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Nutritional and energy differences between *Rattus norvegicus* and *Mus musculus* of different ages

Diferenças nutricionais e energéticas entre *Rattus norvegicus* e *Mus musculus* de diferentes idades

Diferencias nutricionales y energéticas entre *Rattus norvegicus* y *Mus musculus* de diferentes edades

Rodney Murillo Peixoto Couto
Master in Animal Science and Technology
Institution: Universidade Estadual Paulista (UNESP)
Address: Ilha Solteira - São Paulo, Brazil
E-mail: couto.murillo@gmail.com

Priscilla Soares dos Santos
PhD in Ecology and Conservation
Institution: Universidade Federal de Mato Grosso do Sul (UFMS)
Address: Campo Grande - Mato Grosso do Sul, Brazil
E-mail: priscillasoares1989@gmail.com

Antônio Elivelton Paiva de Oliveira
Master's degree in Ecology
Institution: Universidade Federal do Pará (UFPA)
Address: Belém - Pará, Brazil
E-mail: elivelton99oliveira@gmail.com

Daniel Montanher Polizel
Doctor in Animal Nutrition and Production
Institution: Universidade Estadual Paulista (UNESP)
Address: Botucatu - São Paulo, Brazil
E-mail: daniel.polizel@unesp.br

Maiara Cabrera Miguel
Master’s in Animal Biology
Institution: Universidade Federal de Mato Grosso do Sul (UFMS)
Address: Campo Grande - Mato Grosso do Sul, Brazil
E-mail: maiaramiguel05@gmail.com

Rosemeire da Silva Filardi
PhD in Animal Science
Institution: Universidade Estadual Paulista (UNESP)
Address: Ilha Solteira - São Paulo, Brazil
E-mail: rosemeire.filardi@unesp.br

Antônio Carlos de Laurentiz
Doctor of Animal Science
Institution: Universidade Estadual Paulista (UNESP)
Address: Ilha Solteira - São Paulo, Brazil
E-mail: antonio.laurentiz@unesp.br

ABSTRACT
The present study provides a comparative evaluation of the carcasses of frozen prey, with a specific focus on rodents, which are conventionally used as a food source for carnivores kept in captivity. However, there is a noticeable deficiency in recent studies characterizing the nutritional values of these prey in detail, as most previous investigations have relied on single-sample sizes. Furthermore, there exists a range of prey sizes from two commonly utilized rodents for carnivorous diets, each containing different nutritional information. The objective of this work was to assess the differences between Mercois (*Rattus norvegicus*) and mice (*Mus musculus*) within the same weight range, aiming to provide technical information for professionals in the field of animal nutrition who need to formulate balanced diets and may choose between these two species. Between sizes BP3 (4.1 to 6 g) and BP9 (36 to 50 g), the weights of the rodents are equivalent, differing only by the age of the two species. For this study, 56 individuals of each species were used to measure calcium, phosphorus, moisture, mineral matter, fat, protein, and energy. Mice exhibited superior values compared to Mercois, except for moisture, where the inverse was observed. This information facilitates the formulation of diets tailored to the individual needs of carnivorous animals.

Keywords: carnivore diet formulation, comparative physiology, bromatological analysis, nutritional ecology.

RESUMO
O presente estudo apresenta uma avaliação comparativa das carcaças de presas congeladas, com foco específico nos roedores, que são convencionalmente utilizados como fonte de alimentação para carnívoros mantidos em cativeiro. No entanto, observa-se uma defasagem de estudos recentes que caracterizem de forma detalhada os valores nutricionais dessas presas, sendo a maioria das investigações anteriores baseada em amostras de tamanho único. Ademais, há uma diversidade de tamanhos de presas de dois roedores comumente utilizados como alimentação para carnívoros, contendo diferentes informações nutricionais. O objetivo deste trabalho foi avaliar as diferenças existente entre mercois (*Rattus norvegicus*) e camundongos (*Mus musculus*) de um mesmo intervalo de peso, a fim de fornecer informações técnicas para profissionais que atuam no campo da nutrição animal, os quais necessitam formular dietas equilibradas e podem optar entre essas duas espécies. Entre os tamanhos BP3 (4,1 a 6 g) e BP9 (36 a 50 g), os pesos dos roedores são equivalentes, diferenciando-se apenas pela idade das duas espécies. Para este estudo, foram utilizados 56 indivíduos de cada espécie, para mensurar cálcio, fósforo, umidade, matéria mineral, gordura, proteína e energia. Os Camundongos apresentaram valores superiores comparados à Mercois exceto em umidade, que ocorreu de forma inversa. Essas informações permitem a elaboração de dietas direcionadas às demandas individuais de animais carnívoros.

Palavras-chave: formulação de dietas carnívoras, fisiologia comparativa, análise bromatológica, ecologia nutricional.

