

**THE INFLUENCE OF MATERNAL PROTEIN INTAKE ON ASPECTS OF  
SEX-SPECIFIC FOETAL AND NEONATAL DEVELOPMENT IN  
*MASTOMYS NATALENSIS***

by

**Caroline Elizabeth Lamb**

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*Dedicated to my parents, with love.*

“Neither rat nor man has achieved social, commercial, or economic stability. This has been, either perfectly or to some extent, achieved by ants and by bees, by some birds, and by some fishes in the sea. Man and the rats are merely, so far, the most successful animals of prey. They are utterly destructive of other forms of life. Neither of them is of the slightest use to any other species of living thing.”

- Hans Zinsser *Rats, Lice and History*, 1934

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Caroline Lamb

SUPERVISOR: Professor R J van Aarde  
Chair of Conservation Ecology  
Department of Zoology and Entomology  
University of Pretoria  
Pretoria 0002  
South Africa

**ABSTRACT**

Captive multi-mammate mice, *Mastomys natalensis*, were paired and kept on one of three treatment diets (low, medium and high protein) in order to assess differential maternal investment in the sexes, and sex-specific resource allocation of offspring. The influence of maternal dietary protein content on maternal reproductive performance, sex-specific body composition of pups and pup growth from birth to weaning was determined. Mothers on the high protein diet were larger than those on the lower protein diets, and produced more male than female offspring. Mothers on the lower protein diets did not produce sex-biased litters. Maternal dietary protein intake did not significantly influence litter size or the interval between litters. Litters produced by mothers on the medium (15%) protein diet were significantly larger than those produced by

mothers on the low (10%) protein diet. There were no sex-specific differences in body size or body tissue composition of pups at birth or at weaning within each treatment group. At weaning, pups in the 20% protein treatment group had proportionately greater amounts of lean tissue and less body lipid reserves than pups in the 10% protein treatment group. Pups in the 20% protein treatment group were also larger, and had faster growth rates, than those in the 10% protein treatment group. Weaned pups in the 15% protein treatment group had the fastest growth rates and greatest energetic contents of all of the treatment groups. These results suggest that larger mothers on the high (20%) protein diet show differential investment in the sexes, not by allocating more resources to individuals of that sex, but by producing more male than female offspring. Maternal dietary protein intake did not appear to influence the reproductive output of mothers, but did affect pup mass, growth rates and body composition which would have implications for their future success and survival.

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## CHAPTER 1

### INTRODUCTION

#### ***Mastomys natalensis* - a pest species**

The multi-mammate mouse, *Mastomys natalensis*, is an opportunistic species and often the first rodent to move into a disturbed area (Meester, Lloyd & Rowe-Rowe 1979; Ferreira & Van Aarde 1996). It is very much an *r*-selected species because of its large population fluctuations and high reproductive rate (See Pianka 1970). Productivity is maximised through reduced size, reduced longevity and survival, early age at sexual maturation, short generation time and low investment per young (Willan & Meester 1989; Leirs 1994). The species is known for its population explosions in many parts of Africa because of its opportunist attributes and its ability to rapidly adapt to man-made environments (Fielder 1988; Leirs 1994). Multi-mammate mice are truly omnivorous, feeding on vegetation, insects and grains, or whatever is available (Fielder 1988). The ability of multi-mammate mice to climb (Taylor 1968) and swim (Fielder 1988) make developing young maize cobs and rice paddies vulnerable to these rodents. Economically, it is the most important of all of Africa's rodent pest species, as it frequently devastates agricultural crops and stored grain products. Control of their numbers is frequently needed in developing countries in Africa where food is scarce and harvests cannot afford to be lost to rodent plagues. In addition these animals carry or transmit diseases harmful to man, like Bubonic plague (Davis 1964) and Lassa virus (Monath *et al.* 1974).

The species studied in the present project is *Mastomys natalensis* (Smith, 1834), and has been distinguished from the morphologically similar species, *M. coucha* based on rainfall and habitat characteristics of the area in which the experimental animals were captured

(Dippenaar, Swanepoel & Gordon 1993). Multi-mammate mice, so called because of their numerous mammae (up to 12 pairs), are prolific breeders producing litters of up to 20 offspring, the average litter size being 7, (Oliff 1953; Meester 1960 ) at an interval of about 33 days (Meester 1960). Within a breeding season in Morogoro, Tanzania, females are able to produce between four to six litters (Leirs 1994) thus indicating great potential for rapid population growth. The young multi-mammate mice also mature quickly, females apparently reaching first oestrus at 104 days (Oliff 1953). However, our own data and that of Meester (1960) show that females can produce the first litter as early as 77 days (indicating first oestrus at 54 days).

Population explosions in the multi-mammate mouse are characterised by an initial rapid population growth when food is abundant, soon after the first rains that follow a drought or extended dry season (Leirs 1994). Breeding occurs soon after the onset of heavy rains (Field 1975; Taylor & Green 1976; Bronner, Rautenbach & Meester 1988; Leirs 1994). In Australia, *Mus musculus* (Mutze 1991) population outbreaks, like those of multi-mammate mice occur at the onset of rains following long periods of drought. It has been argued that during periods of drought, nutrients (including nitrogen) from decaying plant and animal matter accumulate in the soil, resulting in rich vegetative growth immediately after the rains. The mouse populations are able to exploit this nutrient rich food, giving rise to rapid growth and reproduction unhampered by predators, which are slower to respond to the favourable conditions (Mutze 1991). Leirs (1994) showed that growth patterns of multi-mammate mice are also linked to rainfall. Abundant rain early in the breeding season gives rise to early maturation of individuals often resulting in there being more than one generation of reproductive animals in a breeding season (Leirs 1994). Leirs (1994) also found that deteriorating nutritional conditions marked the end of the breeding season, as adults disappear

and the growth of young is retarded. As we cannot change the situation of there being fluctuations in the amounts of cultivated plants and stored products, “attempts to manipulate, or modify, pest populations should be sought in terms of changes in the survival of the very young, specifically in changes in a relative shortage of their food” (White 1978, page 84). Thus an understanding of the reproductive strategies of a species is needed before any form of ecologically viable control is initiated.

The response of breeding to environmental conditions, especially diet, in multi-mammate mice has been of interest to scientists for years, and attempts to link the breeding season to seasonality of food availability have been made with little success (Taylor & Green 1976; Swanepoel 1980; Bronner *et al.* 1988; Leirs *et al.* 1990; Christensen 1993; Leirs 1994; Leirs *et al.* 1996). The present study is aimed at measuring the influence of the protein content of food ingested on the reproductive abilities of multi-mammate mice, as it appears that food quality, as opposed to energy, is limiting in the field (White 1978).

### **Diet and reproduction**

Protein is essential for growth, reproduction and survival (Yom-Tov 1985; White 1993). In rats, *Rattus rattus*, 5 - 10% protein content of food is required for marginal growth and reproduction (Anthony & Edozien 1975; Musten, Peace & Anderson 1974; Nakagawa & Masana 1971). Although the growth rate and ability for young rats and meadow voles, *Microtus pennsylvanicus*, to reach maturity increases with increasing protein intake, there appears to be an upper limit of 25% digestible protein content to the positive effect of dietary protein intake (Edozien & Switzer 1978; Shenk, Elliott & Thomas 1970). In rats maternal protein intake also significantly influences maternal milk output, offspring body mass at birth

and at weaning, but does not appear to influence litter size (Naismith & Morgan 1976; Sasaki, Nakagawa & Kajimoto 1982). The importance of dietary protein intake has been furthermore supported by the role it plays in the selection of dietary items (Bergeron & Jodoin 1987; Bucyanayandi & Bergeron 1990). In hispid cotton rats, *Sigmodon hispidus* (Cameron & Eshelman 1996), and rats, *Rattus rattus* (Nakagawa & Masana 1971), it was the amount of dietary protein, and not calorific intake which limited their growth and reproduction. Meadow voles are also able to select for food of a high protein content irrespective of its calorific value (Bergeron & Jodoin 1987; Bucyanayandi & Bergeron 1990), and weanling rats appear to regulate protein intake by food selection (Musten *et al.* 1974). Diet choice does not appear to be a learned response as protein-depleted rats chose significantly more protein-rich food within the first minute of being given a choice of a variety of foods (Deutsch, Moore & Hendricks 1989). This response was more pronounced in pregnant females as they had a greater demand for protein (Deutsch, Moore & Hendricks 1989). Rats fed on a low calorie diet also displayed a more cost-efficient deposition of protein in the body, than those fed on low protein diets (Coyer, Rivers & Millward 1987).

During the breeding season of wild multi-mammate mice, seeds form an important part of the diet because of their high energetic contents (Field 1975; Taylor & Green 1976). During pregnancy, protein requirements become increasingly important (Field 1975). Seedlings and arthropods, which are rich in protein, are abundant during these periods and multi-mammate mice have been observed to consume significant numbers of them during their reproductive period (Leirs 1994).

## **Reproductive biology of the multi-mammate mouse**

Multi-mammate mice are polyoestrus, the average period between successive oestrous cycles being nine days and pro- and meta-oestrus last for approximately 2 days (Johnson & Oliff 1954). However, some females experience long periods of anoestrus of about 38 days (Johnson & Oliff 1954). *Post partum* oestrus occurs about three days after parturition and most females conceive at this *post partum* oestrus (Johnston & Oliff 1954), indicating that the oestrous cycles of females suckling young do not essentially differ from those without offspring. The gestation period of the multi-mammate mouse is approximately 23 days (Johnston & Oliff 1954). Multi-mammate mice are sexually dimorphic, males being slightly larger than females (Granjon & Duplantier 1993; Leirs 1994). Populations comprise of polygynous groups made up of an adult male and several females and juveniles, where males from different groups compete with each other for mates (Granjon & Duplantier 1993). Since the males are competitive, the stronger, larger males will have most reproductive opportunities as in other polygynous species, like fallow deer, *Dama dama*, where male mating success is related to a large body size (Clutton-Brock *et al.* 1988). In polygynous species females have a relatively equal (and limited) number of reproductive opportunities, irrespective of size or fitness.

## **Maternal investment theories**

Sex-biased parental investment has been of great interest to evolutionary biologists. Parental investment into male and female offspring is expected to differ in polygynous species because males are generally larger than females. Lifetime reproductive success is more variable in males than females because they are more strongly influenced by parental investment (Clutton-

Brock, Albon & Guinness 1981). Differential investment in the sexes can be achieved either by skewing the sex ratio at birth (Trivers & Willard 1973) or by diverting more resources to individual sons than daughters (Maynard Smith 1980). Maynard Smith's (1980) model assumes that the parent achieves differential investment by altering their behaviour according to the sex of their offspring. Thus natural selection would favour the parent that either invests more in offspring of the sex with the lower investment-specific probability of survival, or in offspring of the sex with the greater investment-specific enhancement of fitness, given an equal chance of survival (Maynard Smith 1980). It is assumed that the primary sex ratio is fixed at unity and that parents can recognise the sex of individual offspring (Maynard Smith 1980). Since sexually dimorphic polygynous mammals fulfill the conditions of his model, species like red deer, *Cervus elaphus* (Clutton-Brock *et al.* 1981; 1982), northern elephant seals, *Mirounga angustirostris* (Reiter, Stinson & Le Boeuf 1978) and African elephants, *Loxodonta africana* (Lee & Moss 1986) have been shown to hold true to his predictions. However in other polygynous and sexually dimorphic species like fallow deer, *Dama dama*, and white-tailed deer, *Odocoileus virginianus* (Gauthier & Barrette 1985) there is no evidence of sex-specific differential investment. Evidence used to support the theory of differential investment is usually based on sex-specific differences in birth weights, growth rates, weaning weights, estimates of milk consumption and differences in the fecundity of females that previously raised male versus female offspring (Byers & Moodie 1990). However, mass or growth rate data may not accurately reflect on maternal investment as they could be a consequence of differential allocation of resources to fatty and lean tissue by the two sexes (Lunn & Arnould 1997; Arnould, Boyd & Socha 1996).

Trivers & Willard (1973), on the other hand, suggested that differential investment in the sexes could be achieved through the manipulation of the secondary sex ratio. In

polygynous species, mothers in good condition should produce sons, as males have to compete for mates, and to be a successful competitor they need to be fit and strong. Thus a male with a good start in life would be ensured of many more matings than a given female (Trivers & Willard 1973). A male in good condition at the end of parental investment is expected to out-reproduce a sister in similar condition, while she is expected to out-reproduce him if both are in poor condition (Trivers & Willard 1973). Natural selection should thus favour parental ability to adjust the sex ratio of offspring produced according to the ability of the parent to invest (Trivers & Willard 1973). Bearing in mind that the males in bad condition may not mate at all, one would expect that mothers in bad condition (because of low quality food available) would produce predominantly females; while a mother in good condition (with high quality food available) would produce predominantly males (Trivers & Willard 1973). In grey seals, *Halichoerus grypus* (Anderson & Fedak 1987), red deer, *Cervus elaphus* (Clutton-Brock, Albon & Guinness 1982; 1984; 1986), common opossums, *Didelphis marsupialis* (Austad & Sunquist 1986), and southern elephant seals, *Mirounga leonina* (Arnbom, Fedak & Rothery 1994) larger females in good body condition, tend to produce more males than females. This suggests that in polygynous species, body condition, or size, of the mother during gestation and lactation may determine the sex-ratio of offspring produced. However, in northern elephant seals, *Mirounga angustirostris* (Kretzmann, Costa & Le Boeuf 1993), and Antarctic fur seals, *Arctocephalus gazella* (Arnould *et al.* 1996), the age or size of the mother did not appear to alter the sex-ratio of the pups.

