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# Nutritional Content of Adult Norway Rats for Small Carnivores' Feeding

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## ABSTRACT

Rats are the natural diet of many free-ranging carnivores. They are also fed to small carnivore mammals, raptor birds, and reptiles in captivity as a sole or partial diet, however, little is known about the nutrients that a rat can provide as animal feed. This study aimed to determine the nutritional content of the whole captive-bred Norway rats. A total of 12 randomly selected weaned male and female Wistar Norway rats were fed *ad libitum* with a local dry dog food diet. The rats were weighed weekly until an average weight of 300 g was reached. Biochemical and mineral analyses were carried out for each rat. The results of the study showed significant differences between male and female rats in terms of growth rate, crude protein, total fat, and calcium concentrations. Males presented a faster growth rate and reached the desired weight in around half the time (6 weeks), compared to females (13 weeks). Moreover, males had a higher percentage of crude protein (23.57%) on a fed matter basis, calcium (2.61%), and phosphorus (0.98%). Females showed higher total fat (13.92%) and lower crude protein (19.49%), calcium (0.54%), and phosphorus (0.47%), compared to males. The results of this research may be used to determine whether a whole rat can provide all the necessary nutrients to carnivore animals commonly kept in captivity. Present findings indicated that rats could provide the necessary nutrients, however, if given as a sole diet, they could not be enough to supply the nutritional requirements of animals in the long term.

**Keywords:** Carnivore nutrition, Norway rats, Nutritional Content, Wistar rat

## INTRODUCTION

Nutrition affects the health, growth, reproduction, availability of key nutrients, and longevity of different animals (Moraal et al., 2012; Wilder et al., 2013). Wild animals instinctively know what to hunt or forage for to survive. The way free-ranging animals choose their diet is a very complex behavior as they use a wide variety of morphological, physiological, and anatomical adaptations (Fernández et al., 2021). Wild predators mostly select their prey based on macronutrient composition or selectively feed on specific body parts to obtain the nutrients they need (Kohl et al., 2015). However, captive animals should feed the supplied food, and they mostly suffer from nutrient deficiencies. Since the natural diet of wild animals in a captive feeding situation can rarely be supplied, they are given a substitute diet that causes different diseases related to dietary management (Liesegang et al., 2008).

It has to be taken into consideration that no studies have been conducted so far to investigate the exact requirements of captive wild animals concerning their different existing species due to the difficulty in obtaining a large number of blood, urine, and feces samples. Generally, combinations of the nutritional requirements of well-studied domestic carnivores and certain omnivores (minks and foxes), are established as guidelines to feed wild carnivores in captivity (AZA Small Carnivore TAG, 2011). The cat is typically the model species used to establish nutrient requirement guidelines for captive carnivorous, such as big cats, otters, herpestids, and euplerids (AZA Small Carnivore TAG, 2009; AZA Small Carnivore TAG, 2010), while the dog's nutritional requirements are used as a baseline for slightly omnivorous carnivores, including large canids and mustelids (AZA Small Carnivore TAG, 2011).

Small prey animals, such as mice and rats, which are a common natural food source for most reptiles, raptors, and small mammals, are often fed to captive wildlife, as is the case of zoo animals and exotic pets (Kleiman et al., 2010). This is based on the belief that whole preys are similar to the natural diet of some carnivores and are commonly presumed to meet nutrient requirements as long as nearly all soft tissues and some bones or other calcified tissues are consumed (Dierenfeld et al., 2002). For wild animals deprived of their natural ecosystem and free-ranging habits, in terms of nutrition, a whole vertebrate prey contains essential amino acids, vitamins, and other nutrients; in terms of behavior, it provides an important form of environmental enrichment improving their biological functioning by stimulating their natural foraging behavior (Cooper and Williams, 2014).



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Since the 19th century, Norwegian rats (*Rattus norvergicus*) have become domesticated on a large scale and produced various forms of the laboratory rat, adapting to conditions imposed by humans (Barnett, 2002). This has resulted in a genetically different population from the original wild type, which is more docile and tolerant of greater crowding, rarely attacks or escapes, and produces more pups by mating earlier and having a longer life reproductive period (Barnett, 2002). Therefore, many companies breed rats and other prey animals specifically for food to supply the exotic pet market, zoos, and aquaria (Ballard and Cheek, 2016). These rats and mice can be purchased from these companies at different ages as well as in various sizes, including other vertebrates (Ballard and Cheek, 2016).

