

Maternal dietary protein intake and sex-specific investment in *Mastomys coucha* (Rodentia: Muridae)

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Abstract

We examined data on captive multi-mammate mice (*Mastomys coucha*) to assess differential maternal investment, and sex-specific resource allocation. Differences in maternal size were induced through manipulation of dietary protein in three treatment groups: low (10%), medium (15%) and high (20%) protein diets. Mothers on the 20% protein diet were significantly larger than those on the lower protein diets, and produced significantly more male than female offspring. Mothers on the lower protein diets did not produce sex-biased litters. 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 reserves of all of the treatment groups. Our 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.

Key words: maternal investment, sex ratio, body tissue composition, pup growth, *Mastomys coucha*

INTRODUCTION

Sex-biased parental investment has been of great interest to evolutionary biologists. In 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 females when in poor body condition (Trivers & Willard, 1973; Maynard Smith, 1980). This 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). 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. However, the predictions of differential investment in the sexes do not hold for Antarctic fur seals *Arctocephalus gazella* (Arnould,

Boyd & Socha, 1996) or northern elephant seals *Mirounga angustirostris* (Kretzmann, Costa & Le Boeuf, 1993).

Males of polygynous species are generally larger than females at birth, and have faster growth rates during maternal investment. Several scientists interpreted this as proof that males are more costly to produce and raise than females (Kovacs & Lavigne, 1986; Trillmich, 1986; Anderson & Fedak, 1987; Boyd & McCann, 1989). 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 in her offspring. In fact, they showed that although male Antarctic fur seal 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). In polygynous males, size is thought to determine reproductive success, while female 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, and females would be expected to deposit fatty tissue to be used during energetically stressful periods (gestation and lactation).

Considering that maternal size may affect the sex

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ratio of offspring produced, we aimed to manipulate maternal body size of the sexually dimorphic polygamous multi-mammate mouse *Mastomys coucha* 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 the closely related species *M. natalensis*, males are larger than females (Granjon & Duplantier, 1993; Leirs, 1994). Since males are competitive (Granjon & Duplantier, 1993), stronger, larger males should have greater reproductive opportunities (see Clutton-Brock *et al.*, 1988). Polygynous females have a relatively equal (and limited) number of reproductive opportunities, irrespective of size or fitness. Mothers maintained on a high nutritional plane should be larger than those on a lower nutritional plane and we expected that the former group may produce more male than female offspring.

This paper addresses the influence of dietary protein intake on the relative reproductive investment of *M. coucha* mothers to each sex, and thus contributes to our understanding of current maternal investment theories.

MATERIALS AND METHODS

Thirty breeding pairs of multi-mammate mice were maintained in the experimental holding facilities (25 °C, 14L:10D) of the University of Pretoria. All holding and handling procedures were sanctioned by the ethical committee of the Faculty of Biological and Agricultural Sciences of the University. Mouse pellets (Epol Animal Feed Manufacturers, 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), and fed mouse pellets *ad libitum* until day 50. As the earliest recorded first oestrus cycle for *M. coucha* is at 54 days (pers. obs.), these offspring were then randomly paired (not with siblings) and assigned to 1 of the experimental groups (high, medium or low protein diets) at the age of 50 days. Twenty breeding pairs were established on each of the treatment groups.

The experimental diets (made up by the Animal Nutrition and Animal Products Institute, Irene, South Africa) were designed so that all nutritional components, except digestible protein content, which varied according to treatment level, remained constant (Table 1). The treatments had an optimal digestible protein content of 15% (medium), 10% (low) and 20% (high). Levels were selected 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 10 g (*c.* 123 kJ) of the relevant diet each day, and all diets provided sufficient energy for growth and reproduction (Perrin & Clarke, 1987). Each parental pair was weighed (Ohaus Precision Advanced Balance, 8 Ohaus Corporation) at 3-day intervals throughout the