The assumptions of Trivers and Willard's (1973) hypothesis are that (i) the condition of young at the end of maternal investment should reflect the condition of the mother during investment, (ii) the condition of offspring at the end of maternal investment should endure into adulthood, and (iii) any slight advantages in offspring condition should assist male

reproductive success more than females (Trivers & Willard 1973).

It must be noted that this differential investment in the sexes is not controlled consciously by the parent, but could possibly be a result of the differing abilities of male and female pups to utilise the maternal resources available to them (Kovacs & Lavigne 1986). Males are exposed to more testosterone *in utero* (Rigaudiere 1979). Thus because of the greater growth-promoting properties of androgens, in comparison to oestrogens (MacGillivray 1986) male foetuses may have faster growth rates than females.

### **Sex ratio adjustment theories**

Although there have been numerous findings both supporting and opposing Trivers & Willard's theory (1973) on sex ratio adjustment, there has been no empirical evidence for a mechanism to explain the observed trends. However, several mechanisms have been proposed (Krackow 1995). Sex ratio adjustment prior to conception implies active manipulation by the mother (in order to minimise energetic waste). However, a shift in sex ratio after conception suggests differences in the susceptibility of the offspring to uterine conditions or nutrient availability (Arnbom *et al.* 1994). Post-conceptual adjustment of sex ratios through sex-specific foetal absorption or differential mortality of embryos may be due to food stress (Rivers & Crawford 1974; Myers 1978; Meikle & Drickamer 1986; Clutton-Brock 1991; Krackow 1992; Meikle & Thornton 1995). Sex-specific differential mortality may result from different requirements of the sexes, males possibly requiring more energy than females to support their faster growth rates (Arnbom *et al.* 1994). Sex-specific embryo mortality has also been linked to steroid hormone levels during pregnancy, which was reflected in a reduction in litter size of golden hamsters, *Mesocricetus auratus* (Huck, Lisk & McKay 1988). Low

levels of progesterone during pregnancy in golden hamsters resulted in increased embryo mortality (Huck *et al.* 1988) possibly because progesterone is important in the maintenance of pregnancy (Heap 1982).

Other suggested mechanisms of sex ratio control are thought to occur before implantation. The sex ratio at birth of Norway rats, *Rattus norvegicus*, appears to be influenced by the time in the oestrous cycle that copulation and insemination take place (Hornig & McClintock 1994; 1996). Similar findings have been recorded for white-tailed deer, *Odocoileus virginianus* (Verme & Ozoga 1981) and golden hamsters, *Mesocricetus auratus* (Huck, Seger & Lisk 1990). High concentrations of testosterone and oestrogen at conception can lead to a male-biased sex ratio, while high concentrations of gonadotropin and steroids result in significantly more female than male offspring (James 1992; 1996). Male-biased sex ratios in dominant mothers (*e.g.* red deer, *Cervus elaphus*, Clutton-Brock *et al.* 1984) may be due to high testosterone levels associated with dominance and aggression (James 1992).

### **Pup energetic contents and differential investment**

The predictions that under conditions of nutritional stress, mothers would differentially invest in female offspring, or produce a female-biased sex ratio, are also based on the assumption that males are energetically more expensive to produce than females. Males of polygynous species are generally larger than females at birth, and have faster growth rates. Several scientists interpreted this as proof that males are more costly to produce than females (Kovacs & Lavigne 1986; Trillmich 1986; Anderson & Fedak 1987; Boyd & McCann 1989; Ono & Boness 1996). However, Arnould *et al.* (1996) suggested that body mass did not necessarily indicate energy intake of the pup, and thus the investment of the mother into her offspring. In

fact, they showed that although male Antarctic fur seal, *Arctocephalus gazella*, pups were heavier at birth than females, the gross energy content of the males and females were similar because females contained a higher proportion of fatty tissue than males (Arnould *et al.* 1996). Female hispid cotton rats also had significantly more fatty tissue than males (Cameron & Eshelman 1996). The age and nutritional status of individuals also affects its body composition (lean tissue and adipose stores) and thus body weight (Robbins 1983; Worthy & Lavigne 1983; Price & White 1985; Oftedal, Bowen & Boness 1993; Allaye Chan-McLeod, White & Holleman 1994). In polygynous males, size is thought to determine reproductive success, while in females success is dependent on survival during gestation and lactation (Arnould *et al.* 1996). Thus, males are more likely to deposit lean muscle for bulk and size, while females would be expected to deposit fatty tissue to be used during energetically stressful periods (gestation and lactation). These findings served as motivation to use body tissue composition analyses as an indication of maternal energetic investment in the present study.

## **Aims of the study**

The objectives of this study are essentially two-fold. First, considering that maternal size may affect the sex ratio of offspring produced, the aim was to manipulate maternal body size of the sexually dimorphic polygynous multi-mammate mouse through the modification of dietary protein content. Body size and body tissue composition are known to be influenced by dietary protein intake (Nakagawa & Masana 1971; Cameron & Eshelman 1996; Van Lunen & Cole 1996). In multi-mammate mice, males are larger than females (Granjon & Duplantier 1993; Leirs 1994). Since the males are competitive (Granjon & Duplantier 1993), the stronger, larger males should have most reproductive opportunities (Clutton-Brock *et al.* 1988). Polygynous females on the other hand have a relatively equal (and limited) number of reproductive opportunities, irrespective of size or fitness. If mothers maintained on a high nutritional plane are larger than those on a lower nutritional plane, then the former group should produce more male than female offspring. Thus, the objective of this study was to address the influence of dietary protein intake on the reproductive investment of female multi-mammate mice to each sex, thus contributing to our understanding of current maternal investment theories.

Second, bearing in mind that protein intake affects reproduction (Nakagawa & Masana 1971; Musten *et al.* 1974; Anthony & Edozien 1975; Yom-Tov 1985; White 1993), I aimed at determining the influence of maternal dietary protein intake on maternal reproductive output (litter interval, litter size and litter sex ratio), pup growth rate and pup body tissue composition (at birth and at weaning). Thus the influence of too little, and an excess of, dietary protein on reproduction in multi-mammate mice could be determined and compared to the recommended, or optimal, level of protein in the diet. This information could then be used to improve our understanding of the dynamics of rodent population explosions in response to food quality by

studying the influence of aspects of dietary protein intake on reproductive output and reproductive investment in the multi-mammate mouse.

### **Objectives**

- To determine the effects of maternal dietary protein content on reproductive performance (litter interval, litter size and litter sex ratio) of the sexually dimorphic multi-mammate mouse.
- To ascertain the influence of maternal dietary protein content on sex-specific mass and body composition (protein, fat, moisture and ash contents) at birth and at weaning, and sex-specific growth from birth to weaning.

### **Key questions**

- How does maternal dietary protein content affect reproductive output, reflected in the litter interval, litter size and litter sex ratio?
- How does maternal dietary protein content affect sex-specific mass and growth rates of offspring from birth to weaning?
- How does maternal dietary protein content affect body composition of male and female newborn pups and weanlings ?

## CHAPTER 2

### MATERIALS AND METHODS

#### **Establishment of the breeding colony**

Thirty breeding pairs of multi-mammate mice were caught in Pretoria (Proefplaas, University of Pretoria), paired, and placed in cages 42 x 25 x 13cm with woodshavings as bedding, and a brown glass jar as a “nesting box”. The mice were maintained in the experimental holding facilities of the University of Pretoria. All holding and handling procedures were in accordance to those sanctioned by the ethical committee of the Faculty of Biological and Agricultural Sciences of the University. The ambient temperature was kept at about 25°C with a 14L:10D photo period regime. Mouse pellets (Epol Animal Feed Manufacturers, Box 19096, Pretoria West, South Africa) and water were provided *ad libitum*. Litters produced by these breeding pairs were removed from the parents at weaning age (21 days *post partum*, Meester 1960), and fed mouse pellets *ad libitum* until Day 50 *post partum*. As the earliest recorded first oestrous cycle for multi-mammate mice is at 54 days *post partum* (Meester 1960; own observations) these offspring were then randomly paired (not with siblings) and assigned to one of the treatment groups (high, medium, or low protein diets) at the age of 50 days. A total of 20 breeding pairs were established on each of the treatment groups. Only data from those pairs with an uninterrupted series of litters (*i.e.* neither of the parents died, and none of their litters were killed during the course of the experiment) were used for the calculation of maternal reproductive output (litter interval, litter size and litter sex ratio), pup growth rates, and pup body tissue compositions.

## Experimental diets

The experimental diets (made up by the Animal Nutrition and Animal Products Institute, Private Bag X2, Irene, 0062, South Africa) were designed so that all nutritional components, except digestible protein content, which varied according to treatment level, remained constant (Table 1). The control feed had an optimal protein content of 15%; the experimental low had 10%; and the experimental high had 20%. Protein levels were selected so as to provide adequate protein to support pregnancy (Richardson *et al.* 1964; Field 1975), while still providing a nutritional protein stress on those animals on the 10% protein diet. Animals received 10g (approximately 123 kJ) a day of the relevant pellets, which provides sufficient energy for growth and reproduction (Perrin & Clarke 1987). Each parental pair was weighed (Ohaus Precision Advanced Balance, © Ohaus Corporation) at 3 day intervals throughout the experimental period so as to monitor their response to diet quality. In addition, parental females were weighed immediately after having given birth (*partum*). Only data from multiparous females was used. The second to the fourth litters were left with the parents until they were weaned on Day 21 *post partum*.

**Table 1:** Percentage composition (by weight) and nutritional content of experimental feed.

<b>Protein Diet</b>	<b>10%</b>	<b>15%</b>	<b>20%</b>
<b>Ingredients (%)</b>			
Maize meal	67	51	38
Wheaten bran	16	24	25
Lucerne	10	10	10
Fish meal	0	8	15
Sunflower oil cake	0	0	4
Feed lime	3	2.5	2
Monocalcium phosphate	2	0.6	0.8
Molasses	0.64	4	5
Synthetic lysine	0.4	0.3	0.4
Fine salt	0.25	0.25	0.3
Mineral Premix	0.75	0.75	0.75
<b>Nutritional Content<sup>a</sup></b>			
Protein	10.21	15	20
Fat	3.45	3.88	4.35
Fibre	6	6.58	7.35
Calcium	1.6	1.55	1.7
Phosphorus	0.7	0.7	0.95
Met-cys	0.37	0.53	0.71
Lysine	0.65	0.98	1.4
Treonine	0.35	0.55	0.73
Digestible Energy (kJ/g)	12.3	12.3	12.3

<sup>a</sup> Feed constitution and nutrient analyses were conducted at Irene Animal Nutrition and Products Institute.

## **Reproductive output**

As parental pairs were not separated during the experimental period, the intervals between litters could be calculated from the dates of successive litters of each experimental pair. On the day that any female gave birth, offspring were sexed and weighed and sequential litter number recorded. From this data, litter sex ratio, litter size and litter mass for each parental female on any treatment could be calculated.

## **Sex-specific pup growth**

Offspring from the second to the fourth litters were sexed and weighed at birth. Only two randomly selected males and two randomly selected females from each litter were left with the breeding pair to be raised, in order to control for the possible effect of litter size on pup growth rates from birth to weaning. These remaining offspring were then weighed every three days until Day 21 *post partum* (weaning day) in order to record the sex-specific growth rates of individuals raised by parents on diets of different quality. At 21 days *post partum*, weaned offspring were weighed and killed using Ethyl Ether (NT Laboratory Supplies {Pty} Ltd, South Africa), and kept at -40°C (Labex Low Temperature Freezer) for later analysis of body tissue composition.

## **Body tissue composition**

Offspring from the fifth and sixth litters were sexed, weighed and killed using Ethyl Ether on the day of birth, and kept at -40°C to be used for newborn body tissue analyses. For all of the

body tissue analyses of both newborn and weaned pups, carcasses were freeze-dried (Sigma MDC5E) and then ground finely (IKA Labortechnik A 10, Janke & Kunkel, Germany) and put into labelled poly-tops (NT Laboratory Supplies {Pty} Ltd, South Africa). From each litter, offspring were randomly allocated to each of the analyses (fat, protein, ash and moisture), so that at least 20 newborn and 20 weaned individuals of each sex from each of the treatment diets were assigned to a specific analysis. From the weaned carcasses, the same individuals were used for both fat and protein analyses so that the total energetic contents of pups could be determined. The energetic contents of newborn pups could not be determined as the individual carcasses were too small to be used for more than one analysis. Energetic values of weaned pups were estimated indirectly for the weaned samples from the proximate compositions, using caloric equivalents of 9.4kcal/g for lipid and 5.65kCal/g for protein (Pike & Brown 1975). The fat content of the dried body weight was determined using the Ether Extract Method (Method 24.005 in the Official AOAC 1980). The relative amount of crude protein (nitrogen x 6.25; Kerley & Erasmus 1991) in the newborn and weaned pups was determined using the Dumas Combustion method (Leco Africa, Pty Ltd) of nitrogen analysis. Samples were dried (Protea Laboratory Equipment, South Africa) at 100°C for 12 hours in order to determine their moisture content, and then burned (Nabertherm D-2804, Germany) for 24 hours at 550°C in a muffle furnace in order to calculate ash content. Fat, protein and ash contents are expressed as a percentage of pup dry weight.