Generally, rats can be bought as males or females without any distinction. However, some studies have shown that testosterone plays a crucial role in the development of muscle tissue by influencing protein metabolism (Tipton, 2001; Bell, 2018) and bone mass development (Quirós et al., 2020), while estrogen affects body fat distribution and storage (O'Sullivan, 2009; Lizcano and Guzmán, 2014; Bell, 2018). Rats produce low levels of testosterone and estrogen at birth, which slowly increase as they reach puberty (Bell, 2018). In a study by Bell (2018), testosterone and estrogen concentrations were recorded in both males and females from birth. While both genders showed low concentrations of testosterone during the first 19 days of age, testosterone increased dramatically in males as they aged 40-60 days. Similar to testosterone, both sexes produce low levels of estradiol, an estrogen steroid hormone, which then increases in females at puberty and during every estrus cycle. As reported, after a peak of estradiol concentration around day 15 in both sexes, estradiol concentration increased only in females until day 39 and it then begins to fluctuate every 4 to 5 days. It could be concluded that whole post-pubertal male rats have higher protein values due to increased testosterone, which contributes to differences in musculature. On the other hand, estrogen in females enhances their body fat.

Although rats are used so extensively around the world for this purpose and fed to many species of animals, little information is available on the nutritional content of the whole captive-bred Norway rats and whether they can provide all the necessary nutrients to the feeding animal (Kleiman et al., 2010). Therefore, this study aimed to examine the values of moisture, crude protein, total fat, ashes, and minerals found in a whole Norway domesticated rat.

## MATERIALS AND METHODS

### Ethical approval

The rats in the current research were treated and euthanized following the guidelines passed by the institutional ethics committee for the care of animals and approved by the Postgraduate Bioethics Committee of the Faculty of Veterinary Medicine and Zootechnics at the certificate reference code of EEPVirtual.51.2020. The selection of the experimental animals and use of materials was authorized by the Institutional Committee for the Care and Use of Laboratory Animals of the Faculty of Chemical Sciences and Pharmacy at certificate reference code of CICUAL-CCQQF-03-2020. Both committees are part of the University of San Carlos of Guatemala, in Guatemala City, Guatemala.

### Experimental sites

The current study was performed in three different research institutes, all located inside the main campus of the University of San Carlos of Guatemala, in Guatemala City, Guatemala. The sample selection, maintenance, feeding and weighing, and euthanasia of the experimental animals were done in the Amarilis Saravia Bioterium, in the pharmacology research area of the Faculty of Chemical Sciences and Pharmacy. The proximal chemical analysis was carried out in the Laboratory of Bromatology of the Faculty of Veterinary Medicine and Zootechnics. Finally, the chemical analysis of ash was performed in the Soil, Water, and Plant Analysis Laboratory of the Faculty of Agronomy.

### Sample selection

A total of 12 captive-bred Norwegian weaned rats of the *Rattus norvergicus albinus* species, Wistar breed, were used in this study. Six males and six females were randomly selected from 12 different litters that were available at the time of the study, with an approximate weight of 50-80 g and an age range of 20-25 days. The rats of the same sex were put together as an experimental group to avoid unwanted reproduction and prevent depression due to social isolation.

### Maintenance of experimental animals

Each group was placed in transparent plastic boxes of 12 liters (40 × 28 × 10 cm), with an aluminum lid of 1 mm diameter galvanized wire mesh with openings of 12 × 12 mm, on which the food and the water bottle were placed to avoid contamination with the animals' wastes. Animals were maintained in an air-conditioned room with a constant temperature of 21-23°C, a 12-hour day-night cycle, 45% humidity, and controlled ventilation. Box and bedding changes were made every three days. The boxes were disinfected with soap and chlorine before their use. Thin pine chips from a local sawmill were used as a substrate, fumigated with the pyrethroid cypermethrin (Dismetrina 25 EC, formulated by


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Disagro in Escuintla, Guatemala), and dried in the sun for 4 days to eliminate probable harmful microorganisms and parasites.