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

	Protein diet		
	10%	15%	20%
Ingredients			
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	3.0	2.5	2.0
Monocalcium phosphate	2.0	0.6	0.8
Molasses	0.64	4.00	5.00
Synthetic lysine	0.4	0.3	0.4
Fine salt	0.25	0.25	0.30
Mineral Premix	0.75	0.75	0.75
Nutritional content^a			
Protein	10.21	15.00	20.00
Fat	3.45	3.88	4.35
Fibre	6.00	6.58	7.35
Calcium	1.60	1.55	1.70
Phosphorus	0.70	0.70	0.95
Met-cys	0.37	0.53	0.71
Lysine	0.65	0.98	1.40
Treonine	0.35	0.55	0.73
Digestible energy (kJ/g)	12.3	12.3	12.3

^a Feed constitution and nutrient analyses were conducted at Irene Animal Nutrition and Products Institute.

study to monitor their response to diet quality. In addition, parental females were weighed at *partum*. Only data from multi-parous females were used and the second to the fourth litters were left with the parents until they were weaned on day 21 *post partum*.

Reproductive output

As parental pairs were not separated during the study, 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. From these 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 2 males and 2 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 3 days until day 21 *post partum* (weaning day) to record the sex-specific growth rates of individuals raised by parents on diets of different quality. At 21 days, weaned offspring were weighed and then 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 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). From each litter, offspring were randomly allocated to each of the analyses (fat, protein, ash and moisture). Energetic values of weaned pups were estimated indirectly for the weaned samples from the proximate compositions, using caloric equivalents of 9.4 kcal/g for lipid and 5.65 kcal/g for protein (Pike & Brown, 1975). The fat content of the dried body weight was determined using the ether extraction method (Horwitz, 1980: method 24.005). The relative amount of crude protein (nitrogen $\times 6.25$; 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 h to determine their moisture content, and then burned (Nabertherm D-2804, Germany) for 24 h at 550°C in a muffle furnace to calculate ash content. Fat, protein and ash contents are expressed as a percentage of pup dry weight.

Statistical analyses

The effect of treatment diet on both litter size and litter mass (as a proportion of maternal *partum* mass) was determined using 2-way analyses of variance with repeated measurements (ANOVAR) (Sokal & Rohlf, 1995) to account for the successive litters produced by each breeding mother. An ANOVAR was used to determine the influence of diet on litter interval.

Two approaches were used to determine 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 male and female biased litters out of those in which 1 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 birth 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 2-way ANOVARS (diet and sex) were calculated for each age class (3 day intervals from day 0 to day 21) within each treatment group.

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 3 dietary protein groups were determined for both males and females using 2-way ANOVARS. In all circumstances, Tukey's multiple comparison test was used to determine *post hoc* differences in ANOVAR results. All mean values are followed by 1 standard error of the mean.

RESULTS

Reproductive output

Maternal dietary protein intake did not significantly influence litter size (Table 2). Litter mass was not significantly affected by maternal dietary protein intake (Table 2). However, when each litter mass was divided by the maternal *partum* mass, mothers on the 15% dietary treatment produced significantly heavier litters for their body size than those on the 10% treatment group (Table 2). These results suggest that mothers on the 15% protein diet invested proportionately more into litter mass. Litter mass on the 20% treatment group did not differ significantly from either of the other treatment groups. Diet did not significantly influence the interval between successive litters (Table 2).

Table 2. Litter variables (mean \pm SE) at birth of *Mastomys coucha* mothers on diets with differing levels of protein. Values in parentheses, sample size. * = $P < 0.05$. Values with the same superscripts in a row are not significantly different (Tukey's multiple comparison test)

Litter variable	Percentage dietary protein			ANOVAR <i>F</i> -value
	10	15	20	
Litter size	7.96 \pm 0.37 (17)	8.16 \pm 0.42 (16)	7.56 \pm 0.47 (17)	$F_{(2,22)} = 2.46$
Litter mass (g)	15.72 \pm 0.80 (19)	17.47 \pm 1.35 (14)	17.24 \pm 1.43 (17)	$F_{(2,21)} = 2.70$
Litter mass/maternal mass	0.34 \pm 0.01 ^a (18)	0.38 \pm 0.09 ^b (15)	0.35 \pm 0.09 ^{ab} (17)	$F_{(2,20)} = 3.57^*$
Litter interval (days)	32.40 \pm 2.10 (16)	35.74 \pm 5.33 (16)	40.42 \pm 4.46 (17)	$F_{(2,24)} = 0.61$