### **Statistical analyses**

Litter size and litter mass (both total litter mass, and litter mass as a proportion of maternal *partum mass*) differences between diet treatment groups were analysed using two-way

analyses of variance, ANOVA (Sokal & Rohlf 1995), to detect possible effects of litter sequence (*i.e.* the second, third or fourth litter) and diet. A two-way ANOVA (diet and lactational status) was performed on the litter interval data. Lactational status was defined as whether a female was either lactating at the time of conception of the following litter, or not. In addition, the influence of sex ratio (recorded as male-biased, female-biased or non-biased litters) and diet on the subsequent litter interval was determined using a two-way ANOVA.

Two approaches were used to determine any treatment effect on sex ratio. The first was used to detect any overall deviation from unity within each treatment, and the second was used to detect whether there were equal numbers of female and male biased litters out of those in which one sex predominated. In both instances *G*-tests (log-likelihood test) with Williams's correction factor (Sokal & Rohlf 1995) were used for each treatment group.

Analysis of covariance, ANCOVA (Sokal & Rohlf 1995), was used to detect the influence of maternal dietary protein intake on the age of mothers at the time of their first litters (using maternal *partum mass* as the covariate). A similar ANCOVA was performed on the maternal *partum mass* data, with age being accounted for as the covariate.

To determine the influence of maternal diet and offspring sex on pup mass, two-way ANOVA's (diet and sex) were calculated for each age class (three day intervals from Day 0 to Day 21) within each treatment group. 57 randomly selected individuals of each sex in each treatment group were used for these analyses in all age classes. After these calculations, pups of each sex and in each treatment group were randomly selected, without replacement, for each age class to be used in weighted linear regression analyses in order to satisfy the assumption of independence. Weighted linear regression analyses were used to describe pup growth for each of the treatment diets (Kleinbaum *et al.* 1998) using SAS (SAS Institute Inc. 1989). *F*-tests were used to assess statistical differences between regression slopes.

Any differences between the relative proportions of fat, protein, ash and moisture for both newborn and weaned pups (and energy content for weaned pups) on the three dietary protein groups were determined for both males and females using two-way ANOVA's. Significant differences in energetic contents of weaned pups between treatment groups were tested, using body mass as the covariate, with an ANCOVA. In all circumstances, Tukey's Multiple Comparison Test was used to determine *post-hoc* differences in ANOVA and ANCOVA results. All mean values are followed by one standard error of the mean, unless otherwise stated.

## CHAPTER 3

### RESULTS

#### REPRODUCTIVE OUTPUT

Litter size was not affected by litter sequence ( $F_{[4,172]} = 0.45, P > 0.05$ ) therefore all litters were pooled to determine the influence of maternal dietary protein intake on litter size. No significant differences were observed (Table 2). Litter sequence also did not influence litter mass ( $F_{[4,174]} = 0.51, P > 0.05$ ). Litters produced by mothers on the optimal (15%) protein diet were significantly heavier than those produced by mothers on the low (10%) protein diet (Table 2). Similar results were obtained when each litter was divided by the maternal *partum mass* (Table 2), thus giving an indication of maternal investment into litter mass. Although litters born to mothers on the 20% diet were heavier than those born to mothers on the 10% diet, the differences were not significant (Table 2). Results from two-way ANOVA's show that neither diet ( $F_{[2,211]} = 0.56, P > 0.05$ ) nor lactational status ( $F_{[1,211]} = 1.17, P > 0.05$ ) significantly influenced the interval between successive litters. All litters (irrespective of lactational status) were pooled for further analysis (Table 2). Neither sex ratio, recorded as male-biased, female-biased or non-biased litters ( $F_{[2,135]} = 0.67, P > 0.05$ ) nor diet ( $F_{[2,135]} = 0.56, P > 0.05$ ) influenced the subsequent interval to the next litter (Table 3). When results were pooled for all three diets, there was still no significant influence of sex ratio on the interval to the following litter. However, the interval following male-biased litters was longer than that subsequent to female-biased and non-biased litters (Table 3).

**Table 2:** Litter variables (mean  $\pm$  standard error) at birth of *Mastomys natalensis* mothers on diets with different levels of protein. The values in brackets represent sample size. \* denotes significance at the 95% level, \*\* denotes significance at the 99% level. Values with the same superscripts in a row are not significantly different (Tukey's Multiple Comparison Test).

Litter Variable	Percentage Dietary Protein			F - value
	10	15	20	
Litter size	7.86 $\pm$ 0.26 (70)	8.51 $\pm$ 0.27 (61)	7.85 $\pm$ 0.29 (61)	$F_{[2,189]} = 1.86$
Litter mass (g)	15.89 $\pm$ 0.50 <sup>a</sup> (69)	18.47 $\pm$ 0.69 <sup>b</sup> (61)	18.11 $\pm$ 0.84 <sup>ab</sup> (61)	$F_{[2,188]} = 4.42^*$
Litter mass / Maternal mass	0.35 $\pm$ 0.01 <sup>a</sup> (69)	0.40 $\pm$ 0.01 <sup>b</sup> (59)	0.36 $\pm$ 0.01 <sup>ab</sup> (61)	$F_{[2,186]} = 5.69^{**}$
Litter interval (days)	32.52 $\pm$ 1.25 (83)	31.16 $\pm$ 1.56 (69)	33.22 $\pm$ 1.42 (61)	$F_{[2,214]} = 0.52$

**Table 3:** Litter intervals (mean  $\pm$  standard error), in days, subsequent to male-biased, female-biased and non-biased litters produced by *Mastomys natalensis* mothers on different dietary protein levels. The values in brackets represent sample size. \* denotes significance at the 95% level.

Diet	Litter Bias			<i>F</i> - value
	male-biased	female-biased	non-biased	
10%	32.9 $\pm$ 1.87 (25)	31.6 $\pm$ 2.75 (21)	29.6 $\pm$ 5.58 (9)	$F_{[2,52]} = 0.26$
15%	28.8 $\pm$ 2.14 (18)	31.2 $\pm$ 1.90 (22)	46.9 $\pm$ 4.45 (9)	$F_{[2,46]} = 0.36$
20%	33.0 $\pm$ 2.06 (28)	24.5 $\pm$ 3.74 (8)	71.2 $\pm$ 1.08 (4)	$F_{[2,37]} = 2.25$
Pooled	31.9 $\pm$ 1.18 (71)	24.5 $\pm$ 0.92 (131)	28.9 $\pm$ 2.82 (22)	$F_{[2,98]} = 2.07$

Overall sex ratio of offspring produced on the 10% diet was 51.5% males (283/550) and did not differ from unity (Table 4). Sex ratio of offspring produced on the 15% diet also did not deviate from unity (49.7% males; 257/517; Table 4). However 56.0% of pups produced on the 20% diet were males (268/479). This ratio deviated significantly from unity (Table 4). Among sexually unbalanced litters on 10%, 15% and 20% diets ( $n = 60, 49$  and  $52$ , respectively), 53.3%, 46.9% and 71.2% were biased toward males (Table 5). The sex ratio of pups produced on the 20% diet differed significantly from unity (Table 5), whilst there were no significant deviations for those on either the 10% or 15% diets (Table 5).

Mothers fed on the high protein diet were significantly older than mothers on low or optimal protein diets when they produced their first litters (Table 6). These mothers were also significantly heavier, when age was used as the covariate, than both the optimal and low protein mothers when they produced their first litters (Table 6). The age and mass of mothers at the time of their first litters were not significantly different for the low and optimal treatments (Table 6).

**Table 4:** Number of males and females produced per litter (mean  $\pm$  standard error) and sex ratio of offspring produced by *Mastomys natalensis* mothers on different dietary protein levels. The values in brackets represent sample size. \*\* denotes significance at the 99% level.

	Percentage Dietary Protein		
	10	15	20
Males	4.04 $\pm$ 0.20 (70)	4.25 $\pm$ 0.24 (61)	4.39 $\pm$ 0.21 (61)
Females	3.81 $\pm$ 0.23 (70)	4.26 $\pm$ 0.20 (61)	3.46 $\pm$ 0.21 (61)
Ratio M:F	283:267	257:260	268:211
$G_{adj}$ - value	0.47	0.02	6.79**

**Table 5:** Proportion of male-biased litters (as a percentage of total number of unbalanced litters) produced by *Mastomys natalensis* mothers on different dietary protein levels. \*\* denotes significance at the 99% level.

Number of litters	Percentage Dietary Protein		
	10%	15%	20%
< 50% M	28	26	15
> 50% M	32	23	37
% male-biased	53.33	46.94	71.15
$G_{adj}$ - value	0.26	0.18	9.52**

**Table 6:** Maternal attributes (mean  $\pm$  standard error) immediately following the birth of the first litters of *Mastomys natalensis* mothers on diets with different levels of protein. The values in brackets represent sample size. \*\* denotes significance at the 99% level; while \* denotes significance at the 95% level. Values with the same superscripts in a row are not significantly different (Tukey's Multiple Comparison Test).

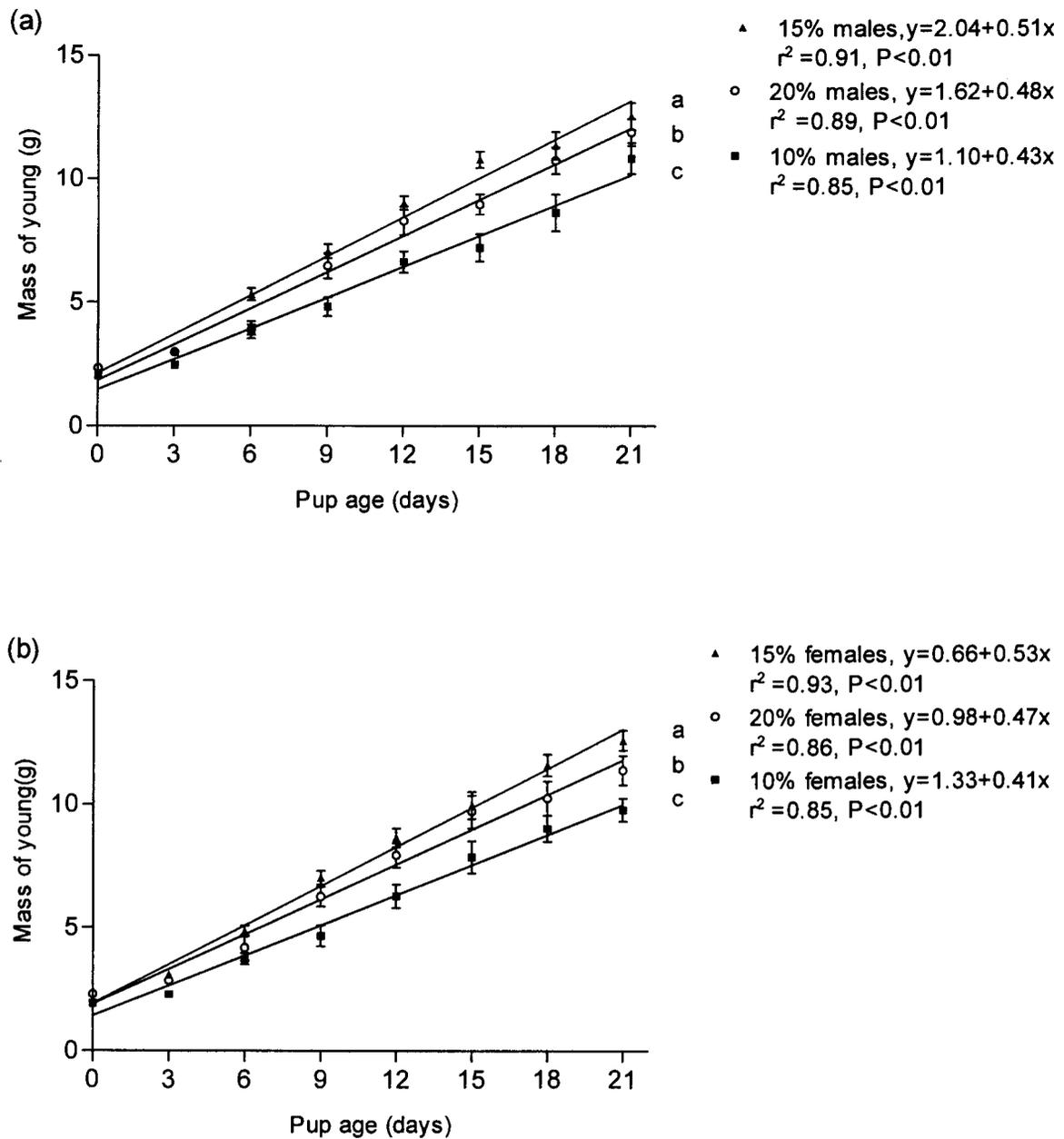
Maternal attribute	Percentage Dietary Protein			ANCOVA <i>F</i> - value
	10	15	20	
Age at birth of first litter (days)	114.4 $\pm$ 8.13 <sup>a</sup> (19)	120.2 $\pm$ 14.27 <sup>a</sup> (17)	209.1 $\pm$ 20.24 <sup>b</sup> (18)	$F_{2,50} = 7.03^{**}$
Maternal <i>partum</i> mass (g)	38.7 $\pm$ 1.20 <sup>a</sup> (19)	37.8 $\pm$ 1.52 <sup>a</sup> (17)	44.7 $\pm$ 0.99 <sup>b</sup> (61)	$F_{2,50} = 3.85^*$

## SEX-SPECIFIC PUP GROWTH

Results from the two-way ANOVA's (diet and sex) on pup mass show that sex-specific differences in pup mass only occur at weaning, Day 21 *post partum*, and only in the 20% protein group (Table 7). Pups born on the high (20%) protein treatment were significantly larger than those born on the low (10%) and optimal (15%) protein treatments only at birth (Table 7). With age, the pups in the optimal (15%) protein treatment group became the largest out of the treatment groups, with those on the 10% treatment group consistently being the smallest (Table 7). These findings are further demonstrated in the regression analyses (Fig. 1) where pup growth rates (slopes of linear regression lines) were highest on the optimal (15%) protein treatment, and lowest on the low (10%) protein treatment for both males (treatment differences:  $F_{[2,541]} = 34.59, P < 0.01$ ) and females (treatment differences:  $F_{[2,498]} = 36.33, P < 0.01$ ). Growth rates of male and female pups born to mothers on 10% (sex-specific differences:  $F_{[1,389]} = 0.004, P > 0.05$ ), 15% (sex-specific differences:  $F_{[1,329]} = 0.24, P > 0.05$ ) and 20% (sex-specific differences:  $F_{[1,329]} = 0.31, P > 0.05$ ) protein diets did not differ from each other.

