turn, must be compared with the elevated energy expenditure of males searching for receptive females, in competition with other males.

A conclusive analysis of sexual dimorphism in body size should address these differences in the nature and magnitude of nutritional requirements, and the ease with which they can be met by individuals of each sex. Further studies must, therefore, include measures of metabolic demands and their variation with season and reproductive status, if these issues are to be satisfactorily resolved.

## Conclusions

1). Though analyses of gross composition revealed significant differences between the 'natural' diets used in the present study, comparison with data presented by other workers demonstrates that the variations within groups of potential prey species are as great as, or greater than, those between the diets themselves. Thus, while it may be said that each of the diets used in the present study reflects the composition of prey items generally available to wild mink, none should be considered as typical of any particular prey type or group.

2). The composition of commercially available feeds was found to differ substantially from that of the 'natural' diets, containing a higher proportion of lipid and less protein, though similar in energy content. This finding supports the assertion that the use of such feeds does not provide a reliable means of estimating the nutritional requirements of animals preying upon species available in the field.

3). As studies of prey-handling indicated that carcase utilization was virtually complete, it may be assumed that minced whole-animal preparations are comparable in composition to those parts of a carcase normally consumed by wild mink. Their use in laboratory feeding trials is, therefore, a valid procedure.

4). While variations in feed intake between diets were comparatively small, most subject animals underwent a significant gain in body weight during each trial, indicating that the _ad libitum_ feeding regime may lead to consumption significantly higher than the maintenance requirement, particularly in animals presented with rations of high energy content.

5). The 'natural' diets differed little in digestibility, although nutrient metabolism varied according to the composition and quantity of feed consumed. Changes in nitrogen metabolism were, however, directly related to the stage of the moult and appear to be independent of the level of intake.

6). The absence of notable sex differences in nutrient metabolism suggests, in conjunction with 1) and 5) above, that consideration of the differences in size and type of prey taken by males and females in the wild is of little consequence in evaluating the nutritional significance of extreme size dimorphism. Of greater relevance is the hypertrophy of the alimentary tract in individuals from free-living populations; it is particularly marked in females, revealing that the smaller sex may have relatively greater difficulty in meeting their metabolic requirements than do males. This may reflect a differential in foraging costs associated with the predation of numerous prey items of relatively small size.

7). The 'natural' diets, fed _ad libitum_, are inferior to commercial rations in promoting a fast rate of weight gain and high adult body weight. Sex differences in the nutrient metabolism of kits are negligible, but the restriction in growth rate is greater in males, leading to reduced size dimorphism in animals maintained on such diets.

The above findings may be considered to be of general application to studies in the feeding ecology of generalist predators. They demonstrate that future work on sexual size dimorphism in carnivores should be directed towards evaluating sex differences in the maintenance requirements of animals living in the field and, in particular, to the costs of reproduction and alternative foraging strategies.

# Appendices

## Appendix A

## Percentage Composition of Rations Obtained from Local Mink Farm

| | Jan-April | May-June | July-August | Sept-Nov |
|---|---|---|---|---|
| Fish frames (various spp.) | 83.5 | 80.5 | 86.0 | 76.0 |
| Oats | 6.0 | 6.0 | 6.0 | 12.0 |
| Chicken fat | 4.0 | 5.0 | 5.0 | 5.0 |
| Yeast | 2.0 | 2.0 | 0.5 | 0.5 |
| Vitamins | 0.5 | 0.5 | 0.5 | 0.5 |
| Wheatgerm | 4.0 | - | - | - |
| Soya meal | - | 2.0 | 2.0 | 3.0 |
| Dried blood | - | - | - | 3.0 |
| Sugar | - | 2.0 | - | - |
| Skimmed Milk Powder | - | 2.0 | - | - |

# Appendix B

## Derivation of the Principal Axis Equation Describing the Regression of Metabolisable Energy Intake on Body Weight (Fig. 3:10)