Table 3. Two-way ANOVAR results (diet effect and sex effect) of pup masses, in grams (mean \pm SE) produced by *Mastomys coucha* mothers on diets with differing levels of protein. Sample size, 15 in each of the groups. * $P < 0.05$, ** $P < 0.01$. 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 ANOVAR <i>F</i> -value	
	10		15		20		Diet	Sex
	Males (g)	Females (g)	Males (g)	Females (g)	Males (g)	Females (g)		
0	2.02 \pm 0.07 ^{a,b}	1.99 \pm 0.06 ^a	2.30 \pm 0.06 ^{b,c}	2.24 \pm 0.06 ^{b,c}	2.39 \pm 0.05 ^c	2.30 \pm 0.06 ^{b,c}	$F_{(2,35)} = 7.47^{**}$	$F_{(1,35)} = 1.68$
3	2.68 \pm 0.13 ^a	2.60 \pm 0.09 ^a	3.23 \pm 0.07 ^b	3.09 \pm 0.09 ^b	3.11 \pm 0.14 ^b	2.86 \pm 0.58 ^b	$F_{(2,35)} = 12.39^{**}$	$F_{(1,35)} = 0.31$
6	3.76 \pm 0.19 ^{ac}	3.88 \pm 0.20 ^a	4.98 \pm 0.70 ^b	5.00 \pm 0.20 ^b	4.50 \pm 0.30 ^b	4.34 \pm 0.28 ^{b,c}	$F_{(2,35)} = 8.61^{**}$	$F_{(1,35)} = 0.49$
9	5.20 \pm 0.28 ^{a,c}	5.25 \pm 0.30 ^a	6.68 \pm 0.31 ^b	6.71 \pm 0.27 ^b	6.19 \pm 0.42 ^b	5.91 \pm 0.34 ^{b,c}	$F_{(2,35)} = 8.00^{**}$	$F_{(1,35)} = 0.24$
12	6.55 \pm 0.37 ^{a,b}	6.48 \pm 0.35 ^b	8.57 \pm 0.35 ^c	8.51 \pm 0.37 ^c	7.75 \pm 0.47 ^c	7.41 \pm 0.38 ^{a,c}	$F_{(2,35)} = 9.06^{**}$	$F_{(1,35)} = 0.29$
15	7.66 \pm 0.47 ^{a,c}	7.66 \pm 0.45 ^a	10.06 \pm 0.40 ^{b,d}	9.99 \pm 0.38 ^{b,d}	9.18 \pm 0.46 ^{b,d}	8.86 \pm 0.43 ^{b,c,d}	$F_{(2,35)} = 9.54^{**}$	$F_{(1,35)} = 0.40$
18	8.96 \pm 0.56 ^a	8.67 \pm 0.65 ^a	11.31 \pm 0.40 ^b	11.12 \pm 0.41 ^b	10.32 \pm 0.50 ^b	9.75 \pm 0.39 ^{a,b}	$F_{(2,35)} = 9.33^{**}$	$F_{(1,35)} = 0.76$
21	10.00 \pm 0.55 ^a	9.92 \pm 0.47 ^a	12.37 \pm 0.38 ^b	12.05 \pm 0.38 ^b	11.92 \pm 0.45 ^b	11.22 \pm 0.36 ^{a,b}	$F_{(2,35)} = 7.97^{**}$	$F_{(1,35)} = 5.40$

Overall sex ratio of offspring produced on the 10% diet was 51.5% males (283/550) and did not differ from unity ($G = 0.47$, d.f. = 1, $P > 0.05$). Sex ratio of offspring produced on the 15% diet also did not deviate from unity (49.7% males; 257/517; $G = 0.02$, d.f. = 1, $P > 0.05$). However 56.0% of pups produced on the 20% diet were males (268/479). This ratio deviated significantly from unity ($G = 6.79$, d.f. = 1, $P < 0.01$). 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. The proportion of male-biased litters on the 20% diet differed significantly from unity ($G = 9.52$, d.f. = 1, $P < 0.01$), whilst there were no significant deviations for those on either the 10% ($G = 0.26$, d.f. = 1, $P > 0.05$) or 15% ($G = 0.18$, d.f. = 1, $P > 0.05$) diets.