**Table 7:** Two-way ANOVA results (diet effect and sex effect) of pup masses, in grams (mean  $\pm$  standard error) produced by *Mastomys natalensis* mothers on diets with different levels of protein. The sample size is 57 in each of the groups. \*\* denotes significance at the 99% level; while \* denotes significance at the 95% level. Values with the same superscripts in a row are not significantly different (Tukey's Multiple Comparison Test).

Age (days)	Pup masses (g) according to maternal dietary protein intake (%)						Two-way ANOVA results	
	10%		15%		20%		<i>F</i> -value (diet)	<i>F</i> -value (sex)
	Males (g)	Females (g)	Males (g)	Females (g)	Males (g)	Females (g)		
0	2.09 $\pm$ 0.04 <sup>a</sup>	2.08 $\pm$ 0.03 <sup>a</sup>	2.23 $\pm$ 0.03 <sup>b</sup>	2.26 $\pm$ 0.04 <sup>b</sup>	2.39 $\pm$ 0.04 <sup>c</sup>	2.28 $\pm$ 0.04 <sup>c</sup>	$F_{[2,336]} = 23.96^{**}$	$F_{[1,336]} = 1.05$
3	2.64 $\pm$ 0.07 <sup>a</sup>	2.69 $\pm$ 0.06 <sup>a</sup>	3.20 $\pm$ 0.06 <sup>b</sup>	3.21 $\pm$ 0.06 <sup>b</sup>	3.18 $\pm$ 0.08 <sup>b</sup>	3.02 $\pm$ 0.09 <sup>b</sup>	$F_{[2,336]} = 32.60^{**}$	$F_{[1,336]} = 0.27$
6	3.94 $\pm$ 0.11 <sup>a</sup>	3.99 $\pm$ 0.11 <sup>a</sup>	5.13 $\pm$ 0.12 <sup>b</sup>	5.14 $\pm$ 0.11 <sup>b</sup>	4.86 $\pm$ 0.17 <sup>c</sup>	4.75 $\pm$ 0.16 <sup>c</sup>	$F_{[2,336]} = 42.73^{**}$	$F_{[1,336]} = 0.02$
9	5.34 $\pm$ 0.15 <sup>a</sup>	5.32 $\pm$ 0.14 <sup>a</sup>	6.97 $\pm$ 0.15 <sup>b</sup>	6.98 $\pm$ 0.14 <sup>b</sup>	6.47 $\pm$ 0.22 <sup>c</sup>	6.44 $\pm$ 0.20 <sup>c</sup>	$F_{[2,336]} = 49.31^{**}$	$F_{[1,336]} = 0.01$
12	6.67 $\pm$ 0.19 <sup>a</sup>	6.66 $\pm$ 0.18 <sup>a</sup>	8.74 $\pm$ 0.19 <sup>b</sup>	8.76 $\pm$ 0.18 <sup>b</sup>	8.12 $\pm$ 0.24 <sup>c</sup>	8.04 $\pm$ 0.22 <sup>c</sup>	$F_{[2,336]} = 56.53^{**}$	$F_{[1,336]} = 0.02$
15	7.84 $\pm$ 0.23 <sup>a</sup>	7.78 $\pm$ 0.21 <sup>a</sup>	10.31 $\pm$ 0.21 <sup>b</sup>	10.27 $\pm$ 0.18 <sup>b</sup>	9.53 $\pm$ 0.25 <sup>c</sup>	9.39 $\pm$ 0.23 <sup>c</sup>	$F_{[2,336]} = 65.84^{**}$	$F_{[1,336]} = 0.19$
18	8.85 $\pm$ 0.26 <sup>a</sup>	8.79 $\pm$ 0.24 <sup>a</sup>	11.51 $\pm$ 0.23 <sup>b</sup>	11.40 $\pm$ 0.20 <sup>b</sup>	10.63 $\pm$ 0.26 <sup>c</sup>	10.34 $\pm$ 0.23 <sup>c</sup>	$F_{[2,336]} = 63.10^{**}$	$F_{[1,336]} = 0.65$
21	10.23 $\pm$ 0.25 <sup>a</sup>	9.99 $\pm$ 0.22 <sup>a</sup>	12.55 $\pm$ 0.23 <sup>b</sup>	12.21 $\pm$ 0.19 <sup>b</sup>	12.15 $\pm$ 0.25 <sup>b</sup>	11.57 $\pm$ 0.22 <sup>c</sup>	$F_{[2,336]} = 55.05^{**}$	$F_{[1,336]} = 4.38^*$



**Figure 1:** Growth rates for (a) male and (b) female offspring of *Mastomys natalensis* reared by females on diets with different protein contents. Means  $\pm$  standard errors of the means (based on individuals randomly selected, without replacement, for each age class) and linear regressions are shown. The slopes of lines with the same superscripts are not significantly different (*F*-test).

## BODY TISSUE COMPOSITION

Results from two-way ANOVA's (diet and sex) showed that there were no sex-specific differences in ash ( $F_{[1,112]} = 1.16, P > 0.05$ ), moisture ( $F_{[1,113]} = 0.60, P > 0.05$ ), fat ( $F_{[1,114]} = 1.14, P > 0.05$ ) and protein ( $F_{[1,114]} = 1.81, P > 0.05$ ) contents of newborn or weaned (ash:  $F_{[1,112]} = 0.42, P > 0.05$ , moisture:  $F_{[1,113]} = 0.51, P > 0.05$ , fat:  $F_{[1,114]} = 0.04, P > 0.05$ , protein:  $F_{[1,114]} = 0.48, P > 0.05$  and energy:  $F_{[1,106]} = 0.44, P > 0.05$ ) pups (see Appendix 1 and 2), thus the sexes were pooled for further diet-specific analyses. Moisture and fat contents of newborn pups did not differ between treatments (Table 8). Ash contents of pups born on the 10% protein diet were larger than those of pups on the 15% and 20% protein diets. At birth, pups born to mothers on the 15% protein diet had higher body protein contents than pups on the 10% and 20% protein treatment diets (Table 8).

At weaning pup ash, moisture and protein contents increased with increasing maternal dietary protein intake, while pup fat contents decreased significantly with increasing maternal protein intake (Table 8). Pups with the highest energetic values were born to the optimal (15%) protein treatment group, while pups with the lowest energetic values were born to the low (10%) protein treatment group (Table 8). Pups born to the optimal (15%) protein diet had the highest energetic values because they had relatively high fat and protein contents (and were also the largest of the weaned pups), while pups born to the high (20%) protein diet had low fat contents despite the large amounts of lean tissue (Table 8). Even when pup mass was taken into account, the energetic values of pups born to the medium (15%) protein diet were significantly greater than pups born to the high (20%) protein diet, which in turn were significantly greater than pups born to the low (10%) protein diet (ANCOVA:  $F_{[2,108]} = 98.65, P < 0.01$ ).

**Table 8:** Body tissue analyses (mean  $\pm$  standard error) of newborn and weaned pups (males and females pooled) produced by *Mastomys natalensis* mothers on diets with different levels of protein. The values in brackets represent sample size. \*\* denotes significance at the 99% level. Values with the same superscripts in a row are not significantly different (Tukey's Multiple Comparison Test).

Variable	Percentage Dietary Protein			F - value
	10	15	20	
<b>Newborn pups <sup>†</sup></b>				
Moisture (% wet body mass)	84.9 $\pm$ 0.27 (40)	84.7 $\pm$ 0.23 (39)	85.0 $\pm$ 0.26 (39)	$F_{[2,115]} = 0.48$
Ash (% dry body mass)	11.5 $\pm$ 0.16 <sup>a</sup> (40)	11.0 $\pm$ 0.13 <sup>b</sup> (39)	10.6 $\pm$ 0.15 <sup>b</sup> (39)	$F_{[2,115]} = 9.24^{**}$
Protein (% dry body mass)	65.4 $\pm$ 0.41 <sup>ab</sup> (40)	67.0 $\pm$ 0.65 <sup>b</sup> (40)	64.7 $\pm$ 0.47 <sup>a</sup> (40)	$F_{[2,117]} = 5.05^{**}$
Fat (% dry body mass)	14.0 $\pm$ 0.70 (40)	14.1 $\pm$ 0.73 (40)	13.5 $\pm$ 0.52 (40)	$F_{[2,117]} = 0.25$
<b>Weaned pups</b>				
Moisture (% wet body mass)	69.9 $\pm$ 0.17 <sup>a</sup> (40)	71.1 $\pm$ 0.35 <sup>b</sup> (40)	72.7 $\pm$ 0.23 <sup>c</sup> (39)	$F_{[2,116]} = 30.35^{**}$
Ash (% dry body mass)	10.1 $\pm$ 0.13 <sup>a</sup> (40)	10.9 $\pm$ 0.21 <sup>b</sup> (39)	11.9 $\pm$ 0.16 <sup>c</sup> (39)	$F_{[2,115]} = 28.85^{**}$
Protein (% dry body mass)	51.9 $\pm$ 0.63 <sup>a</sup> (40)	60.9 $\pm$ 0.96 <sup>b</sup> (40)	62.0 $\pm$ 0.62 <sup>b</sup> (40)	$F_{[2,117]} = 54.29^{**}$
Fat (% dry body mass)	30.7 $\pm$ 0.86 <sup>a</sup> (41)	25.8 $\pm$ 1.01 <sup>b</sup> (40)	20.7 $\pm$ 0.68 <sup>c</sup> (41)	$F_{[2,119]} = 34.66^{**}$
Energy (kJ / pup)	70.1 $\pm$ 2.74 <sup>a</sup> (32)	90.6 $\pm$ 1.94 <sup>b</sup> (40)	76.7 $\pm$ 1.94 <sup>a</sup> (40)	$F_{[2,109]} = 22.81^{**}$

<sup>†</sup> Newborn pup energetic contents could not be determined as individuals were too small to be used for more than one analysis.

## CHAPTER 4

### DISCUSSION

Protein intake affects body tissue composition and body size (Nakagawa & Masana 1971; Cameron & Eshelman 1996; Van Lunen & Cole 1996). In addition body size in polygynous species affects the sex of offspring (Anderson & Fedak 1987; Clutton-Brock *et al.* 1982; 1984; 1986; Austad & Sunquist 1986; Arnbom *et al.* 1994). Thus the present study was directed at inducing differences in maternal body size by manipulating dietary protein intake, and assessing the consequences thereof for reproductive variables. In accordance with Trivers and Willard (1973), large multi-mammate mouse mothers on the high protein diet differentially invested more in male than female offspring, not by allocating more resources to male pups as predicted by Maynard Smith (1980), but by producing more male-biased litters.

In addition, diet quality (including digestible protein content) influences reproductive ability and reproductive rate (Cameron & Eshelman 1996; Nakagawa & Masana 1971). Thus through the manipulation of dietary protein content we can contribute to our understanding of the dynamics of rodent population fluctuations in response to food quality, specifically digestible protein content, as opposed to just food availability.

#### REPRODUCTIVE OUTPUT

##### **Maternal mass and age at first litter**

In the present study maternal protein intake affected age at first conception, with females on a high (20%) protein diet breeding much later than those on lower (10% and 15%) protein

diets. It could be argued that because protein is essential in supporting adequate growth, especially in rapidly growing young organisms (White 1978), an excess would only enhance the benefits. However, protein intake in excess no longer become beneficial after a certain level (Edozien & Switzer 1978; Van Lunen & Cole 1996).

Research has shown that it is not just the size of the animal that influences the onset of puberty, but also the ratio of fatty to lean tissue (Frisch 1984). Low amounts of energy impair reproductive activity by the suppression of the luteinizing hormone - releasing hormone (LHRH) and luteinizing hormone pulse frequency necessary for ovarian follicles to grow to the preovulatory stage (Schillo 1992). Also, as dietary protein intake increases, increased amounts of energy are required to accompany the increased energy demands for protein synthesis (Campanile *et al.* 1998). Thus increasing dietary protein levels without simultaneously increasing energy intake results in an energy deficiency.