**Feeding and weighing**

All rats were fed the commercial dry food for adult dogs of the brand Rufo by Concentrados Aliansa, made in Guatemala *ad libitum*. This feeding was chosen because small producers of rats and mice in Guatemala commonly use dry commercial dog food to feed rodents sold as prey that are the most inexpensive ones. Rufo and Rambocan made by Concentrados Aliansa, a Central American feed manufacturer based in Guatemala, El Salvador, Honduras, and Costa Rica, are the most used ones for this purpose since the price range from 1.00 to 2.00 US Dollars per kilogram. The average quantity of food provided daily was a one-quarter cup, about 30 g of dry dog food, per rat during the first and second week. This amount increased to half a cup, about 60 g, during weeks three and four. From week five, each rat daily received one cup, about 120 g. All rats were fed until they reached the minimum weight of 300, which took 6 weeks for males and 12 weeks for females. Drinking water was filtered from the tap and placed in rodent water bottle dispensers. The animals were weighed without *nil per os* (NOP) on the first day of every week from the beginning of the experiment until the day of the euthanasia to observe the weight gain. The weights were obtained using a digital kitchen scale with a removable plastic container without the need for anesthesia or restriction of movement. On a weighing day, a physical health examination was also performed to follow the guidelines of the bioethics committee.

**Euthanasia**

Upon reaching an average weight of 300 g, the rats were euthanized by the researcher according to the guidelines recommended in the IUCN code of ethics and the AVMA guide for the euthanasia of animals. According to Flecknell (2009), lidocaine in rodents should not exceed 10 mg/kg for local and regional anesthesia. With this in mind, an overdose of 1 ml of Lidocaine HCL 2% was injected intraperitoneally into each rat to provide analgesia and anesthesia. Subsequently, intrathecal injection of 0.6 ml of Lidocaine HCL 2% was used through the foramen magnum using a 1 mL syringe, with a 25 gauge, and 16 mm needle. In order to gain proper access to the foramen magnum, the rat's head was bent down about 45 degrees with one hand while the needle was inserted with the skillful hand into the cerebello-medullary cistern through the atlantooccipital junction (Zolhavarieh et al., 2011). Lidocaine causes a relatively rapid loss of cerebrocortical function (brain death) when administered intrathecally to anesthetized animals and leaves relatively low tissue residues and is not expected to pose hazards to scavenging animals that might feed on the carcass (AVMA, 2020). The bodies were identified, individually placed in airtight bags whole, and frozen for 2 months until the laboratory of bromatology reopened its doors to the public after the COVID-19 lockdown of 2020.

**Biochemical analysis**

The proximate composition analysis was used to determine the percentage of moisture (dry matter), crude protein, ether extract (total fat), crude fiber, ash (total minerals), and nitrogen-free extract (NFE). The analysis was performed for each rat to see if there were marked differences in the nutritional values between individuals. To determine the amount of moisture, oven drying methods were used to obtain the partial and total dry matter. The rats were thawed at room temperature and chopped into pieces with a kitchen ax to reduce the drying time. The pieces were spread on a tempered glass tray and introduced into a forced draft oven, where they remained for 72 hours at 60°C until around 80% of the moisture was removed, obtaining partial dry matter (Gregg, 2016). The samples were weighed daily to see the reduction in humidity. The partial dry matter was ground to a particle size ≤ 2 mm in a hammer mill to obtain a homogeneous sample and stored in individual airtight containers. Subsequently, to obtain the total dry matter, 5 g of the homogeneous sample of each rat were placed in a draft air oven at 105°C for 24 hours (Gregg, 2016).

The macronutrient values were determined following the Association of Official Analytical Chemists methods (AOAC, 2019), except for ether extract. Crude protein determination was carried out using AOAC 976.05 (Kjeldahl Method), with a Kjeldahl Auto 1030 analyzer using 1 g of the partial dry matter sample. The extraction of fat was carried out with 2 g of partial dry matter in accordance with the Randall technique using a Soxhlet SER 148/6 solvent extractor (Gregg, 2016). The crude fiber was obtained using 1 g of the remnants of the ethereal extract according to AOAC 962.09 (Ceramic Fiber Filter Method) in a Fibertec System I. Total ash determination was conducted according to AOAC 942.05, where 5 g of the partial dry matter was introduced into a muffled furnace and heated to 600°C for 2 hours. Finally, to obtain the amount of nitrogen-free extract, the formula introduced by Gregg (2016) was applied: NFE = 100 – (Crude protein % + Total fat % + Crude fiber % + Ash %).