Given:-

Mean body weight $\bar{X}$ = 0.085

Mean ME intake $\bar{Y}$ = 3.133

Variance about $\bar{X}$ S.x = 0.029

Variance about $\bar{Y}$ S.y = 0.018

Covariance S.xy = 0.020

(i) Derivation of latent roots (eigenvalues)

$$D = \sqrt{(S.y + S.x)^2 - 4(S.y \times S.x - S.xy^2)}$$

$$= \sqrt{(0.018 + 0.029)^2 - 4(0.018 \times 0.029) - 0.020^2)}$$

$$= 0.0415$$

$$\text{and } E.x = \frac{S.x + S.y - D}{2}$$

$$= \frac{0.029 + 0.018 - 0.0415 = 0.0028}{2}$$

$$\text{and } E.y = \frac{S.x + S.y + D}{2} \qquad = 0.0442$$

(ii) Derivation of slope and principal axis equation

$$\text{The slope 'b'} = \frac{S.xy}{E.y - S.x} = \frac{0.020}{0.0442 - 0.018} = 0.762$$

and the equation of this axis is $y = \bar{Y} + b(x - \bar{X})$

$$= 3.068 + 0.762x$$

(iii) Derivation of confidence limits

The 95% confidence limits about the slope 'b' are derived as follows:-

$$\text{Let } H = \frac{F(0.05)[1,\ n-2]}{[(E.y/E.x) + (E.x/E.y) - 2](n-2)}$$

$$= \frac{4.20}{[(0.0442/0.0028) + (0.0028/0.0442) - 2] \times 28}$$

$$= 0.0108$$

$$\text{and } A = \sqrt{\frac{H}{(1-H)}} = \sqrt{\frac{0.0108}{(1 - 0.0108)}} = 0.0109$$

$$\text{then } L1 = \frac{b - A}{1 + bA} = \frac{0.762 - 0.0109}{1 + (0.762 \times 0.0109)} = 0.745$$

$$\text{and } L2 = \frac{b + A}{1 - bA} = \frac{0.762 + 0.0109}{1 - (0.762 \times 0.0109)} = 0.779$$

## Appendix C

## Assay of Urea- and Creatinine-Nitrogen in 'Fresh' and Preserved Urine Samples

### Aims

To determine the proportion of urine Nitrogen contained in creatinine, and to evaluate the losses of total urine Nitrogen occurring in the period between collection and assay.

### Methods

Urine samples were collected from one adult male mink housed in a metabolism cage and fed *ad libitum* on a ration of slaughter-house by-products (c.f. section 3:4.1), over a period of two days. Clean collecting bottles containing approximately 0.5 ml of toluene were placed beneath the urine tray each morning and assays performed in triplicate on sub-samples of the contents, at 1 hr. and 24 hr. after collection. To simulate the conditions which obtained for collections made during feeding trials, the samples were stored at room temperature (approximately 15 °C) in un-stoppered collecting flasks, for the intervening period.

Determinations of urine creatinine content were made using the method of Bonsnes and Taussky (1945), as described in Varley *et al* (1980). Urine samples of 1 ml were diluted to 100 ml with de-ionised water and aliquots of 3 ml placed in tubes, to which were added 1 ml of 40-mM picric acid, followed by 1 ml of 750-mM Sodium hydroxide solution. Absorbance was measured at 500 nm after 15 min. Standard curves prepared using a 10-mM solution of creatinine sulphate were found to be linear for concentrations from 24 - 120-uM, and determinations of urine creatinine made on the basis of direct proportionality.

Urine urea Nitrogen was assayed according to the procedures detailed in section 3:4.4.2.

## Results and Discussion

The urine Nitrogen content of 'fresh' samples, analysed within 1 hr. of collection, was found to be 2.87 mg N/ml, declining to 2.47 mg N/ml after 24 hr., a fall of approximately 13.9%. The values recorded for creatinine were 0.389 and 0.208 mg N/ml for fresh and preserved urine, respectively, indicating losses of approximately 47%.

These results suggest that significant losses of urine Nitrogen occur during storage under the conditions described; for the samples analysed in the present investigation, these losses constituted 17.89% of the Nitrogen contained in urea and creatinine, combined. It is to be noted, however, that the assays of urine urea Nitrogen carried out during feeding trials yielded values considerably higher than those given above, ranging from 27.66 mg N/ml for trials with E-MIX, to 37.45 mg N/ml with the standard rations. Total urine Nitrogen losses may, therefore, be closer to the value of 13.9% obtained for urea Nitrogen, given that creatinine constituted a comparatively small proportion of the total Nitrogen content.

Losses of this magnitude do not fully account for the descrepancies in Nitrogen balance determinations noted in sections 3:5.6 and 4:3.5, however, and a satisfactory explanation for these anomalous results remains to be established.

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