RESUMEN
El presente estudio presenta una evaluación comparativa de las carcasas de presas congeladas, con especial énfasis en los roedores, los cuales son convencionalmente utilizados como fuente de alimentación para carnívoros mantenidos en cautiverio. No obstante, se observa una escasez de estudios recientes que caractericen de manera detallada los valores nutricionales de estas presas, ya que la mayoría de las investigaciones previas se basan en muestras de un único tamaño. Asimismo, existe una diversidad de tamaños de presas de dos roedores comúnmente utilizados como alimento para carnívoros, los cuales presentan diferentes composiciones nutricionales. El objetivo de este trabajo fue evaluar las diferencias existentes entre ratas (*Rattus norvegicus*) y ratones (*Mus musculus*) dentro de un mismo intervalo de peso, con el fin de proporcionar información técnica a los profesionales que actúan en el ámbito de la nutrición animal, quienes necesitan formular dietas equilibradas y pueden optar entre estas dos especies. Entre los tamaños BP3 (4,1 a 6 g) y BP9 (36 a 50 g), los pesos de los roedores son equivalentes, diferenciándose únicamente por la edad entre ambas especies. Para este estudio, se utilizaron 56 individuos de cada especie para la determinación de calcio, fósforo, humedad, materia mineral, grasa, proteína y energía. Los ratones presentaron valores superiores en comparación con las ratas, excepto en el contenido de humedad, el cual mostró un comportamiento inverso. Estos resultados permiten la formulación de dietas ajustadas a las demandas individuales de animales carnívoros.

Palabras clave: formulación de dietas para carnívoros, fisiología comparada, análisis bromatológico, ecología nutricional.

1 INTRODUCTION

Rodents have been widely used as a food source for carnivores kept in captivity (BIRD & HO, 1976). Recently, due to their use as experimental animals, an increasing number of studies have focused on the reproduction, health, and welfare of these species (NEVES, 2013). Such characteristics make these animals easy to manage, requiring little space and exhibiting a high reproductive rate under controlled conditions, in addition to the availability of literature, such as breeding manuals, which facilitates their production (NEVES, 2013). However, the scientific literature reveals a scarcity of studies addressing the nutritional values of these prey, with most previous investigations being outdated and limited to a single size or weight (BIRD & HO, 1976; DIERENFELD *et al*., 2002).

The energy value of a food refers to the amount of energy it provides to the organism after digestion and metabolism. This information is essential to ensure that animals receive adequate energy to meet their metabolic needs, as well as to promote growth, reproduction, and production (NRC, 2012). Despite size variations, an adult rat can weigh up to ten times more than an adult mouse (NEVES, 2013). At certain life stages, however, both species may exhibit similar weights, with the difference being only in age. Considering this, in animal feeding, particularly within weight ranges that these species share at some point in their lives, uncertainties arise regarding the choice of prey to be used. Between sizes BP3 (4.1–6 g) and BP9 (36–50 g), rodent weights are equivalent, differing only in age between the two species.

Thus, for a carnivorous animal feeding on prey within this weight range (4.1 g to 50 g), options include rats or mice. However, questions often arise as to which alternative would be more advantageous. This study aims to evaluate the nutritional and energetic differences between rats and mice, considering sizes from BP3 (4.1–6 g) to BP9 (36–50 g).