**Mineral analysis**

For the chemical analysis of minerals, 5 g of the partial dry matter obtained during the biochemical analyses were used to calculate the percentage of macroelements (phosphorus, calcium, potassium, and magnesium) and values in parts per million (ppm) of microelements (copper, zinc, iron, and manganese) for each rat. The destruction of the organic


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matter, for the removal of interferents or water, was performed by the Dry Combustion Method, where the samples were subjected to a high temperature of 450°C in a muffle furnace for 4 hours until they were incinerated (Schumacher, 2002). The minerals were recovered with 1N HCl and then the determinations were made. For phosphorus, the quantification was carried out using the Ultraviolet Reflectance analysis with a Perkin Elmer Lambda 11 UV/VIS Spectrophotometer made in Germany (Kulkarni et al., 2014). The rest of the minerals were determined by Atomic Absorption Spectroscopy (AAS), using a Perkin Elmer AAnalyst 100 Spectrometer made in the United States (JoVE Science Education Database, 2022).

### Statistical analysis

Sex differences in biochemical and mineral content and comparisons between them were analyzed using the Mann-Whitney U statistic or the Student's t-test. The means and population standard deviation were calculated for each nutrient of each sample using a population size (N) of 6 for each sex. The confidence intervals were analyzed using the online confidence interval calculator software MathsIsFun.com v0.912 (Pierce, 2021).

## RESULTS

### Weight gain

The results of weight gain are shown in Graph 1. There was a great difference between the growth rate of the males and the females. All the males reached the desired weight (around 300 g) in only 6 weeks from the start of the experiment (between 62 and 67 days of age) while the group of females took more than twice the time (13 weeks, between 111 and 116 days of age). The heaviest rats on their last day of life were the female rat Number 5 with 360 g and the male rat Number 5 with 349 g. All the rats reached values above 300 g except for one female rat (Number 6), maintaining 273 g from week 12 to week 13. The lightest male rat was Number 3 with 303 g.

### Biochemical analysis

The results of the biochemical analysis are presented in Table 1. All data except moisture and dry matter content (DMC) is presented on both a dry and fed matter basis. On a dry matter basis, data contains 0% water. On a fed basis, the data shown includes the moisture percentage. Males presented a higher percentage of moisture and ash than females. They also had more protein (68.64%) and lower lipids (16.55%) than females (51.35% and 36.60%, respectively). Both sexes showed no significant difference in carbohydrate percentage ($p < 0.05$). The confidence intervals, shown in parenthesis, were also smaller in males, indicating more variations between the values of the females.

### Mineral analysis

The results of the mineral analysis are presented in Table 2. All the data was obtained based on the partial dry matter of the bromatological analysis. Regarding mineral analysis, the greatest difference between the two groups was found in males as they showed five times more calcium (2.61%) than females (0.54%). While females presented higher levels of potassium and copper, males had a greater quantity of the other minerals. The confidence intervals showed a very small variation in most minerals, except for iron in both males and females at 150.83 ± 23.4 ppm and 114.17 ± 30.9 ppm, respectively.

### Requirements for small carnivores

To ensure the nutritional content of whole Norway rats can meet the dietary requirements of some small carnivore animals normally kept in captivity, the necessary information was gathered from multiple sources and summarized in Table 3 for the convenience of the reader. As reported by AZA Raptor TAG (2010), there are no documented dietary needs for raptors or vultures; therefore, target nutrient ranges are developed using a mix of the requirements of a strict carnivore (domestic cat) and big poultry (turkey). Bigger carnivores, such as big cats, larger canids, and hyenas were not included due to their size and the number of rats needed to fulfill their daily intake of nutrients. Carnivore reptiles, such as lizards and snakes, were excluded since no nutritional requirements were found.