Rats fed on a high fat diet come into oestrus significantly earlier than those on an isocaloric, yet low fat diet (Frisch, Hegsted & Yoshinga 1975). Breeding activity in multi-mammate mice apparently correlates with the build-up of fat reserves and increased amounts of seeds in the diet (Field 1975). Seeds, containing large amounts of fat, are ingested and would therefore provide additional energy to support reproduction (Field 1975). Although I did not record the amount of food eaten by the experimental animals in this study, other experiments have shown that animals adjust their food intake according to the protein content of the diet (Edozien & Switzer 1978). For example, domestic pigs (Ferguson & Gous 1997) and rats (Sasaki *et al.* 1982) increase ingestion rates as dietary protein content decreases. This directly results in a higher body lipid content as the excess calories ingested are deposited as fat (Ferguson & Gous 1997). I suggest that those mothers on the low (10%) protein diet increased ingestion rates so as to maximise protein intake. This would result in them also ingesting

excess calories leading to a build up of fat stores. The high fat to lean tissue ratio which may have resulted apparently enabled them to come in to oestrus as early as those mothers on the optimal (15%) protein diet. However, further body tissue analyses of mothers on the different protein diets would confirm this.

To explain the delayed onset of reproduction in the mothers on the high (20%) protein diet in my experiment and in other studies with excess protein intake, a similar argument has been used. Pigs (Van Lunen & Cole 1996) and rats (Edozien & Switzer 1978) on high protein diets decrease their total food consumption, resulting in an overall decrease in energy intake and thus lessened fat deposition. In rats, as in the present study, dietary protein content has a positive influence on lean tissue deposition, but a negative effect on fat deposition (Coyer *et al.* 1987). In ruminants, high protein diets, or protein supplements actually stimulate body fat mobilization (Robinson 1996) thus delaying first oestrus (Sinclair, Broadbent & Hutchinson 1994).

In ruminants, excess dietary protein intake also results in an associated decrease in progesterone concentrations (Robinson 1996). Since progesterone is important in the maintenance of pregnancy (Heap 1982), the recorded delay in the age of first conception in females on the high protein diet could also have been because of insufficient concentrations of progesterone for the maintenance of pregnancy. Excess energy ingested is deposited as fat while protein in excess of the body's requirements is deaminated and excreted, resulting in a further loss of energy (Van Lunen & Cole 1996).

Mothers on the high (20%) protein diet were significantly larger at the time of their first litter, than those on the lower (10 & 15%) protein diets, suggesting that the amount of bulky lean tissue deposition may have increased with increasing dietary protein intake. In pigs (Van Lunen & Cole 1996) and rats (Edozien & Switzer 1978) lean tissue deposition increases with

increasing protein uptake, but only up to a certain limit. The existence of a species-specific optimal lipid to protein ratio (Van Lunen & Cole 1996) or an optimal energy to protein ratio for maximal growth and reproduction means that protein ingested in excess is wasted (Edozien & Switzer 1978).

### **Litter interval and litter size**

The multi-mammate mouse is an opportunistic, *r*-selected species (Willan & Meester 1989) and females appear to breed maximally. This is reflected in the relatively constant litter size and litter interval between the three treatment groups in the present experiment. The short interval (approximately 32 days) between litters is possible because of the existence of a *post partum* oestrus in the species (Johnston & Oliff 1954). Thus in most cases mothers were still lactating when they conceived their following litter, even when protein was at sub-optimal levels in the diet.

It has been argued that because male offspring are more costly to produce than females, mothers producing sons would have a longer interval before reproducing again than mothers that had produced daughters (Birgersson 1998). This would influence the future reproductive cost of the mother (Birgersson 1998). Polygynous species producing singletons like fallow deer, *Dama dama* (Birgersson 1998), and African elephants, *Loxodonta africana* (Lee & Moss 1986) do have a longer interval before reproducing again after having produced a son. This reduces future reproductive potential of those mothers having invested in more sons. In the present study, however, the frequency of reproduction (indicated by litter interval) was not impaired by the production of male-biased litters.

### **Sex-specific pup birth mass**

In multi-mammate mice litter size and the intervals between litters were not significantly influenced by maternal diet. Similar results were found in rats (Naismith & Morgan 1976; Sasaki *et al.* 1982) and common opossums, *Didelphis marsupialis* (Austad & Sunquist 1986), where protein supplementation did not affect the size of litters, but just pup size. In these trials, pup mass at birth increased significantly with increasing maternal dietary protein intake, although there were no sex-specific differences within each of the diets.

### **Sex ratio**

The lack of sex-specific differences in body mass of newborn pups on any of the maternal diets suggests that there was no differential investment in the sexes, at the level of the individual by multi-mammate mouse mothers. However, within overall litters, only mothers on the 20% protein diet displayed differential investment in male offspring, not by investing more into the individual pups, but by producing more males than females. These mothers on the high protein diet, producing predominantly male-biased litters, were larger than those on the lower protein diets (even when age was taken into account). Protein-supplemented common opossum mothers also produce male-biased litters, with offspring of both sexes being of similar masses (Austad & Sunquist 1986).

Polygynous species with large litter sizes present problems when considering the Trivers and Willard (1973) hypothesis, as mothers can potentially alter the litter interval, litter size, pup size and sex ratio simultaneously in order to maximise reproductive effort. However, there is no testable hypothesis which includes all of these variables (Krackow 1997). As there

was no apparent manipulation of litter interval and litter size according to diet, I shall discuss the observed differences in pup mass and litter sex ratio in response to maternal dietary protein intake in multi-mammate mice.

In order to apply Trivers & Willard's theory to data, three assumptions should ideally be satisfied. First, the condition of young at the end of the period of maternal investment should reflect the condition of the mother during investment. Second, the condition of offspring at the end of parental investment should endure into adulthood, and third, any slight advantages in offspring condition should assist male reproductive success more than females (Trivers & Willard 1973). In rats, the condition of young at the end of parental investment has been shown to reflect the condition of the mother during parental investment (Allen & Zeman 1971). In this experiment, pups born to mothers on low protein diets were significantly smaller than those born to mothers on the higher protein diets. Although pups born on the 15% protein treatment group were larger than those born to the 20% protein treatment group, at weaning the differences were not significant. This suggests that multi-mammate mouse pups, at the end of the period of parental investment, reflect the condition of their mothers during parental investment.

There is no evidence for multi-mammate mice showing that the condition of pups at the end of parental investment determines the condition at adulthood. However studies on rats have shown that the effects of poor maternal diet prenatally are irreversible and permanent (Allen & Zemen 1971), while impaired growth resulting from postnatal restrictions only are often compensated for when sufficient resources become available (Sikes 1996). Although northern grasshopper mice, *Onychomys leucogaster*, have been shown to display compensatory growth (Sikes 1996), in this study, energetic restrictions were imposed both pre- and postnatally ensuring that the negative consequences of an inadequate maternal diet to the offspring would

endure into adulthood, leaving little chance for any compensatory growth. Violations of Trivers and Willard's (1973) second assumption usually occur only when limitations on maternal resources are only imposed during postnatal care (Sasaki *et al.* 1982; Sikes 1996). If the dietary stresses are imposed prenatally, like in the present study, the assumption generally holds (Sasaki *et al.* 1982; Sikes 1996).

The third assumption, states that males would be differentially advantaged in reproductive success by slight advantages in condition. As males compete to inseminate females, any advantages in condition should be more beneficial to males than females (Trivers & Willard 1973). In multi-mammate mice, males compete with each other (Granjon & Duplantier 1993), thus any advantages accrued from being born to a mother in good condition would affect their reproductive success more than females. In wild house mice, *Mus musculus domesticus*, small differences in offspring body weight affected male fitness more than that of females (Krackow 1993).

If slight advantages in condition enhance the reproductive success of males more than females, then in contrast, adverse environmental conditions should negatively affect males more than females. In Senegal, although the sex ratio of multi-mammate mice was equal at birth, at adulthood populations were female-biased (Granjon & Duplantier 1993). Thus adult male multi -mammate mice have a lower survivorship than females (Granjon & Duplantier 1993; Leirs 1994) and would therefore suffer more from inadequate conditions. Further studies investigating whether males born to high protein treatment diets do indeed sire more offspring than males born to low protein treatment diets are required. This would provide insight into the assumption that the reproductive output of males born to high protein treatment diets is enhanced by the additional maternal dietary protein during the period of maternal investment.

## Possible sex ratio adjustment mechanisms

When considering a possible mechanism for the skewed sex ratio observed in the high protein group in the present study, sex-specific mortality *in utero* is unlikely as there was no decrease in litter size in this group. Clutton-Brock *et al.* (1984) also reported that the observed variation in sex ratio of red deer was unlikely to be due to differences in foetal mortality, as there was no corresponding change in calving rate between the dominant subordinate hinds. However, post-implantation manipulation of sex ratio cannot be totally ruled out in the present study as Hornig & McClintock (1994) found that in laboratory-kept Norway rats, *Rattus norvegicus*, both balanced and sex-biased litters had the same number of offspring at birth even though there were post-implantation losses of offspring in the biased litters. The observed sex ratio bias occurred when the uterine horns were crowded and pup loss inevitable. To benefit from the situation, mothers could selectively abort female offspring in order to maximise reproductive effort and produce more of the sex that would most likely produce the most grand-offspring (Hornig & McClintock 1994). In litters where all implanted embryos survived to birth (*i.e.* there were no additional placental scars), there were no biased sex ratios. However, litters with biased sex ratios (all were male-biased) had more placental scars than offspring produced, suggesting selective resorption of female embryos (Hornig & McClintock 1994). Foetal resorption in multi-mammate mice does occur, as the number of placental scars of reproducing females is often greater than the number of offspring produced (Duplantier, Granjon & Bouganaly 1996). Thus further studies on the selective resorption of embryos in multi-mammate mice would assist in discovering the mechanism for the observed skewed sex ratios observed in litters produced by mothers on the high (20%) protein diet. Wauters *et al.* (1995) showed that the male-biased litters produced by roe deer, *Capreolus capreolus*, mothers in

good condition were not because of differences between male and female juvenile mortality (because there were no additional placental scars to indicate embryo resorption) and suggested that the sex ratio adjustment occurred before implantation.

Nutritional state affects progesterone levels in ruminants, there being a decrease in progesterone concentrations with excess dietary protein intake (Robinson 1996). James (1992) suggested that at conception, high concentrations of testosterone and oestrogen lead to a male-biased sex ratio, while high concentrations of gonadotropin and progesterone result in significantly more female than male offspring (James 1992; 1996). Thus if excess dietary protein intake results in decreased levels of progesterone, this may lead to male-biased litters as recorded in the present study on multi-mammate mice.

## **PUP GROWTH FROM BIRTH TO WEANING**

### **Sex-specific pup growth**

Although it is expected that mothers of polygynous species differentially invest more in male than female offspring (Maynard Smith 1980), other studies show that, like the present study, there are no sex-specific differences in pup growth rates before weaning in northern elephant seals, *Mirounga angustirostris* (Kretzmann *et al.* 1993), hispid cotton rats, *Sigmodon hispidus* (Cameron & Eshelman 1996), Antarctic fur seals, *Arctocephalus gazella* (Lunn & Arnould 1997) and southern elephant seals, *Mirounga loenina* (McCann, Fedak & Harwood 1989; Campagna *et al.* 1992). However, in species such as grey seals, *Halichoerus grypus* (Kovacs & Lavigne 1986), Galapagos fur seals, *Arctocephalus galapagoensis* (Trillmich 1986), and African elephants, *Loxodonta africana* (Lee & Moss 1986), males are larger at birth and grow

faster than females.

Despite no sex-specific differences in mass or growth from birth to Day 18 *post partum*, at weaning (Day 21) males were significantly heavier than females in the high (20%) treatment group. In some polygynous species, like fallow deer, *Dama dama* (Birgersson, Tillbom & Ekvall 1998), pronghorn sheep, *Antilocapra americana* (Byers & Moodie 1990) and southern elephant seals (McCann *et al.* 1989) sex-specific differences in size only occur later in life, males and females being similar in size during the period of maternal investment.

### **Maternal diet and pup growth**

The nutritional content of the mothers diet has direct consequences for the offspring, as the nutrients ingested by the mother are passed directly in to the milk (Hammond & Diamond 1994). Cows on high protein diets (19% digestible protein) produce milk with accordingly higher protein contents (Komaragiri & Erdman 1997) as the nitrogen content of milk is determined by maternal protein intake (Naismith & Morgan 1976). Thus those pups raised by mothers on low protein diets were effectively on a low protein diet themselves, while those being nursed by mothers on the high protein diet were receiving surplus protein.

It is expected that pup growth would increase with increasing dietary protein, because protein ingestion favours lean tissue deposition (Coyer *et al.* 1987) and growth is often limited by protein intake (Cameron & Eshelman 1996). The growth rates of multi-mammate mouse pups on the high (20%) protein diet were actually slower than those on the medium (15%) protein diet. Physiologically, there is an upper limit (at about 25% dietary protein) to the positive effect of dietary protein in other species such as rats (Edozien & Switzer 1978), meadow voles (Shenk *et al.* 1970) and pigs (Van Lunen & Cole 1996). Excessively low and

excessively high amounts of protein intake result in a low response in growth to food intake (Van Lunen & Cole 1996). At low protein to energy ratios there is insufficient protein intake for nitrogen deposition, and at high protein to energy ratios the efficiency of tissue deposition is low due to the loss of energy for deamination and elimination of excess protein (Van Lunen & Cole 1996). In addition, the multi-mammate mouse mothers may have adjusted their food intake according to their diet, increasing ingestion rates at low dietary protein levels, and decreasing intake at high protein levels (Cameron & Eshelman 1996; Van Lunen & Cole 1996), thus directly influencing the resources available to the suckling pups.