3 MATERIAL AND METHODS

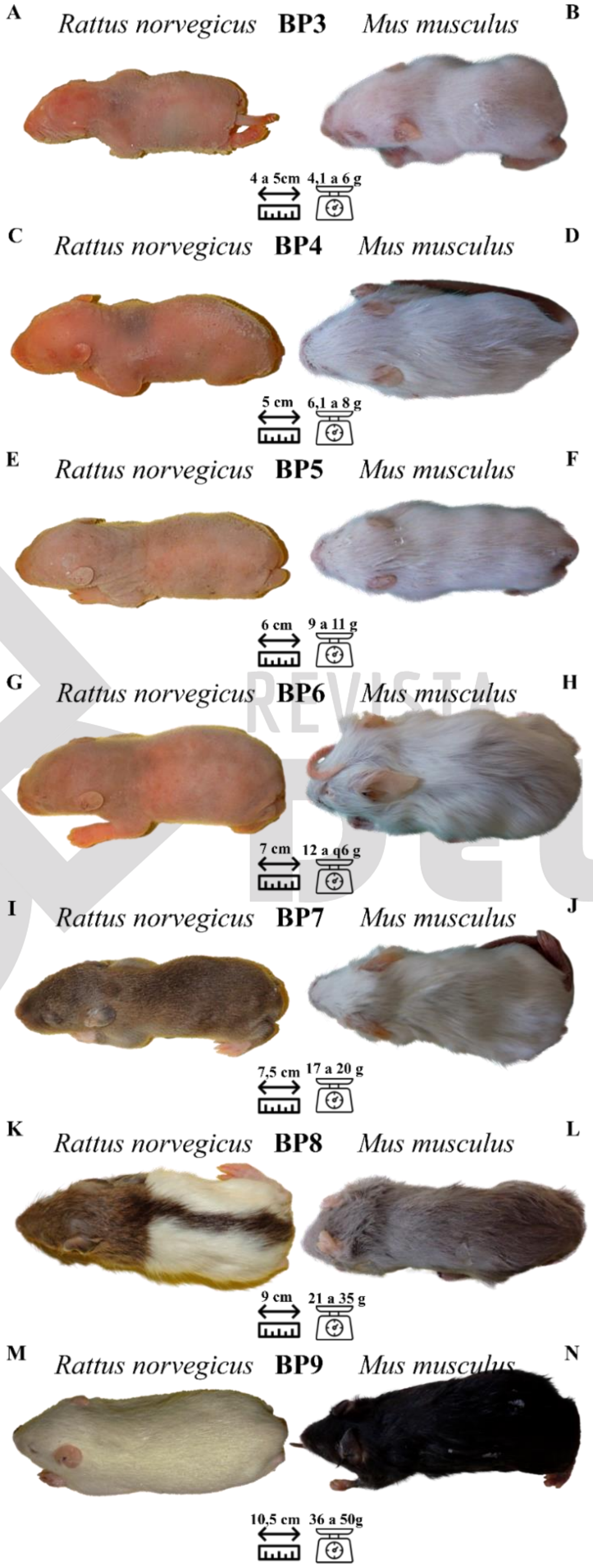
The study was conducted at the Pantanal Animal Facility, which aims to provide whole frozen prey for the feeding of captive carnivorous animals. Two prey species were used: Mouse (*Mus musculus*; n = 56) and Rat (*Rattus norvegicus*; n = 56) (Table 2). They were categorized by weight classes; however, some weight ranges are shared between the two species at certain life stages (between BP3 and BP9) (Figure 1).

Table 1. Age and weight of rodents used for the experiment. [1] weaning age; [2] age at which sexual maturity is reached.

Description	weight	Rat	Mouse
BP3	4,1 to 6g	1 day	7 days
BP4	6,1 to 8g	3 days	10 days
BP5	9 to 11g	7 days	15 day
BP6	12 to 16g	12 days	21 days[1]
BP7	17 to 20g	17 days[1]	30 days
BP8	21 to 35g	21 days	45 days[2]
BP9	36 to 50g	30 days	60 days

Source: Prepared by the authors.

Figure 1. Sizes of the Mercois and mice used for the experiment.



Source: Prepared by the authors

All animals were maintained in temperature-controlled environments according to the species' requirements. The rodents belong to the BP® lineage. This lineage consists of isogenic rodents, which are populations genetically uniform, with genetic similarity ranging from 95% up to 99%. They were genetically selected to be precocious animals with high fertility, produced solely to serve as food for carnivorous animals. The animals were fed twice daily with species-specific feed. Water was provided ad libitum via automatic drinkers. For euthanasia, the animals were subjected to a pre-slaughter stunning procedure, followed by rapid freezing in an ultra-low temperature freezer.

For each weight category, eight individuals per species were analyzed. After euthanasia, all prey items were first cut into smaller pieces to enhance drying efficiency, weighed, and dried in forced-air circulation ovens at 65–70°C, not exceeding 75°C. Weights were recorded before and after drying. Drying was carried out for 72 hours, after which the samples were ground in a stainless-steel ball mill to prevent contamination, especially by iron, zinc, and copper, with a capacity of 30 g per batch. Each sample was homogenized and stored in hermetically sealed zip-lock bags labeled with material information.

In the bromatology laboratory, samples were analyzed to quantify dry matter, crude protein, ether extract, and minerals following the procedures of Silva & Queiros (2006). Calcium (Ca) and phosphorus (P) contents were also measured. Based on the bromatological composition, gross energy values (kcal/kg) were estimated using the formula:

$$\text{Gross Energy (kcal/g)} = \text{Ether Extract (g)} \times 9\text{ kcal} + \text{Protein (g)} \times 4\text{ kcal} + \text{Carbohydrates (g)} \times 4\text{ kcal.}$$

4 RESULTS AND DISCUSSION

The results revealed consistent differences in bromatological composition and energy value between rats and mice across the different evaluation points (BP3–BP9). Overall, significant variations were observed in calcium, phosphorus, water, mineral matter, ether extract, crude protein, and metabolizable energy contents, with distinct patterns between species and among the analyzed periods. Statistical analyses indicated that several of these differences were significant, reflecting progressive changes in body composition and energy content throughout development, as detailed below (Table 2).