According to Table 3, the crude protein percentage of the male rats (23.57%) in the current study on a fed matter basis is within the minimum range of daily needs of most small mammals and the bird of prey (Andean condor), except for domestic cats and otters, which need at least 26% and 24% of crude protein, respectively. Female rats are a better option when it comes to crude fat requirements for most animals. Otters also require a higher percentage of fat (15-30%) than the fat provided by female rats (13.92%). For mink and foxes, the fat percentage was not mentioned in the studies by NRC (1982) about their nutritional requirements, and their study was the only one found in this subject area. The mineral requirements for most of the animals in Table 3 can be supplied with a whole rat. Male rats are best to fulfill calcium, phosphorus, and manganese requirements, while female rats are a better option to provide the potassium, magnesium, copper, and iron nutritional needs. Cats and dogs require higher levels of potassium (0.60%) and zinc (75


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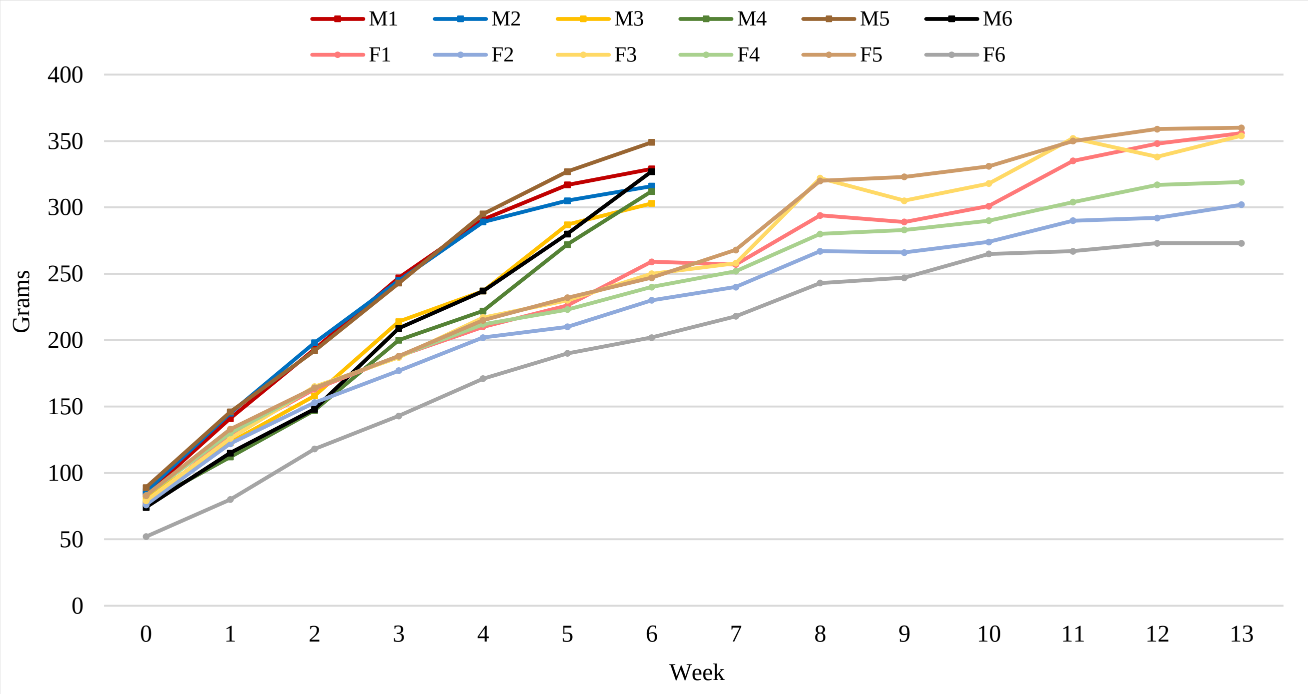
ppm and 80 ppm, respectively) than those provided by rats. The Andean condor needs higher levels of copper (> 9 ppm), zinc (> 75 ppm), and especially manganese, which is more than 67 ppm.