Mothers fed on the 20% protein diet were significantly older (209 ± 20.2 days, $n = 18$) than mothers on 10% (114 ± 4.1 days, $n = 19$) or 15% (120 ± 14.3 days, $n = 17$) protein diets when they produced their first litters (ANCOVA: $F_{(2,50)} = 7.03$, $P < 0.01$). These mothers were also significantly heavier (44.7 ± 1.0 g, $n = 18$), when age was used as the covariate, than both the 15% (37.8 ± 1.5 g, $n = 17$) and 10% (38.7 ± 1.2 g, $n = 19$) protein mothers when they produced their first litters (ANCOVA: $F_{(2,50)} = 3.85$, $P < 0.05$). The age and mass of mothers at the time of their first litters were not significantly different for the 10% and 15% treatments.

Sex-specific pup growth

Results from the two-way ANOVARs (diet and sex) on pup mass show that there are no sex-specific differences in pup mass from birth to weaning in any of the dietary treatment groups (Table 3). Pups born on the 20% protein treatment were significantly larger than those born on the 10% protein treatment for most of the period of maternal investment (Table 3). Although pups in the 20% treatment group were larger than those in the 15% treatment group at birth, with age, the pups in the 15% protein treatment group became the largest out of all of the treatment groups, with those on the 10%

treatment group consistently being the smallest (Table 3).

Body tissue composition

Results from two-way ANOVARs (diet and sex) showed that there were no sex-specific differences in ash ($F_{(1,40)} = 0.10$, $P > 0.05$), moisture ($F_{(1,40)} = 0.09$, $P > 0.05$), fat ($F_{(1,43)} = 1.37$, $P > 0.05$) and protein ($F_{(1,43)} = 0.21$, $P > 0.05$) contents of newborn or weaned (ash: $F_{(1,38)} = 0.25$, $P > 0.05$, moisture: $F_{(1,38)} = 0.39$, $P > 0.05$, fat: $F_{(1,38)} = 0.01$, $P > 0.05$, protein: $F_{(1,43)} = 0.04$, $P > 0.05$ and energy: $F_{(1,33)} = 0.26$, $P > 0.05$) pups, thus the sexes were pooled for further diet-specific analyses. Moisture, protein and fat contents of newborn pups, as a proportion of body mass, did not differ between treatments (Table 4). However, ash contents of pups born on the 10% protein diet were greater than those of pups on 20% protein diet (Table 4).

At weaning, ash, moisture and protein content of pups, as a proportion of body mass, increased with increasing maternal dietary protein intake, while the fat content of pups decreased significantly with increasing maternal protein intake (Table 4). Pups from mothers on the 15% protein treatment group were born with the highest energetic values, while pups from mothers on the 10% protein diet were born with the lowest energetic values (Table 4). Pups in the 15% protein treatment group had the highest energetic values because they had relatively high fat and protein contents as well as being the largest of the weaned pups, while pups from mothers on the 20% protein diet had low fat contents (Table 4).

DISCUSSION

Protein intake affects body tissue composition and body size (Nakagawa & Masana, 1971; Cameron & Eshelman, 1996; Van Lunen & Cole, 1996), and body size in polygynous species affects the sex of offspring (Clutton-Brock *et al.*, 1982; 1984; 1986; Austad & Sunquist, 1986;

Table 4. Body tissue analyses (mean \pm standard error) of newborn and weaned pups produced by *Mastomys coucha* mothers on diets with differing levels of protein. The values in brackets represent sample size. * $P < 0.05$, ** $P < 0.01$. Values with the same superscripts in a row are not significantly different (Tukey's multiple comparison test)