Table 2. Nutritional and energy values of mice and mice of varying ages and the same weight range. CA = Calcium; P = Phosphorus; AW = Moisture, MM = Mineral Matter; EE = Fat; PB = Protein; Kcal/g = Energy; Bold highlighting = significant or equal differences; Green = significant differences and Blue = equal.

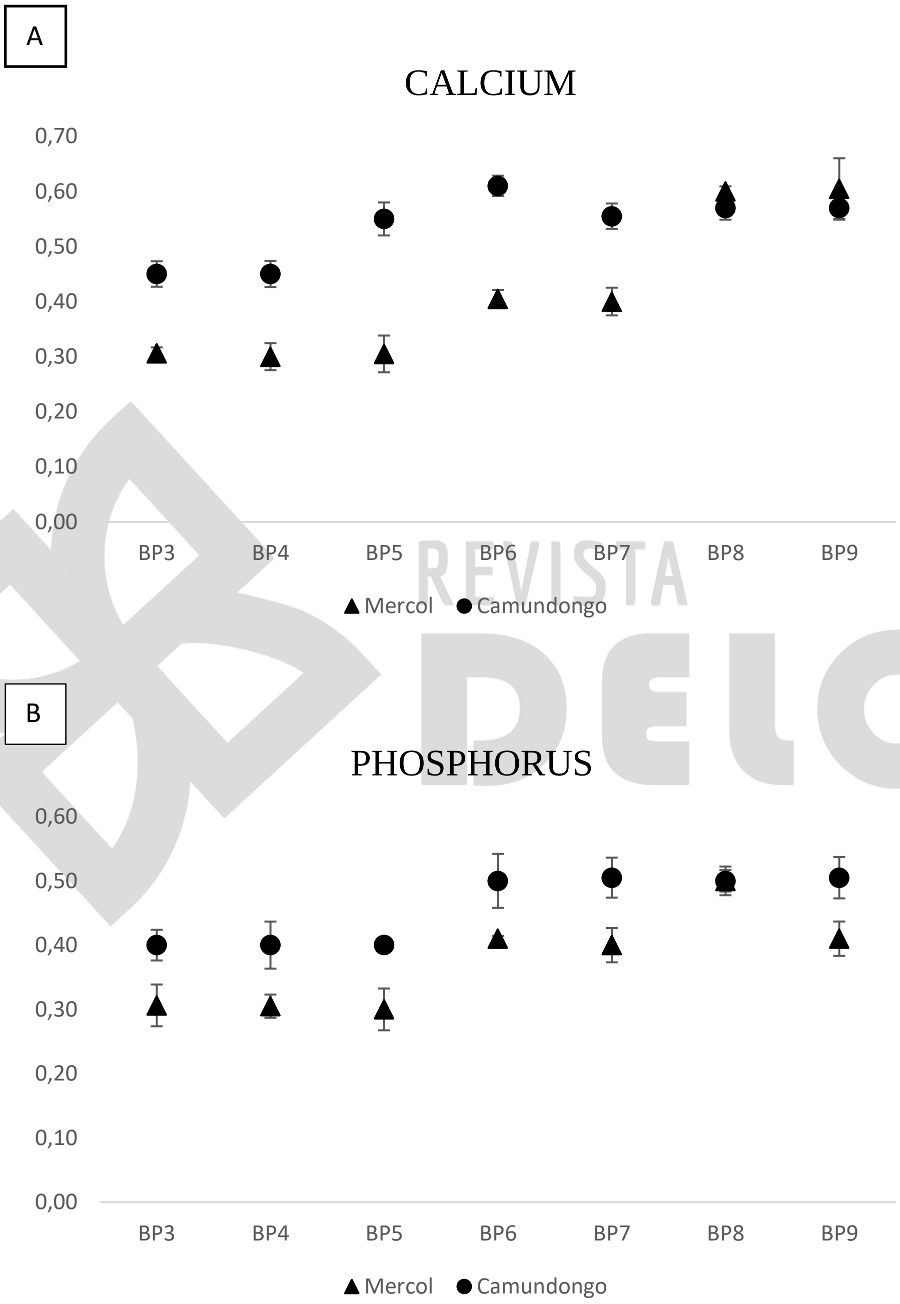
		BP3	BP4	BP5	BP6	BP7	BP8	BP9
CA (%)	**Rat**	0,31 ± 0,01	0,30 ± 0,02	0,31 ± 0,03	**0,41 ± 0,02**	**0,40 ± 0,03**	**0,60 ± 0,01**	**0,61 ± 0,06**
	Mouse	**0,45 ± 0,02**	**0,45 ± 0,02**	**0,55 ± 0,03**	**0,61 ± 0,02**	**0,56 ± 0,02**	**0,57 ± 0,02**	**0,57 ± 0,02**
	Teste	W(1, 14)= 53	W(1, 14)= 56	W(1, 14)= 57	W(1,14)= 43.5	W(1,14)= 48	W(1,14)= 31	W(1,14)= 27.5
	Value of P	p<0.05	p<0.05	p<0.05	p>0.05	p>0.05	p>0.05	p>0.05
P (%)	**Rat**	0,31 ± 0,02	0,31 ± 0,04	0,30 ± 0,01	0,41 ± 0,04	0,40 ± 0,03	**0,50 ± 0,02**	0,41 ± 0,03
	Mouse	**0,40 ± 0,03**	**0,40 ± 0,02**	**0,40 ± 0,03**	**0,5 ± 0**	**0,51 ± 0,03**	**0,50 ± 0,02**	**0,51 ± 0,03**
	Teste	W(1, 14)= 61.5	T(10.22)= 6.579	W(1, 14)= 64	W(1,14)= 64	T(13.68)= 7.413	T(12.964)= -0.380	T(13.525)= 6.417
	Value of P	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
AW (%)	**Rat**	**83,25 ± 0,80**	**83 ± 1**	**80,25 ± 1,21**	**77,35 ± 1,01**	**74,75 ± 2,02**	69 ± 1,25	**72,25 ± 1,15**
	Mouse	81,10 ± 1,19	74,10 ± 0,78	69 ± 1,22	70,40 ± 0,61	71,10 ± 0,78	**71,25 ± 1,13**	68,25 ± 1,31
	Teste	T(12.257)= -4.060	T(13.187)= -19.497	T(13.998)= -13.769	T(12.85)= -14.565	W(1,14)= 0.10	T(13.858)= 3.368	T(13.756)= -6.362
	Value of P	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
MM (%)	**Rat**	**1,99 ± 0,09**	**2,02 ± 0,09**	**2 ± 0,07**	2,01 ± 0,05	2,01 ± 0,08	**3,10 ± 0,13**	**3 ± 0,10**
	Mouse	**2,03 ± 0,09**	**2 ± 0,08**	**2,025 ± 0,12**	**2,95 ± 0,08**	**3,03 ± 0,11**	3,03 ± 0,10	**3 ± 0,09**