**Graph 1.** Male and female rats' weight gain in grams. M: Males, F: Female

**Table 1.** Biochemical analysis of the adults Norway rats

| Nutrient (%) | Males - Dry | Males - Fed | Females - Dry | Females - Fed |
|---|---|---|---|---|
| Moisture | --- | 65.68 ± 0.66 | --- | 62.09 ± 1.63 |
| DMC | 34.32 ± 0.66 | --- | 37.91 ± 1.63 | --- |
| Crude protein | 68.64 ± 1.35 | 23.57 ± 0.79 | 51.35 ± 2.30 | 19.49 ± 1.35 |
| Total fat | 16.55 ± 1.48 | 5.67 ± 0.41 | 36.60 ± 2.68 | 13.92 ± 1.38 |
| Crude fiber | 1.78 ± 0.42 | 0.61 ± 0.15 | 1.82 ± 0.28 | 0.69 ± 0.10 |
| Ash | 11.11 ± 0.65 | 3.81 ± 0.22 | 9.48 ± 2.22 | 3.57 ± 0.77 |
| NFE | 1.93 ± 1.10 | --- | 1.66 ± 1.67 | --- |

All data are means; a confidence interval of 95%, N: 6 for each sex. DMC: Dry matter content, NFE: Nitrogen-free extract

**Table 2.** Mineral analysis of the adults Norway rats

| Mineral | Males | Females |
|---|---|---|
| Calcium (%) | 2.61 ± 0.41 | 0.54 ± 0.06 |
| Phosphorus (%) | 0.98 ± 0.13 | 0.47 ± 0.05 |
| Potassium (%) | 0.25 ± 0.02 | 0.46 ± 0.07 |
| Magnesium (%) | 0.11 ± 0.01 | 0.07 ± 0.02 |
| Sodium %() | 0.26 ± 0.02 | 0.20 ± 0.01 |
| Copper (ppm) | 3.67 ± 1.51 | 8.00 ± 0.00 |
| Zinc (ppm) | 74.17 ± 5.38 | 52.17 ± 3.28 |
| Iron (ppm) | 150.83 ± 23.40 | 114.17 ± 30.90 |
| Manganese (ppm) | 7.50 ± 2.00 | 3.67 ± 0.60 |

All data are means ± the margin of error with a confidence interval of 95%, N: 6 for each sex.



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**Table 3.** Nutritional content of Norway rats and carnivore requirements

| | Nutritional content | Carnivore mammal nutritional requirements | | | | | | | | Birds of prey nutritional requirements |
|---|---|---|---|---|---|---|---|---|---|---|
| **Nutrient** | **Norway rat** | **Domestic cat [1]** | **Domestic dog [2]** | **Otters [3]** | **Herpestids/ Euplerids [4]** | **Omnivore mustelids[5]** | **Carnivore mustelids [6]** | **Mink [7]** | **Foxes [8]** | **Andean condor [9]** |
| CP (%) | 23.57 M<br>19.49 F | 26.00 | 18.00 | 24.00-32.50 | 19.70-32.50 | 17.50-26.00 | 19.70-32.50 | 21.80-26.00 | 19.70 | > 20.00 |
| Fat (%) | 5.67 M<br>13.92 F | 9.00 | 5.50 | 15.00-30.00 | 9.00-30.00 | 5.00-8.50 | 9.00-30.00 | --- | --- | > 10.00 |
| Ca (%) | 2.61 M<br>0.54 F | 0.60 | 0.50 | 0.60-0.80 | 0.29-1.00 | 0.50-1.20 | 0.50-1.00 | 0.30 | 0.60 | 0.80-2.50 |
| P (%) | 0.98 M<br>0.47 F | 0.50 | 0.40 | 0.60 | 0.26-0.80 | 0.50-1.00 | 0.50-0.80 | 0.30 | 0.40 | 0.39-0.72 |
| K (%) | 0.25 M<br>0.46 F | 0.60 | 0.60 | 0.20-0.40 | 0.40-0.60 | 0.40-0.60 | 0.40-0.60 | --- | --- | 0.40-0.67 |
| Mg (%) | 0.11 M<br>0.07 F | 0.04 | 0.06 | 0.04-0.07 | 0.03-0.08 | 0.04-0.06 | 0.03-0.08 | --- | --- | 0.04-0.06 |
| Na (%) | 0.26 M<br>0.20 F | 0.20 | 0.08 | 0.04-0.06 | 0.05-0.40 | 0.04-0.30 | 0.05-0.40 | 0.50 | 0.50 | 0.10-0.13 |
| Cu (ppm) | 3.67 M<br>8.00 F | 5.00 | 7.30 | 5.00-6.25 | 5.00-8.80 | 6.00-12.40 | 5.00-8.80 | --- | --- | > 9.00 |
| Zn (ppm) | 74.17 M<br>52.17 F | 75.00 | 80.00 | 50.00-94.00 | 50.00-94.00 | 50.00-120.00 | 50.00-94.00 | --- | --- | > 75.00 |
| Fe (ppm) | 150.83 M<br>114.17 F | 80.00 | 40.00 | 80.00-114.00 | 80.00-114 | 30.00-90.00 | 80.00-114.00 | --- | --- | > 80.00 |
| Mn (ppm) | 7.70 M<br>3.67 F | 7.60 | 5.00 | 5.00-9.00 | --- | --- | --- | --- | --- | > 67.00 |