Variable	Percentage dietary protein			ANOVAR F -value
	10	15	20	
Newborn pups				
Moisture (% wet body mass)	84.75 \pm 0.41 (18)	84.63 \pm 0.29 (21)	85.09 \pm 0.30 (18)	$F_{(2,43)} = 0.91$
Ash (% dry body mass)	11.25 \pm 0.21 ^a (17)	10.94 \pm 0.19 ^{a,b} (21)	10.72 \pm 0.16 ^b (19)	$F_{(2,43)} = 3.19^*$
Protein (% dry body mass)	65.20 \pm 0.11 (20)	65.21 \pm 0.29 (15)	65.08 \pm 0.64 (22)	$F_{(2,46)} = 2.17$
Fat (% dry body mass)	13.90 \pm 1.06 (19)	13.94 \pm 0.90 (19)	13.37 \pm 0.71 (17)	$F_{(2,46)} = 0.17$
Weaned pups				
Moisture (% wet body mass)	69.88 \pm 0.24 ^a (22)	70.83 \pm 0.55 ^b (21)	72.69 \pm 0.38 ^c (19)	$F_{(2,41)} = 17.20^{**}$
Ash (% dry body mass)	10.18 \pm 0.16 ^a (21)	10.58 \pm 0.35 ^b (21)	11.89 \pm 0.23 ^c (20)	$F_{(2,41)} = 11.33^{**}$
Protein (% dry body mass)	52.22 \pm 0.73 ^a (23)	61.08 \pm 1.09 ^b (24)	61.84 \pm 0.74 ^b (24)	$F_{(2,46)} = 32.97^{**}$
Fat (% dry body mass)	30.21 \pm 1.10 ^a (22)	25.63 \pm 1.29 ^b (23)	21.95 \pm 0.86 ^c (21)	$F_{(2,41)} = 15.70^{**}$
Energy (kJ/pup)	68.35 \pm 3.30 ^a (20)	90.62 \pm 2.29 ^b (24)	76.70 \pm 2.36 ^a (22)	$F_{(2,36)} = 9.37^{**}$

Anderson & Fedak, 1987; 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 the respective reproductive variables.

Reproductive output

As there were no sex-specific differences in body mass of newborn pups on any of the maternal diets, we suggest that there was no differential investment in the sexes, at the level of the individual. However, in accordance with Trivers & Willard (1973), large multimammate mouse mothers on the high (20%) 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. 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 *Didelphis marsupialis* mothers also produce male-biased litters, with offspring of both sexes being of similar masses (Austad & Sunquist, 1986).

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, Nakagawa & Kajimoto, 1982) and common opossums (Austad & Sunquist, 1986), where protein supplementation did not affect the size of litters, but just pup size. In our trials, pup mass at birth increased with increasing maternal

dietary protein intake, although there were no sex-specific differences within each of the diets.

Although there have been numerous findings both supporting and opposing Trivers & Willard's (1973) theory of sex ratio adjustment, there has been no unequivocal evidence for a mechanism to explain the observed trends. However, several mechanisms have been proposed (see Krackow, 1995 for a review), which can come into effect at or after conception. The first, and most conventional argument, concerns a post-conception adjustment of the sex ratio by sex-specific foetal absorption or differential mortality of embryos, particularly during food stress (e.g. in wild mice: Rivers & Crawford, 1974; Krackow, 1992; Meikle & Thornton, 1995) or in mothers in poor body condition (Clutton-Brock, 1991). In our trials sex-specific mortality *in utero* is unlikely as there was no resulting decrease in litter size in the 20% protein group where we observed skewed sex ratios.

Other suggested mechanisms involved in sex ratio adjustment include: (1) the timing of insemination; (2) the influence of hormones at the time of implantation on the ultimate sex ratio (see James, 1992). It has been reported that nutritional state affects progesterone levels in ruminants, with a decrease in progesterone concentrations associated 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 gonadotrophin 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 we have recorded in

the present study on *M. coucha*. Reduced progesterone levels may also explain the recorded delay in the age of first conception in females on the high (20%) protein diet, as progesterone is important in the maintenance of pregnancy (Heap, 1982).