It is more difficult for mothers storing energy as lean tissue to mobilise protein stores than it would be to mobilise fatty tissue (Komaragiri *et al.* 1998). Although pups born on the high protein treatment group were larger than pups born on the lower protein treatment groups, with time, they could not maintain fast growth rates (Table 7). Mothers on the high protein diet apparently could not mobilise sufficient energy to support the fast growth rates of their pups as their energetic demands increased during late lactation. As in hispid cotton rats (Cameron & Eshelman 1996), in the present study the growth rates of pups born to the high (15% and 20%) protein treatment groups were greater than those of pups born to the low (10%) protein treatment group. Pigs on low protein diets have slow growth rates because of their poor food conversion efficiency (Van Lunen & Cole 1996) as seen in the pups born to mothers in the low protein treatment group.

Although multi-mammate mouse pups born on the 15% protein diet were larger than pups born on the 20% protein diet at weaning, these differences were not significant. Similar results were found for rats fed on diets with 18% and 36% digestible protein contents, there being no significant differences in mass between rat pups on the two diets (Sasaki *et al.* 1982). These sets of results both support the suggestion of an upper limit to the positive effect of

increasing dietary protein levels on pup growth.

## **BODY TISSUE COMPOSITION**

### **Sex-specific body tissue composition at birth**

Sex-specific growth rates, or body size data are not ideal for making inferences on sexually biased investment because of the difficulty in separating the influences of the mother and the pup itself on pup growth (Birgersson *et al.* 1998). Faster growth rates in males during the period of maternal investment could be a result of a greater efficiency of energy conversion in males (Clutton-Brock, Albon & Guinness 1985; Ono & Boness 1996) or differential allocation of resources in males (Arnould *et al.* 1996) and not differential maternal investment.

The findings of Arnould *et al.* (1996) have emphasised the importance of quantitatively measuring the energetic investment of mothers into pups, and not making inferences from body size and growth data. However, in contrast to their findings and those of Van Lunen & Cole (1996) where female pigs deposited more fat than males, we find that multi-mammate mice do not show any sex-specific differences in body tissue composition at birth or at weaning. Other polygynous species like northern elephant seals, *Mirounga angustirostris* (Kretzmann *et al.* 1993) also show no sex-specific differences in body tissue composition of pups. Thus maternal investment, in terms of energy, is similar for the sexes irrespective of diet. In addition, it is more difficult for mothers of large litters to preferentially allocate resources to individuals of a particular sex than it is for mothers of singletons or small litters, like Antarctic fur seals (Arnould *et al.* 1996).

### **Maternal diet and body tissue composition at birth**

Maternal diet does not appear to influence the body composition of the multi-mammate mouse at birth. Protein metabolism during pregnancy is under hormonal rather than dietary control, ensuring that sufficient protein is available to support rapid foetal growth irrespective of maternal diet (Naismith & Morgan 1976). Protein metabolism during pregnancy is biphasic by nature, during the early stages of pregnancy (anabolic phase) the mother builds up a nitrogen reserve to be used in the final stages of pregnancy (catabolic phase) when the rapid growth of the foetus demands increased protein for build up of lean tissue. Maternal protein intake cannot inhibit the catabolic phase of protein metabolism during pregnancy (Naismith & Morgan 1976). Unlike multi-mammate mice, the protein composition of rat foetuses at birth was higher in pups born to mothers on protein supplemented diets during pregnancy (Naismith & Morgan 1976).

### **Maternal diet and sex-specific body tissue composition at weaning**

In the weaned pups, again there were no sex-specific differences in body tissue composition, suggesting that Maynard Smith's (1980) prediction that mothers invest more into individual male than female offspring, does not apply in the multi-mammate mouse. Results from the energetic contents (per animal) of weaned pups show that weanlings in the 10% and 20% protein treatment groups have similar energetic values, despite the significant differences in body masses. This further demonstrates the importance of using energy values in addition to body weights as a measure of investment. Proportionally more of the energy received by weanlings was deposited as fat in the low (10%) protein treatment group, while those in the

high (20%) protein treatment group deposited proportionally more lean tissue. Since fatty tissue has a higher energetic value per gram than protein (Pike & Brown 1975) pups of differing masses can still be energetically similar. Lean tissue deposition increases with increasing protein to energy ratios of food intake (Van Lunen & Cole 1996). Thus weaned pups on the 20% diet preferentially deposit lean tissue which is important for increased bulk and size, and would therefore be more beneficial to males who have to compete for mates. Although weaned pups in the 20% protein treatment group contained more lean tissue than those in the 15% protein treatment group, the differences were not significant. Maternal dietary protein intake is reflected in the milk of cattle ( Carlsson & Pehrson 1994). However, high levels of urea in blood and milk are not always used in the production of milk proteins (Campanile *et al.* 1998), indicating that excess dietary protein ingested by mothers is not necessarily transferred on to pups. Pups would thus benefit from high levels of maternal dietary protein, but would suffer if there is an excess, as this surplus would only be wasted.

### **Differential resource allocation according to available nutrients**

Antarctic fur seals with increased amounts of lean tissue enjoy increased reproductive success, suggesting that selection would favour rapid lean tissue deposition, especially in males McCann (1980). Multi-mammate mice born to high protein diets contained large amounts of lean tissue. In contrast, pups on the low protein diet preferentially deposit fatty tissue which is easily mobilised in times of nutritional stress (Komaragiri, Casper & Erdman 1998) and would thus enhance the chance of survival of these pups especially in times of low food quality. Survival is more important for animals born to a low protein diet, while in pups born to high protein diets increased competitive ability is advantageous. *Mastomys erythroleucus*

individuals accumulate lipids early on in the dry season, as these fats become an essential source of energy and “metabolic” water later on in the dry season when food and water are limiting (Hubert & Demarne 1981), thus emphasising the importance of survival strategies.

Fat deposition is also energetically more efficient than lean tissue deposition in domestic animals (Blaxter 1989). Physiologically, offspring in the low treatment group may have deposited proportionally more fat than those in the optimal (15%) and high (20%) treatment groups because of increased ingestion by their mothers in order to maximise protein intake. In this way surplus energy is ingested, which then stimulates lipogenesis (Allaye Chan-McLeod *et al.* 1994) and may have resulted in increased fat deposition of offspring. In addition a lower protein intake is also compensated for by increased efficiency of nitrogen recycling (Van Soest 1994). In calves (Donnelly & Hutton 1976) and white-tailed deer, *Odocoileus virginianus* (Holter & Hayes 1977), increased protein intake results in decreased fat deposition, as seen in the weaned pups in the high treatment group. In fact, as maternal dietary protein intake increased, so the fat composition of weaned offspring decreased, and with this there was a corresponding increase in the pup moisture content. At weaning those pups born to mothers on the 15% protein diet had significantly greater energetic contents than those born to mothers on the 20% protein diet because they had significantly greater percentages of fat and relatively equal proportions of lean tissue, resulting in them being both large and energy rich.

The relative contribution of moisture to body weight in weaned pups is less than that of newborns, while the fat composition is higher in weaned pups than newborns, on each of the diets. As offspring mature, the amount of body fat increases while the moisture content decreases (Spray & Widdowson 1950), as water in animal cells is replaced by fat (Worthy & Lavigne 1983). In weaned pups there appears to be an inverse relationship between body fat and moisture content with increasing maternal dietary protein intake. High moisture contents

indicate large amounts of lean tissue, as muscle contains significantly more water than fat (Whittlemore 1993), while increased amounts of fatty tissue results in decreased amounts of tissue moisture, as the fat displaces cell water. Increased protein ingestion influences intracellular osmotic concentration (Withers 1992) which could result in dehydration if additional water is not taken in. The additional uptake of water maintains homeostasis. This accounts for the greater moisture contents of those pups born to mothers on the high protein diet than those on the lower protein diets.

## CHAPTER 5

### CONCLUSION

#### **Optimal protein diets**

Increasing maternal protein intake beyond a particular level thus does not necessarily benefit multi-mammate mouse offspring. Although multi-mammate mouse pups were able to use the increased maternal protein intake for increased lean tissue deposition, the high maternal protein diet did not benefit the growth rate or weanling mass when compared to weanlings on the medium (optimal) protein diet. Weaned pups of mothers on the high (20%) protein diet had significantly lower amounts of energy than weaned pups born to medium (15%) protein diets. This suggests that either mothers on high protein diets were investing less energy into individual pups because of reduced food intake, or that the energetic transfer from mother to pup was inefficient because of their inability to convert excess protein ingested into usable energy without waste. Excessively high protein diets have also been shown to have negative effects in rats where the incidence of kidney tumours and lesions was greater in animals on high (36% digestible protein) protein diets (Sasaki *et al.* 1982). This also suggests that other implications of high protein diets may influence the health and perhaps reproductive potential of mothers.

The medium (15%) protein diet was chosen as the “control” diet in the present study as it contained the recommended amount of protein to support growth and reproduction in rodents (Richardson *et al.* 1964). Various aspects of the results of this study support the motivation that of all the diets chosen, the 15% protein diet was the optimal diet. The growth rate from birth to weaning of pups born to the 15% treatment group was the greatest, and the

body size (from Day 6 to Day 18 *post partum*) of male and female pups on the 15% protein diet was greater than that of those on the other treatment diets. The energetic contents of weaned pups in the 15% protein treatment group was significantly greater than that of pups in both the 10% and 20% protein treatment groups. As all of the maternal diets were isocaloric, the results suggest that the assimilation of energy was more efficient in pups born in the 15% protein treatment group. These results also suggest that mothers on the 15% protein diet were able to maximally invest in their offspring by optimally converting the ingested food into nutrients that could be readily used by the suckling pups. Mothers on the low (10%) protein diet did not receive sufficient amounts of protein to support the rapid growth of their pups from birth to weaning. Thus pups born to these mothers had slower growth rates, a smaller body size because of reduced lean tissue deposition, and low energetic contents.

### **Maternal dietary protein intake and sex ratio theory**

The size of multi-mammate mouse mothers was significantly influenced by the experimental manipulation of maternal dietary protein content. Mothers in the high (20%) protein treatment group were significantly larger than mothers in both the 10% and 15% protein treatment groups. These larger mothers produced significantly more male than female pups, these pups being significantly larger than those pups born to mothers on the low (10%) and medium (15%) protein diets at birth. This supports Trivers and Willard's (1973) hypothesis that in polygynous species larger mothers (on the high protein diet) should produce significantly more males. Also, since size (and thus lean tissue deposition) benefits males more than females, reproductive success in males being highly variable and dependant on body size, the high protein diet should also favour male-biased litters. Accordingly, the only treatment group showing sex-specific

differences in pup size was the high protein group, where males were significantly larger than females, but only at weaning. No other evidence for differential investment in the sexes was observed, thus suggesting that multi-mammate mice do not show any energetically biased maternal investment in individuals of either sex.

### **Implications for pest management**

The sex ratio skews observed in the present study also have implications for the management of multi-mammate mice. The multi-mammate mouse is an economically important pest species because of its frequent population explosions (Leirs 1994) which often result in massive loss of cultivated crops and stored grain products throughout Africa (Fielder 1988; Leirs 1994). Population outbreaks of multi-mammate mice in Africa (Leirs 1994) and wild house mice in Australia (Mutze 1991) often occur during the first rains, following a drought. During the dry period, preceding the first rains, nutrients (including nitrogen) from dead and decaying organic matter build up in the soil. At the onset of the rainy season the vegetative growth, rich in nutrients, and arthropods with high protein contents, are exploited by the mice. The favourable dietary conditions, and the absence of significant numbers of predators, enable rapid growth and reproduction of the mice. The present study has shown that a high protein (nitrogen) content of food skews the sex ratio towards males at birth. Since male multi-mammate mice have larger home range sizes than females (Christensen 1996), it is suggested that males are the primary dispersers. Thus more males produced at the beginning of the breeding season would enable the population to disperse more quickly, colonising new habitats and could account for the observed multi-directional mass dispersal during rodent outbreaks (Christensen 1996).

In addition, our results support the observations that multi-mammate mice are able to exploit inhospitable areas because of their ability to reproduce maximally even under poor (10%) dietary protein conditions, maternal energetic investment into pups being optimal at moderate (15%) dietary protein levels.

### **Motivation for further studies**

The findings of the present study have shown that multi-mammate mice are able to skew the sex ratio of offspring produced, in response to a high protein diet. However, from these findings, several further questions still have to be answered concerning the specific mechanism causing the sex ratio skew. The production of male-biased litters could be a result of the hormonal environment in the uterus at the time of conception (James 1992; 1996; Robinson 1996). A study measuring hormone (particularly progesterone) concentrations of mothers on the different protein diets at the time of fertilization should be undertaken to determine the influence of hormone levels, if any, on the sex ratio of offspring produced.

It is still not clear whether the biased sex ratios observed were as consequence of differential sex-specific mortality *in utero*. In order to clarify this, the number of placental scars should be compared to the number of newborn young in the first litters of mothers on the different protein diets. Although there were no resulting reductions in litter size in those litters producing skewed sex ratios in the present study, other studies have shown that post-implantation embryo mortality does not necessarily result in an alteration of the litter size (Hornig & McClintock 1994).