	Teste	T(13.914)= 22.054	T(13.886)= 0.447	T(11.181)= 0.500	T(10.377)= 25.226	T(12.802)= 22.037	T(13.039)= -1.507	T(13.803)= -1.128
	Value of P	p>0.05	p>0.05	p>0.05	p<0.05	p<0.05	p<0.05	p>0.05
EE (%)	**Rat**	4,07 ± 0,42	6,15 ± 0,46	6,10 ± 0,60	8 ± 0,39	**8 ± 1,22**	**11 ± 0,90**	**10,4 ± 1,57**
	Mouse	**9 ± 0,38**	**10,25 ± 1,02**	**10 ± 0,62**	**9 ± 0,32**	**8,05 ± 0,37**	8,05 ± 0,24	**12,25 ± 1,98**
	Teste	T(13.902)= 24.922	T(9.754)= 10.054	T(13.970)= 12.884	T(13.949)= 4.916	T(8.242)= 0.138	W(1,14)= 0.10	T(13.31)= 1.888
	Value of P	p<0.05	p<0.05	p<0.05	p<0.05	p>0.05	p<0.05	p>0.05
PB (%)	**Rat**	11,29 ± 0,82	9,25 ± 1,33	11,25 ± 1,59	12,3 ± 0,91	13,10 ± 1,19	**15,8 ± 1,89**	15,25 ± 1,59
	Mouse	**14,25 ± 1,55**	**14,20 ± 0,47**	**15,25 ± 1,31**	**16 ± 1,42**	**16,10 ± 0,93**	**17,10 ± 0,33**	**18,25 ± 0,92**
	Teste	T(10.614)= 4.971	W(1, 14)= 64	T(13.497)= 5.379	W(1,14)= 64	T(13.221)= 5.877	W(1,14)= 44	T(11.23)= 4.344
	Value of P	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p>0.05	p<0.05
Kcal/g (%)	**Rat**	82,75 ± 2,92	106 ± 7,17	124 ± 9,32	134,50 ± 7,61	152 ± 8,62	**182 ± 5,76**	153 ± 10,04
	Mouse	**146 ± 7,74**	**197,50 ± 8,38**	**194,50 ±7,86**	**154 ± 5,13**	**166 ± 7,81**	140 ± 9,70	**169,50 ± 7,63**
	Teste	T(8.946)= 22.054	T(13.672)= 23.398	T(13.615)= 16.787	T(13.992)= 5.999	T(13.868)= 2.857	W(1,14)= 0.10	T(13.065)= 3.475
	Value of P	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

Source: Prepared by the authors

Calcium concentrations varied among the Rat and Mouse groups, with the highest levels observed in Mice. Mouse sizes BP3, BP4, and BP5 exhibited higher values than Rats. In contrast, sizes BP6, BP7, BP8, and BP9 did not show significant differences between the two groups

(Figure 1A). Regarding phosphorus, higher concentrations were also observed in Mice, except for size BP8, in which no differences were detected (Figure 2B).