Sex-specific pup growth

Although it is expected that mothers differentially invest more in male than female offspring (Maynard Smith, 1980), other studies show that, like ours, there are no sex-specific differences in the growth rate of pups before weaning in northern elephant seals *Mirounga angustirostris* (Kretzmann *et al.*, 1993), hispid cotton rats *Sigmodon hispidus* (Cameron & Eshelman, 1996), Antarctic fur seals, (Lunn & Arnould, 1997) and southern elephant seals *Mirounga loenina* (McCann, Fedak & Harwood, 1989; Campagna *et al.*, 1992). However, in some species, like 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.

Since there were no sex-specific differences in pup weights from birth to weaning, the observed sexual dimorphism in multi-mammate mice at adulthood (Granjon & Duplantier, 1993; Leirs, 1994) must only become evident after the period of maternal investment. In some other 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.

It is expected that pup growth would increase with increasing dietary protein, because protein ingestion favours lean tissue deposition (Coyer, Rivers & Millward, 1987) and growth is often limited by protein intake (Cameron & Eshelman, 1996). In our trials the growth rates of 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) and meadow voles *Microtus pennsylvanicus* (Shenk, Elliott & Thomas, 1970). Excessively low and excessively high amounts of protein intake result in a low response in growth to food intake, because at low protein:energy ratios there is insufficient protein intake for nitrogen deposition, and at high protein:energy ratios the efficiency of tissue deposition is low because of the loss of energy for deamination and elimination of excess protein (Van Lunen & Cole, 1996). In addition, 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). In our trials, pup

growth on the 20% and 15% protein diets was faster than that of pups born to mothers on 10% protein diets, as in hispid cotton rats (Cameron & Eshelman, 1996).

Although pups born to mothers on the 15% diet were larger than 20% pups at weaning, these differences were not significant. Similar results were found for rats fed on diets with 18% and 36% 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 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 differential allocation of resources in males (Arnould *et al.*, 1996) and not differential maternal investment. In contrast to the findings of Arnould *et al.* (1996), we found that *M. coucha* did not show sex-specific differences in body tissue composition at birth or weaning. Other polygynous species like northern elephant seals (Kretzmann *et al.*, 1993) also show no differences in body tissue composition. At birth, maternal diet does not seem to influence the body tissue composition of the multi-mammate mice significantly, possibly because 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).

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 in male than female offspring, may not apply in multi-mammate mice. Our results from the energetic contents (per animal) of weaned pups show that weanlings in the 10% and 20% 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 10% treatment group, while those in the 20% 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. 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.

McCann (1980) showed that Antarctic fur seals with increased amounts of lean tissue enjoyed increased

reproductive success, suggesting that selection would favour rapid lean tissue deposition, especially in males. In contrast, pups on the 10% protein diet preferentially deposit fatty tissue which is easily mobilized 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.

Physiologically, offspring in the 10% treatment group may have deposited proportionally more fat than those in the 15% and 20% treatment groups because increased ingestion by their mothers maximizes protein intake. In this way surplus energy is ingested, which stimulates lipogenesis (Allaye Chan-McLeod, White & Holleman, 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). It has also been shown in calves (Donnelly & Hutton, 1976) and white-tailed deer *Odocoileus virginianus* (Holter & Hayes, 1977), that increased protein intake results in decreased fat deposition, as seen in the weaned pups in the 20% treatment group. At weaning those pups born to mothers on the 15% protein diet had significantly greater energetic values than those born to mothers on the 20% 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.

Increasing maternal protein intake beyond a particular level thus does not necessarily benefit offspring. Although *M. coucha* offspring were able to use the increased protein for increased lean tissue deposition on the high (20%) protein diet, this diet did not benefit the growth rate or weanling mass when compared to weanlings on the medium (15%) protein diet. Weaned pups of mothers on the high (20%) protein diet also had significantly lower amounts of energy than those of mothers on the 15% protein diet, suggesting 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.

CONCLUSION

From our experimental manipulation of 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. This supports Trivers & 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 dependent on body

size, the high protein diet should also favour male-biased litters. No other evidence for differential investment in the sexes was observed, thus suggesting that multi-mammate mouse mothers do not show any energetically biased investment in individuals of either sex.

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