One of the assumptions of Trivers and Willard's (1973) hypothesis of sex ratio adjustment is that the condition of offspring at the end of parental investment should persist

into adulthood. In order to determine whether this indeed holds true for multi-mammate mice, a post-maternal investment study monitoring the growth and body tissue composition of offspring, produced by mothers on the different protein diets, could be carried out well on into the adulthood of the offspring.

It is assumed that male pups born on the high protein diet would be superior competitors to those males born on the lower protein diets because of their additional muscular tissue, and because their mothers actively selected for more male than female offspring. Thus a study could be undertaken, where several marked males born on the different protein diets are left in an enclosure with several females. DNA analyses of both parents and offspring could be used to determine the relationships between them. Those males siring more offspring should thus be the superior competitors with the highest reproductive output. It is expected that healthy males born to large mothers (on the high protein diet) would sire more offspring than males born to mothers on the lower protein diets, as any slight benefits of being born to a mother in good condition would enhance male reproductive success more than that of females. Also, in polygynous species where males compete for mates, stronger and healthier males should have the most reproductive opportunities (Clutton-Brock *et al.* 1988).

In the present study it was assumed that because mothers on the high (20%) protein diet were the largest at the time of breeding, they were the females of superior quality. However, in order to verify this, the body tissue composition of mothers on each of the diets could be analysed. Females of superior quality are likely to contain more energy than those of a lower quality. It would also determine whether mothers on the low (10%) protein diet contain more fatty than lean tissue, and mothers on the high (20%) protein diet contain more lean tissue than adipose tissue, as was assumed in this study.

## CHAPTER 6

### SYNTHESIS

The first aim of the present study was to determine whether maternal dietary protein content influenced the reproductive performance of the multi-mammate mouse. This pest species is known for its frequent population explosions throughout Africa (Fielder 1988; Leirs 1994) because of its ability to breed prolifically and its opportunist attributes. Control of their numbers has frequently been needed, particularly in developing African countries where food cannot afford to be lost to rodent plagues. Before any viable control of a pest species can be initiated, a better understanding of the response of breeding to environmental conditions, especially diet, is required. It appears that food quality, and not specifically energy, is limiting in the field (White 1978). It is also known that rodent population explosions often occur during the first rains that follow a drought (Leirs 1994). Since nutrients from dead and decaying plant and animal matter accumulate in the soil during the dry periods, the vegetative growth following the first rains is often rich in nutrients, particularly nitrogen (Mutze 1991). The present study thus used the experimental manipulation of digestible protein content of maternal diet to determine whether maternal protein intake influenced the reproductive output of the multi-mammate mouse.

The level of protein in the diet of multi-mammate mice does not influence the reproductive output *per se*, since the number of offspring per litter, and the interval between litters is not significantly affected by maternal dietary protein intake. However, results from the present study show that mothers ingesting high levels of protein produce significantly more male than female offspring. In multi-mammate mice, males are also the first to disperse from an area (Christensen 1996). Thus, at the onset of rains following a drought, when levels of

protein in the diet of rodents are high, multi-mammate mouse mothers would produce significantly more males than females. These males, being the primary dispersers, would increase the colonising ability of the population and would explain how quickly and widespread the population becomes during rodent outbreaks observed during such environmental conditions.

The second aim of the present study was to contribute to our understanding of current maternal investment theories by determining the influence of dietary protein intake on the reproductive investment of female multi-mammate mice to each sex. In polygynous species, where reproductive success varies more widely in males than females, and where parental investment influences the reproductive success of their offspring, mothers should invest in males when they are in good body condition, and in females when in poor body condition (Trivers & Willard 1973; Maynard Smith 1980). Maternal body size and body tissue composition are known to be influenced by dietary protein intake (Nakagawa & Masana 1971; Cameron & Eshelman 1996; Van Lunen & Cole 1996). Thus it was expected that multi-mammate mice would differentially invest in male and female offspring, according to their dietary protein intake, in order to maximise future reproductive success. Differential investment in the sexes could have been achieved either by diverting more resources to individual sons than daughters (Maynard Smith 1980) or by skewing the sex ratio at birth (Trivers & Willard 1973).

There is no evidence for differential investment in individual male and female multi-mammate mice according to maternal dietary protein intake. The theory of differential resource allocation is usually based on sex-specific differences in birth weights, growth rates, weaning weights, estimates of milk consumption and differences in the fecundity of females that previously raised more male versus female offspring (Byers & Moodie 1990). In multi-

mammate mice, the absence of sex-specific differences in birth weights and growth rates of pups born to each of the dietary protein groups suggest that mothers are unable to differentially invest in offspring according to their sex, irrespective of diet. Although male pups born to mothers on a high protein diet are larger than female pups at weaning, this provides insufficient evidence for additional resource allocation to male offspring. Pup mass and growth rate data also may not give an accurate indication of maternal investment, as differences between the sexes could be a consequence of differential allocation of resources to fatty and lean tissue by the sexes (Arnould, Boyd & Socha 1996; Lunn & Arnould 1997). In multi-mammate mice there are no sex-specific differences in the deposition of adipose and lean tissue of both newborn and weaned pups, despite maternal dietary protein intake. The energetic contents of weaned pups do not differ between males and females within each protein treatment group suggesting that Maynard Smith's (1980) prediction that mothers invest more energetically into male than female offspring, does not apply in the multi-mammate mouse. The fact that the future reproductive success (indicated by the interval to the following litter) is not impaired by the production of male-biased litters also demonstrates that investing in male offspring is no more costly to the mother than investing in female offspring in multi-mammate mice.

The differences in body tissue composition observed in weaned multi-mammate mice are a consequence of the resources available (maternal diet). Pups in the high protein treatment group contain large amounts of lean tissue, a direct consequence of the high protein diet ingested by their mothers. In multi-mammate mice significant amounts of lean tissue result in increased bulk and size, an advantage for males that have to compete for mates. Pups in the low protein treatment group preferentially deposit adipose tissue which is easily mobilised in times of nutritional stress (Komaragiri, Casper & Erdman 1998) enhancing their chance of survival.

Although the present study shows that multi-mammate mouse mothers do not divert

additional resources to individual male pups when in good condition (indicated by larger maternal size) on high protein diets, they still do invest more into males than females by producing predominantly male-biased litters as predicted by Trivers and Willard (1973).

In polygynous species, differential maternal investment is frequently achieved by the manipulation of the sex ratio of offspring produced (Clutton- Brock *et al.* 1982; 1984; 1986; Austad & Sunquist 1986; Anderson & Fedak 1987; Ambom *et al.* 1994). However, as yet there is no clear evidence indicating a mechanism for sex ratio adjustment. The male-biased sex ratio produced by multi-mammate mouse mothers on a high protein diet could be a result of differential mortality of female offspring *in utero*. However, as there is no change in the size of these biased litters, this suggestion is rejected. High concentrations of testosterone and oestrogen in mothers at conception can lead to male-biased sex ratios, while high concentrations of gonadotropin and progesterone result in significantly more female than male offspring (James 1992; 1996). Since progesterone concentrations decrease with excess dietary protein intake (Robinson 1996), the male-biased litters produced by mothers on the high protein diet in the present study may have been because of inadequate levels of progesterone at conception.

Although the results of the present study have provided additional evidence for maternal investment theory, the need for further investigations into the actual mechanism of sex ratio adjustment has been highlighted. The fact that dietary protein levels are known to influence specific hormone concentrations, and that the levels of these hormones are known to effect the sex ratio of offspring produced, suggest that an experiment where hormone levels are monitored in reproducing mice on different protein diets would be a good starting point for future research.

The findings of the present study have provided additional evidence for the observed

success of the multi-mammate mouse as a colonist species. Its ability to respond favourably to enhanced environmental conditions (represented in the present study by excess dietary protein contents) by producing more of the sex that disperse ensures that they colonise new areas more quickly than in a population with an equal sex ratio of offspring being produced. The present study has also verified some of the opportunistic attributes of the species, in that the reproductive output of females does not decrease even in unfavourable conditions (sub-optimal levels of dietary protein).

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## *SUMMARY*

The objectives of the present study were to determine the effects of maternal dietary protein content on sex-specific reproductive investment of the sexually-dimorphic, polygynous multi-mammate mouse, *Mastomys natalensis*. Female multi-mammate mice were fed one of three diets, namely a low (10%), medium (15%) or high (20%) protein diet.

The reproductive performance of the multi-mammate mouse mothers was assessed from litter interval, litter size and sex ratio data in each of the dietary treatment groups. The sex-specific mass and body tissue composition (protein, fat, ash and moisture contents) of pups born to mothers on the different protein diets was determined at birth and at weaning. Sex-specific pup growth rates from birth to weaning were also recorded for each of the dietary treatment groups.

Maternal dietary protein intake significantly influenced the age and mass of mothers when they produced their first litters. Mothers on the high protein treatment diet were significantly older and larger than those on the lower protein treatment diets.

Although the litter size was not affected by maternal dietary protein intake, litters produced by mothers on the medium (15%) protein diet were significantly heavier than those produced by mothers on the low (10%) and high (20%) protein diets. Maternal diet did not influence the interval between successive litters. In addition, neither lactational status (whether a mother was previously lactating, or not) nor litter sex ratio significantly influenced the subsequent interval to the next litter. These results suggest that in multi-mammate mice, mothers reproduce maximally irrespective of available resources.

The lack of sex-specific differences in pup birth mass suggest that there was no differential investment in the sexes at the level of the individual. However, mothers on the high

(20%) protein diet invested more into male than female offspring, not by diverting additional resources to males, but by producing more male than female pups. These mothers on the high protein diet were larger than those on the lower (10% and 15%) protein diets and produced predominantly male-biased litters. The sex ratio of pups produced by mothers on the medium and low protein diets did not deviate from unity. These results suggest that although maternal dietary protein intake significantly influences the sex ratio of offspring produced, deviations from unity only occur at high dietary protein levels.

There were no sex-specific differences in growth rates of pups from birth to weaning in all three dietary treatment groups. The only sex-specific differences in pup mass occurred at weaning (Day 21 *post partum*) and only in the high (20%) protein treatment group. This suggests that during the period of maternal investment mothers on the low and medium protein diets do not differentially allocate more resources to individuals of a particular sex.

Although pups born to mothers on the high protein diet were significantly larger than those born to mothers on the lower protein diets, these differences did not persist. In fact, with age, pups in the medium (15%) protein treatment group became the largest out of the treatment groups, with the fastest growth rates. Pups in the low (10%) protein treatment group were consistently smaller than pups in the medium (15%) and high (20%) protein treatment groups and had significantly slower growth rates.

In all of the dietary treatment groups there were no significant differences in the body tissue compositions of pups at birth or at weaning. These results confirm that multi-mammate mouse mothers do not preferentially divert resources to individual pups of a particular sex during maternal investment.

Maternal dietary protein intake did not significantly influence the body tissue composition of pups at birth. At weaning, pup ash, moisture and protein contents increased

with increasing maternal dietary protein intake, while pup fat contents decreased significantly. Pups in the medium (15%) protein treatment group had the highest energetic values because of the relatively high body fat and protein contents. Pups in the low (10%) and high (20%) protein treatment groups had similarly low energetic contents. Although pups in the high protein treatment group had large amounts of lean tissue, they had low amounts of fatty deposits, thus accounting for their low energetic contents. Pups in the low protein treatment group had proportionally high amounts of fatty tissue, but because of their low amounts of lean tissue and small body size their overall energetic values were low.

The results from the present study show that maternal dietary protein intake directly influenced the size of mothers at the time at which they produced their first litters, with mothers on a high protein diet being significantly larger than those on lower protein diets. These larger mothers produced significantly more male than female pups. In addition, the only treatment group showing sex-specific differences in pup size was the high protein group, where males were significantly larger than females, but only at weaning. Although mothers on high protein diets produced more male than female offspring, results from pup masses, growth rates and body tissue analyses provide no additional evidence of differential investment in the sexes.

Maternal dietary protein intake did not appear to influence the reproductive rate of multi-mammate mice, with mothers on the different protein diets producing approximately equal numbers of offspring, with similar intervals between litters. Maternal dietary protein intake did affect the mass and body tissue composition of pups, and would thus influence the future survival of offspring. Pups born to mothers on the medium protein diet were large, energy-rich and had the fastest growth rates. Pups in the low protein treatment group were small with little lean tissue deposition, but had proportionally large amounts of fatty tissue, enhancing the probability of them surviving in nutritionally-poor habitats. Pups in the high

protein treatment group were large and had significant amounts of lean tissue deposition, but relatively low fatty tissue deposits. The bulk and size of these pups is expected to enhance their competitive abilities, particularly in males.

The present study showed that female multi-mammate mice are able to differentially invest in offspring in response to dietary protein intake. This is achieved by producing significantly more male than female offspring, and not by preferentially allocating additional resources to males.

## OPSOMMING

Die doel van die huidige studie was om te bepaal wat die effek is van verskillende maternale proteïeninnames op die geslagsspesifieke belegging in nakomelinge. Wyfies van die geslagsdemorfiese, poliginiese Natalse vaalveldmuis, *Mastomys natalensis* is op een van drie verskillende dieettipes, naamlik 'n lae (10%), medium (15%) of 'n hoë (20%) proteïendieet geplaas.