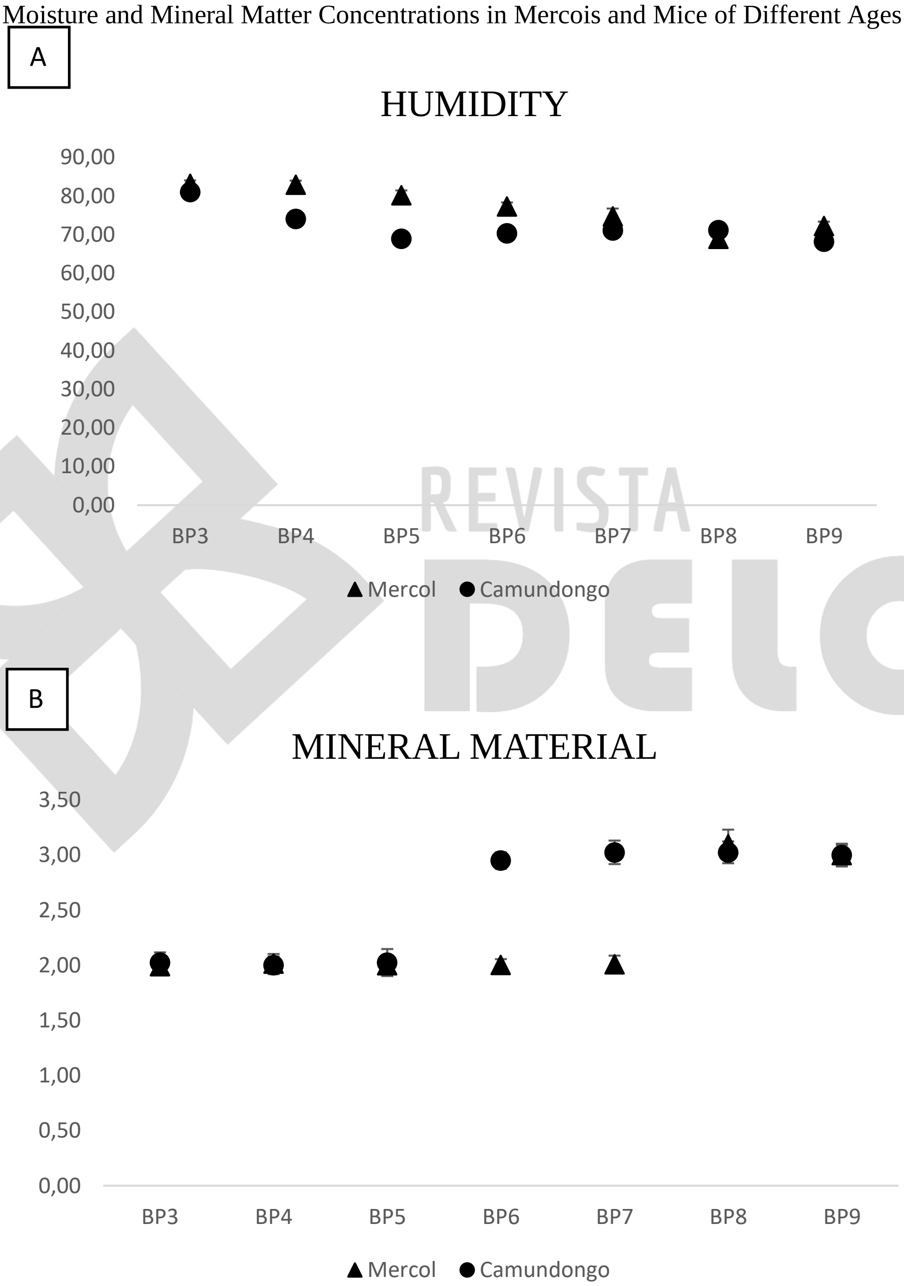
Figure 2. Calcium and Phosphorus Concentrations in Mercois and Mice of Different Ages and Weights.



Source: Prepared by the authors.

Regarding moisture, Rats predominantly exhibited higher levels, except for size BP8, in which the highest concentration was observed in Mice. No differences in mineral matter were observed for sizes BP3, BP4, BP5, and BP9 (Figure 3A). However, the highest mineral matter values were recorded in Mouse sizes BP6 and BP7 and in Rat size BP8 (Figure 3B).