Crude Protein (CP) and Total Fat are presented on a fed matter basis. Minerals are based on a dry matter basis. M: Male rats, F: Female rats.
Nutritional requirements are for adult animals in maintenance. Values are the minimum nutrient requirements in their diet.
[1, 2] AAFCO (2014) Cat and dog food nutrient profile based on dry matter.
[3] AZA Small Carnivore TAG (2009) Otter target nutrient ranges based on dry matter.
[4] AZA Small Carnivore TAG (2011) Mongoose, meerkat, & fossa target nutrient ranges based on dry matter.
[5] AZA Small Carnivore TAG (2010) More omnivorous mustelids (skunk and tayra) target nutrient ranges based on dry matter.
[6] AZA Small Carnivore TAG (2010) More carnivorous mustelids (badger, ferret, fisher, wolverine) target nutrient ranges based on dry matter.
[7, 8] NRC (1982) Mink and Fox Nutrient Requirements based on dry matter.
[9] AZA Raptor TAG (2010) Andean condor target nutrient ranges based on dry matter.


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## DISCUSSION

During the experiment, it was observed that male rats presented a faster growth rate and higher body mass, compared to females. For females, it took 13 weeks to reach the desired weight (approximately 320 g), which was twice the time needed for males (6 weeks). Most adult male mammals are usually larger and heavier than females. Maximum body weight in adult male and female Wistar rats has been reported to be 677.3 ± 9.2 g and 463.3 ± 8.6 g, respectively, and is attained by postnatal day 100 in males and slightly sooner in females (Ghasemi et al., 2021). These differences in weight between males and females have been attributed to being affected by sex hormones as testosterone enhances muscle growth and influences bone density, while estrogens inhibit female growth (Quirós et al., 2020). As reported by Tripton (2001), male rats have a bigger body mass and a faster growth rate due to the effects of testosterone on the regulation of body growth, while estrogens in females suppress growth rate and muscle hypertrophy.

The percentage of macronutrients in the rats of this experiment showed that males had an approximate protein: lipids: carbohydrates ratio of 69P:17L:2C while females had less crude protein and higher values of total fat, 51P:37L:2C (Table 1). It was found that testosterone also improved net muscle protein balance by stimulating muscle protein synthesis, decreasing muscle protein degradation, and improving the utilization of amino acids (Bhasin et al., 2003). Another factor that could be explained by the effect of hormones is the higher values of total fat observed for the female group. The accumulation of crude fat in females begins to increase at puberty and persists throughout adulthood, giving them a higher body fat percentage than males (O'Sullivan, 2009). A low testosterone level or testosterone deficiency deregulates lipid and glucose metabolism, resulting in increased adiposity in the liver and peripheral tissues (Baik et al., 2020). This means that the low concentrations of testosterone in females can be associated with decreased muscle mass and increased fat mass, which was in accordance with the findings of a study by Singh et al. (2003) on mice. Estrogen, as well as testosterone, affects adipocyte physiology. Due to estrogen's ability to reduce postprandial fatty acid oxidation, more fat is stored in the body, explaining the increased body fat in females (O'Sullivan, 2009). The effect of estrogen in males is not like that of females so estrogen is insufficient to promote body fat in males (Bell, 2018).