Die voortplantingsukses van die Natalse vaalveldmuiswyfies is bereken vanaf werpsel interval-, werpselgrote- en geslagsverhoudingsdata versamel vir elk van die groepe onder verskillende dieetbehandelings. Die geslagsspesifieke massa- en liggaamweefselsamestelling (proteïen, vet, as en voginhoud) van die nakomelinge van moeders wat op verskillende proteïendieë geplaas is, is bepaal by geboorte en by spening. Geslagsspesifieke groeitempo's vanaf geboorte tot en met spening is ook bepaal vir elk van die groepe.

Proteïeninname het die ouderdom en massa van moeders ten tyde van die produsering van hul eerste werpsels betekenisvol beïnvloed. Moeders op die hoë proteïenbehandeling was betekenisvol ouer en swaarder as moeders op laer proteïenbehandelings.

Alhoewel die werpselgroottes nie beïnvloed is deur maternale proteïeninname nie, was die werpsels van moeders op die medium (15%) proteïendieet betekenisvol swaarder as die werpsels van moeders op die lae (10%) en hoë (20%) proteïendieet. Maternale dieet het geen invloed gehad op die intervalle tussen opeenvolgende werpsels nie. Daarbenewens het maternale lakteringsstatus (i.e. hetsy voorheen gelakteer al dan nie) en die geslagsverhouding van werpsels geen betekenisvolle invloed gehad op die interval tot daaropvolgende werpsels nie. Hierdie resultate dui daarop dat die Natalse vaalveldmuismoeders maksimaal voortplant ongeag van beskikbare bronne.

Die afwesigheid van geslagsspesifieke verskille in die geboortemassa van nakomelinge dui daarop dat daar nie 'n differensiele belegging in geslagte op individuele vlak is nie. Moeders op die hoë (20%) proteïendieet het egter meer in manlike nakomelinge belê, nie deur addisionele bronne aan manlike nakomelinge te spandeer nie, maar om meer manlike as vroulike nakomelinge te produseer. Moeders op die hoë (20%) proteïendieet was groter as moeders op die laer (10% en 15%) proteïendiete en het grotendeels 'n manlik-bevoordeelde nageslag geproduseer. Die geslagsverhouding van nakomelinge van moeders op die medium en lae proteïendieet het nie van 1:1 afgewyk nie. Hierdie resultate dui daarop dat alhoewel die proteïeninnome van moeders die geslagsverhoudings van nakomelinge betekenisvol kan beïnvloed, afwyking van 1:1 slegs by hoë proteïen innome geskied.

Daar was geen geslagsspesifieke verskille in die groeitempo's van nakomelinge vanaf geboorte tot spening in al drie dieetbehandelingsgroepe nie. Die enigste geslagsspesifieke verskil in nakomelingmassa het plaasgevind by spening (Dag 21 *post partum*) en slegs vir die hoë (20%) proteïenbehandelingsgroep. Dit dui daarop dat moeders op die lae en mediumdiëte gedurende die periode van moederlike belegging, bronne nie differensieel toewys aan individue van 'n spesifieke geslag nie.

Alhoewel nakomelinge van moeders op die hoë proteïendieet betekenisvol swaarder was as nakomelinge van moeders op die laer proteïendiete, was die verskille nie blywend nie. Intendeel, met toename in ouderdom het nakomelinge uit die medium (15%) proteïengroep die swaarste geword van die behandelingsgroepe en die vinnigste groeitempo gehad. Nakomelinge van die lae (10%) proteïenbehandeling was deurgans kleiner as nakomelinge van die medium (15%) en hoë (20%) proteïenbehandelingsgroepe en het betekenisvol laer groeitempo's gehad.

Daar was geen betekenisvolle verskille in die liggaamsweefselamestelling tussen

nakomelinge van die verskillende behandelingsgroepe by geboorte of spening nie. Hierdie resultate bevestig dat Natalse vaalveldmuismoeders geen voorkeur het vir individue van 'n spesifieke geslag met die toewysvan bronne gedurende moederlike belegging nie.

Proteïenname van moeders het nie die ligamsweweefsel samestelling van nakomelinge by geboorte beïnvloed nie. Die vog, as en proteïeninhoud van nakomeling by spening neem toe met toename in maternale proteïenname, terwyl die vetinhoud betekenisvol verlaag. Nakomelinge in die medium (15%) proteïenbehandelingsgroep het die hoogste energiewaarde as gevolg van die relatiewe hoë vet- en proteïeninhoud gehad. Nakomelinge van die lae (10%) en hoë (20%) proteïenbehandelingsgroepe het dieselfde lae energieinhoud gehad. Alhoewel nakomelinge in die hoë proteïenbehandeling baie maerweefsel gehad het, het hulle lae vetneerleggingswaardes gehad, wat die lae energieinhoud verklaar. Nakomelinge uit die lae proteïenbehandelingsgroep het proporsioneel meer vetweefsel gehad, maar as gevolg van die lae hoeveelheid spierweefsel en klein liggaamsgrootte was hul algehele energiewaarde laag.

Die resultate van die huidige studie illustreer dat maternale proteïenname die grootte van moeders by die geboorte van hul eerste werpsels direk beïnvloed, met moeders op 'n hoë proteïendieet betekenisvol swaarder as moeders op laer proteïendieëte. Hierdie groter moeders het betekenisvol meer manlik as vroulike nakomelinge geproduseer. Daarbenewens was die enigste behandelingsgroep wat geslagsspesifieke verskille in nakomelinggrootte aangetoon het die hoë proteïengroep, waar mannetjies groter as wyfies was en ook slegs by spening. Alhoewel moeders uit die hoë proteïengroep meer manlike as vroulike nakomelinge geproduseer het, het resultate van nakomelinggroottes, groeitempo's en ligamsweweefsel samestelling geen bykomstige bewyse gelewer dat moeders differensieel in geslagte belê nie.

Dit wil voorkom asof maternale proteïenname nie die voortplantingstempo van die Natalse vaalveldmuis beïnvloed nie. Moeders op verskillende proteïendieëte produseer ongeveer

dieselfde hoeveelheid nakomelinge met dieselfde intervale tussen werpsels. Maternale proteïenname het wel die massa en liggaamsweefselsamestelling van nakomelinge beïnvloed en sal derhalwe die toekomstige oorlewing van nakomelinge beïnvloed. Nakomelinge van moeders op die medium proteïendieet was groot en het die grootste energiewaardes en vinnigste groeitempo's gehad. Nakomelinge van moeders in die lae proteïengroep was klein met min spierweefselneerlegging, maar het proporsioneel groot hoeveelhede vetweefsel gehad wat hulle waarskynlikheid vir oorlewing in 'n voedingstofbeperkte omgewing verhoog. Nakomelinge in die hoë proteïengroep was groot en het betekenisvol meer neerlegging van spierweefsel gehad, maar relatief min vetneerlegging. Die grootte en massa van hierdie nakomelinge behoort, veral in mannetjies, hul kompeterende vermoëns te verhoog.

Die huidige studie het aangetoon dat Natalse vaalveldmuiswyfies differensieel in nakomelinge kan belê as 'n resultaat van verkillende maternale proteïendieetinname. Dit word bereik deur meer manlike as vroulike individue te produseer, en nie deur voorkeur te gee aan manlike individue met die toewys van bronne nie.

## APPENDIX 1

**Table 9:** Body tissue analyses (mean  $\pm$  standard error) of newborn and weaned male pups produced by *Mastomys natalensis* mothers on diets with different levels of protein. The values in brackets represent sample size. \*\* denotes significance at the 99% level. Values with the same superscripts in a row are not significantly different (Tukey's Multiple Comparison Test).

Variable	Percentage Dietary Protein			F - value
	10	15	20	
<b>Newborn pups <sup>†</sup></b>				
Moisture (% wet body mass)	84.6 $\pm$ 0.40 (20)	84.6 $\pm$ 0.27 (28)	85.1 $\pm$ 0.31 (22)	$F_{[2,67]} = 0.91$
Ash (% dry body mass)	11.0 $\pm$ 0.19 (20)	10.9 $\pm$ 0.17 (27)	10.7 $\pm$ 0.19 (22)	$F_{[2,66]} = 0.56$
Protein (% dry body mass)	65.0 $\pm$ 0.63 (21)	66.0 $\pm$ 0.91 (20)	65.0 $\pm$ 0.53 (20)	$F_{[2,58]} = 0.72$
Fat (% dry body mass)	13.3 $\pm$ 0.83 (20)	13.6 $\pm$ 1.01 (20)	13.4 $\pm$ 0.68 (21)	$F_{[2,58]} = 0.05$
<b>Weaned pups</b>				
Moisture (% wet body mass)	69.9 $\pm$ 0.25 <sup>a</sup> (20)	70.9 $\pm$ 0.51 <sup>b</sup> (20)	72.7 $\pm$ 0.35 <sup>c</sup> (20)	$F_{[2,57]} = 15.10^{**}$
Ash (% dry body mass)	9.8 $\pm$ 0.17 <sup>a</sup> (20)	11.0 $\pm$ 0.30 <sup>b</sup> (19)	11.9 $\pm$ 0.24 <sup>c</sup> (20)	$F_{[2,56]} = 18.91^{**}$
Protein (% dry body mass)	52.2 $\pm$ 0.81 <sup>a</sup> (20)	61.7 $\pm$ 1.33 <sup>b</sup> (20)	61.8 $\pm$ 0.91 <sup>b</sup> (20)	$F_{[2,57]} = 28.06^{**}$
Fat (% dry body mass)	30.5 $\pm$ 1.24 <sup>a</sup> (21)	25.5 $\pm$ 1.39 <sup>b</sup> (20)	21.0 $\pm$ 1.01 <sup>c</sup> (21)	$F_{[2,59]} = 15.20^{**}$
Energy (kJ / pup)	70.3 $\pm$ 4.05 <sup>a</sup> (16)	91.8 $\pm$ 3.01 <sup>b</sup> (20)	77.7 $\pm$ 3.03 <sup>a</sup> (20)	$F_{[2,53]} = 10.67^{**}$

<sup>†</sup> Newborn pup energetic contents could not be determined as individuals were too small to be used for more than one analysis.

## APPENDIX 2

**Table 10:** Body tissue analyses (mean  $\pm$  standard error) of newborn and weaned female pups produced by *Mastomys natalensis* mothers on diets with different levels of protein. The values in brackets represent sample size. \*\* denotes significance at the 99% level. Values with the same superscripts in a row are not significantly different (Tukey's Multiple Comparison Test).

Variable	Percentage Dietary Protein			<i>F</i> - value
	10	15	20	
<b>Newborn pups <sup>†</sup></b>				
Moisture (% wet body mass)	85.2 $\pm$ 0.37 (20)	84.9 $\pm$ 0.44 (11)	84.9 $\pm$ 0.45 (17)	<i>F</i> <sub>[2,45]</sub> = 0.31
Ash (% dry body mass)	11.9 $\pm$ 0.21 <sup>a</sup> (20)	11.0 $\pm$ 0.21 <sup>b</sup> (12)	10.3 $\pm$ 0.24 <sup>b</sup> (17)	<i>F</i> <sub>[2,46]</sub> = 13.82**
Protein (% dry body mass)	65.9 $\pm$ 0.51 <sup>ab</sup> (19)	68.0 $\pm$ 0.91 <sup>b</sup> (20)	64.5 $\pm$ 0.79 <sup>a</sup> (20)	<i>F</i> <sub>[2,56]</sub> = 5.48**
Fat (% dry body mass)	14.6 $\pm$ 1.12 (20)	14.6 $\pm$ 1.09 (20)	13.5 $\pm$ 0.82 (19)	<i>F</i> <sub>[2,56]</sub> = 0.36
<b>Weaned pups</b>				
Moisture (% wet body mass)	69.8 $\pm$ 0.24 <sup>a</sup> (20)	70.9 $\pm$ 0.51 <sup>a</sup> (20)	72.7 $\pm$ 0.35 <sup>b</sup> (20)	<i>F</i> <sub>[2,57]</sub> = 15.05**
Ash (% dry body mass)	10.3 $\pm$ 0.17 <sup>a</sup> (20)	10.8 $\pm$ 0.31 <sup>a</sup> (20)	11.9 $\pm$ 0.22 <sup>b</sup> (19)	<i>F</i> <sub>[2,56]</sub> = 11.22**
Protein (% dry body mass)	51.6 $\pm$ 0.98 <sup>a</sup> (20)	60.0 $\pm$ 1.39 <sup>b</sup> (20)	62.2 $\pm$ 0.87 <sup>b</sup> (20)	<i>F</i> <sub>[2,57]</sub> = 26.08**
Fat (% dry body mass)	30.9 $\pm$ 1.21 <sup>a</sup> (20)	26.2 $\pm$ 1.49 <sup>b</sup> (20)	20.4 $\pm$ 0.93 <sup>c</sup> (20)	<i>F</i> <sub>[2,57]</sub> = 18.52**
Energy (kJ / pup)	70.0 $\pm$ 3.81 <sup>a</sup> (16)	89.3 $\pm$ 2.48 <sup>b</sup> (20)	75.6 $\pm$ 2.47 <sup>a</sup> (20)	<i>F</i> <sub>[2,53]</sub> = 11.89**

<sup>†</sup> Newborn pup energetic contents could not be determined as individuals were too small to be used for more than one analysis.