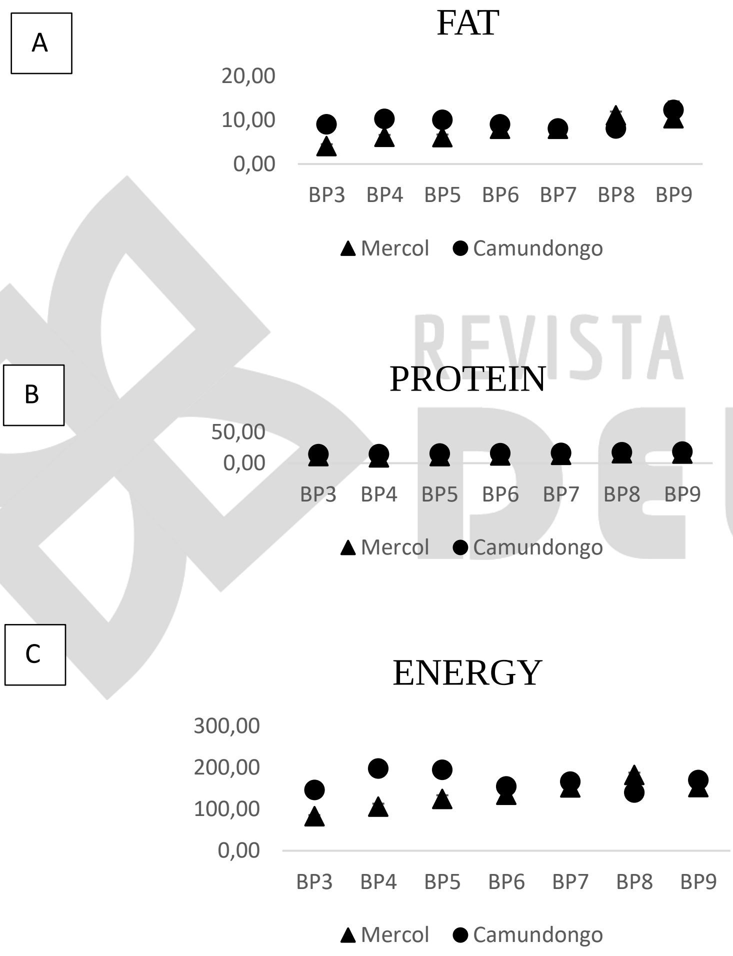
Figure 3. Moisture and Mineral Matter Concentrations in Mercois and Mice of Different Ages and Weights.



Source: Prepared by the authors.

Mice exhibited higher fat content compared to Mercois in sizes BP1 to BP6 and BP8, while no significant differences were observed in BP7 and BP9 (Figure 4A). Regarding protein content, Mice showed a higher proportion, except for size BP8, which did not display significant differences (Figure 4B). Finally, the energy values of Mice were also higher than those of Mercois, except for size BP8, in which Mercois presented higher values (Figure 4C).

Figure 4. Fat, Protein, and Energy Concentrations in Mercois and Mice of Different Ages and Weights.



Source: Prepared by the authors.

To understand the differences recorded between the size classes of both species, it is essential to consider their respective patterns of growth and development.

The Mercol (*Rattus norvegicus*) is a rodent species that can exceed 500 g in body weight. At birth, individuals weigh up to 6 g (BP3) (Figure 1A), begin ingesting solid feed at 14 days of age (BP6) (Figure 1G), and are weaned at approximately 21 days (BP8) (Figure 1K). Males exhibit prolonged reproductive capacity, whereas females enter estrus three hours postpartum, remaining receptive for three days, and subsequently reenter estrus every three days. Females produce an average of 12 offspring per litter, with the potential to reach up to 27 neonates, and possess six pairs of mammary glands. Sexual maturity occurs between 8 and 10 weeks of age.

The Mouse (*Mus musculus*) is a rodent species that may reach up to 50 g (BP9) (Figure 1N). Neonates weigh up to 2.5 g (BP1), begin consuming solid feed at around 12 days of age (BP4) (Figure 1D), and are weaned after 17 days (BP5) (Figure 1F). As in *R. norvegicus*, males have continuous reproductive potential, while females enter estrus three hours after parturition and remain receptive for three days, repeating the cycle every three days. Females produce an average of 15 offspring per litter, with records of up to 34 neonates, and possess five pairs of mammary glands. Sexual maturity is reached between 6 and 8 weeks of age (BP7 and BP8) (Figures 1J and L).

As rodents grow, the proportions of calcium, phosphorus, mineral matter, fat, energy, and protein tend to increase, while moisture decreases inversely (Douglas *et al*., 1994). Considering this, when comparing Mercois and Mice of similar body weights but different ages, it becomes possible to understand the differences recorded in this study.