Regarding the mineral analysis, male and female rats differed significantly in terms of calcium percentage at 2.61% and 0.54%, respectively. Calcium is the most abundant mineral in the body, followed by phosphorus, and greater than 99% of it is stored in bone tissue in vertebrates (Institute of Medicine, 1997). The remainder is present in the blood, extracellular fluid, muscle, and other tissues, where it plays a role in mediating vascular contraction and vasodilation, muscle contraction, nerve transmission, and glandular secretion (Institute of Medicine, 1997). The only study that recorded calcium levels in rats was the one conducted by Sherman and MacLeod (1925). After they recorded the calcium content of the body in different ages of rats of both sexes, the findings indicated the fact that pubertal males have more calcium percentage than females. At 15 and 30 days of age, the females presented slightly higher values of calcium than males, but from 60 days onward, calcium content slowly increased in males, which was even above that of the female rats (Sherman and MacLeod, 1925). As concluded, the gross weight of calcium in the body is higher for males than females since the average weight of the males is greater, but more recent studies have shown other factors like the physiology of calcium absorption that may explain these differences.

Calcium is absorbed by active transport (dependent on Vitamin D) and passive diffusion across the intestinal mucosa (Institute of Medicine, 1997). Intestinal absorption is predicted to be halved in 2 months old females, compared with young males, mainly because vitamin $D_3$ levels are 50% lower in females (Granjon et al., 2016). While this will affect the amount of calcium absorption in the bones, it does not fully explain the reason females in this study had 4 times less calcium than males. Another factor could be the effect of sex hormones, which is similar to the effect they have on body mass, protein concentration, and fat gain. Testosterone is responsible for mediating bone mineral quantity in males by stimulating their body growth and thus increasing bone size and mass, while estrogens in females affect the development of the appendicular skeleton by suppressing their growth (Quirós et al., 2020). Zhang et al. (1999) studied the effects of gonadectomy on bone values in male and female rats and indicated that testosterone and growth hormone are growth-promoting in growing male rats, producing independent effects on bone size and mass. Moreover, they revealed that in growing female rats, estrogen was growth limiting at appendicular sites.

Phosphorus concentrations were higher in males (0.98%) than females (0.47%), which then showed higher levels of potassium (0.46%), compared to the values shown in males (0.25%). Mineral concentrations and the effects of gender on rats have not been widely studied. Variability in trace element composition can be caused due to several possible reasons, including the influence of different dietary trace mineral levels, gender-specific metabolism, varying accuracy of analytical techniques, and contamination of the samples (Dierenfeld et al., 2002). An old study performed by Sherman and Quinn (1926) determined phosphorus content in rats and no other recent studies were found in this area. They recorded that after 15 days of age phosphorus concentrations averaged higher in the males than the females because of their greater average body weights. The concentrations in males and females were the same by day 21 and then females showed higher percentages of phosphorus than males of the same age from day 28 onwards (Sherman and Quinn, 1926).


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This means that age could act as a factor that leads to a higher percentage of phosphorus in males since male rats in this study were 6 weeks younger than females.

## CONCLUSION

Comparisons between the results of this study and the nutritional requirements of certain small carnivores showed that rats could provide the necessary nutrients, however, if given as a sole diet, they could not sufficiently supply the requirements these animals need in the long term. While male rats would be a better option for crude protein and calcium requirements of most carnivore animals, females fulfill best the target nutrient level of total fat. Differences found in growth rate, nutrient, and mineral composition can be attributed to the effect of sex hormones in males and females since testosterone and estrogens impact body growth, fat storage, and bone size and mass. The rat's gender should be taken into consideration since the level of nutrients an animal can obtain will differ if given a male or female rat as feed. Solid conclusions are difficult to make since many factors can affect the nutritional composition of a feed, such as age, diet, and storage time after death. Further studies are needed to be made to see the effect of those variations, as well as the exact nutritional requirements for each species of animal, desired to feed.

## DECLARATIONS

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### Competing interests

The author declares that there are no competing interests.

### Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and or falsification, double publication and or submission, and redundancy have been checked by the author.

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