The disparities observed are attributed to postnatal embryonic development, since neonates present physiological characteristics that differ from those of juveniles and adults (Neves, 2013). Newborn rats, for instance, emerge after a gestation period of approximately 21 days and, at this early stage, lack fur, have closed eyes, and possess a skeletal structure still in formation. As development progresses, ossification occurs, whereby cartilage is converted into bone. Concurrently, fur begins to develop and the skeletal system consolidates, resulting in the physiological traits typical of adulthood. These developmental changes lead to variations in calcium, phosphorus, moisture, mineral matter, fat, protein, and energy throughout the rodents' lifespan, reflecting the influence of age on these parameters (Douglas *et al*., 1994).

While BP6 Mice reach the weaning stage, BP6 Mercois are just beginning hair growth and completing the transition from cartilage to bone (Neves, 2013). Consequently, Mice exhibit higher calcium percentages than Mercois during the early stages (BP3, BP4, and BP5). From BP6 onward, calcium percentages become similar between species, as both have fully formed body structures. Phosphorus levels also tend to be higher in Mice, except at BP8, when proportions are equivalent. Conversely, since moisture is inversely proportional to the other evaluated parameters (Douglas *et al*., 1994), Mercois generally show higher moisture levels than Mice, except at BP8, when Mice display the higher values.

Fat content is similar between both species at BP7 and BP9. Mercois at BP7 begin consuming solid feed but still rely partially on maternal milk, whereas Mice at BP7 are entering reproductive maturity. BP9 Mercois have been weaned for only a few days, still retaining fat reserves accumulated during the milk-feeding stage, while BP9 Mice, both males and females, are at the peak of reproductive age. During reproduction, many animals exhibit a marked tendency to accumulate and store fat reserves (Store, 1991). This phenomenon results from physiological adaptations that ensure survival and reproductive success. Increased fat storage provides an accessible energy source during critical periods such as gestation and lactation, when energy demands are elevated. Additionally, stored fat functions as thermal insulation, protecting adults and their offspring under adverse environmental conditions (Store, 1991).

Reproductive females must accumulate adequate energy reserves to maintain their own health and that of their offspring. This evolutionary strategy, observed across numerous species, underscores the importance of fat reserves in reproductive cycles. Thus, fat accumulation during the breeding season plays a crucial role not only in individual survival but also in reproductive success (Rappolee, 1995; Thorne, 2000; Gentry, 2012; Figueiredo, 2016). In many species, males also accumulate lipids prior to the breeding season, a common strategy among those that reduce food intake during mating. Consequently, these animals rely on stored fat as an adaptive mechanism to sustain energy requirements throughout reproduction (Store, 1991).

Weaned individuals tend to have higher fat reserves because they are transitioning from milk to solid diets, which are often rich in nutrients and calories, while also exhibiting greater capacity for caloric metabolism and deposition (Cattaneo, 1996; Taverner & Campbell, 2000; McCarthy & Roberts, 2004; Pluschke & Wilhelm, 2008).

Mice exhibited higher protein and energy contents overall, except for BP8, where no significant differences were detected in protein levels. In contrast, Mercois showed higher energy values at BP8.

These results provide professionals responsible for formulating carnivore diets with a clearer understanding of the nutritional composition of prey of equivalent weights between Mercois and Mice. It is noteworthy that, in most cases, Mice present superior nutritional values compared to Mercois; however, a lower nutrient level in a given prey type does not imply nutritional deficiency or unsuitability.

Such variations are fundamental for designing customized diets according to each animal's physiological needs. For instance, for individuals prone to obesity that require BP6-sized prey, Mercois are recommended due to their lower fat content compared to Mice. Conversely, for animals recovering from illness, requiring weight gain, or in reproductive phases, the inclusion of BP6 Mice is advisable.

It is important to emphasize that most species excrete excess nutrients, whereas others store them, which may have positive or negative effects. For example, in certain geckos of the genus Phelsuma, females store extraskelletal calcium in endolymphatic sacs to be used for eggshell formation (McKeown, 1993). In tortoises, high-protein diets can lead to negative protein accumulation, as excessive intake of animal-based protein is one of the main factors associated with pyramiding, a condition characterized by abnormal growth of the carapace scutes that form a pyramidal shape due to disproportionate keratin deposition (Heinrich *et al*., 2016).

5 CONCLUSION

This study demonstrated significant differences in the nutritional and energetic values between Mercois and Mice of equivalent body weight. Mice exhibited higher levels of calcium, phosphorus, fat, energy, protein, and mineral matter, whereas Mercois showed higher moisture content. In exceptional cases, the values were comparable, and in certain prey size classes, an inverse pattern was observed, with Mercois displaying higher values than Mice. Therefore, the findings of this study may serve as reference parameters for future research and contribute to improving the nutritional management of carnivorous animals, offering a broader range of possibilities for adapting diets to the specific demands of each species.

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