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The influence of behavioral enrichment on dry food consumption by the black tufted-ear marmoset, *Callithrix penicillata* (Mammalia: Callithricidae): A pilot study

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ABSTRACT. Stereotyped behaviors in captive primates are often caused by unsuitable conditions. Environmental enrichment has been used to reduce these behaviors, and also to increase the frequency of behaviors appropriate to the species. In this pilot study we evaluated whether behavioral enrichment influences food intake by the black tufted-ear marmoset, *Callithrix penicillata* (É. Geoffroy Saint-Hilaire, 1812), by calculating energy maintenance requirements. We evaluated 16 individually housed, healthy adult black tufted-ear marmosets, randomly divided into two treatment groups, one with behavioral enrichment and one without. The enrichment techniques included structural aspects, such as placing fixed and mobile objects in the cage and supplying dry foods in an enriched form, in order to stimulate cognition. Based on the metabolic weight of the animals, we calculated the energy requirements for their maintenance. The animals that received behavioral enrichment consumed more food than those that did not. We also observed that the animals that did not receive enrichment consumed 9.85% less food than had been calculated for energy maintenance requirements, while the animals that received enrichment consumed 24.97% more food than had been calculated. Results indicate that the use of behavioral enrichment items raised the energy requirements of the black tufted-ear marmoset and, therefore, the consumption of dry food, suggesting that environmental enrichment plays a role in stimulating food consumption. This conclusion should alert scientists, technicians and primatologists to the importance of controlling body weight of marmosets when introducing environmental enrichment to avoid overfeeding and obesity. To verify this conclusion, a study is needed with a longer time frame and more parameters, such as behavior observation and body weight.

KEY WORDS. Animal welfare; energy; nutrition, maintenance.

Unsuitable captivity conditions are responsible for the development of stereotyped behaviors such as pacing, rocking from side to side, self-mutilation, inactivity (Boorer 1972, Hart *et al*. 2009), inappropriate sexual, social and maternal behaviors (Davenport 1979) and hypersexuality (Hediger 1969). Frequent stereotyped behaviors observed in primates in social isolation include rocking, self-directed orality, beating themselves up, embracing themselves, bizarre postures, walking the same path for long periods, unnecessary repetitive moves, biting, hyperphagia, and polydipsia (Erwin *et al*. 1973, Mason 1991, Boere 2001). To reduce these abnormal behaviors, and also increase the frequency of behavior appropriate to the species, environmental enrichment has been suggested as an efficient counterbalance (Newberry 1995, Boere 2001, Young 2003). This approach leads to an improvement in the biological functioning of the animal by increasing reproductive success and boosting physical health as a result of changes to its environment (Newberry 1995).

It has been observed that, given the option between foraging for food and receiving food, many species of primates prefer the former (Neuringer 1969). Wild animals spend much of their time in foraging activity (Reinhardt 1993). In nature, the animal searches for foodstuffs that meet its needs and satisfy its palate; in captivity, however, it is restricted to one type of diet. To minimize the stress of animals in captivity, many forms of behavioral enrichment have been used (Newberry 1995). Food-related enrichment presents significant results when more varied food items are offered, and this is further improved when animals need to search for and handle the foods (Pereira *et al.* 1988). The diet can be provided in an interesting and enriching way, stimulating motor and cognitive performance and occupying the captive individual's time (Bloomstrand *et al.* 1986).

The vertebrate metabolism has been widely studied, and knowledge is largely based on the work of Kleiber, conducted in the 30's and 40's. This researcher showed that the relationship between metabolic rate and body mass is not linear, and proposed the exponent of mass 0.75 to express the basal metabolism in relation to body mass in interspecific comparisons (McMahon 1983, Withers 1992). Exploring the concept of metabolic weight, Kleiber (1961) concluded that the basal metabolic rate could be expressed as 70 x $PV^{0.75}$ kcal per day both for a mouse weighing 0.021 kilograms and for a cow weighing 600 kg. The energy requirements for primates are determined by calculating the energy maintenance requirements obtained through metabolic weight of the animal. This equation has been used to calculate the amount of food required for a species and in studies about obesity and calorie restriction for animals in captivity (Raman *et al.* 2007), but there are no studies on the relationship between environmental enrichment and energy requirements.

The black tufted-ear marmoset, *Callithrix penicillata* (Saint-Hilaire, 1812) is an animal used in biomedical research, and present in other sorting centers. Marmosets are easily bred in captivity, giving birth to twins approximately every five months, so that colonies can be obtained with relatively few problems (Vitale & Manciocco 2004). However, in captivity, these animals are socially isolated, and this is considered to trigger stress. Because environmental enrichment is applied to these animals in order to reduce stress in captivity, promote animal well-being and increase reproduction rates (Muhle & Bicca-Marques 2008), we hypothesized that, if environmental enrichment is applied to Callithrichids, their energy requirements would increase. Thus, the aim of this pilot study was to compare the intake of the black tufted-ear marmoset with and without behavioral enrichment and the modulation of consumption by the energy needs for maintenance. We expected that the animals with enrichment would consume more dry food than those without enrichment.

MATERIAL AND METHODS

The experiment was conducted at the Laboratório de Metabolismo Animal, Departamento de Zootecnia, Universidade Federal de Minas Gerais, municipality of Belo Horizonte, state of Minas Gerais, Brazil (19°55'15"S, 43°56'16"W). Sixteen healthy and vaccinated adult black tufted-ear marmosets, male and female, were housed in individual cages made of galvanized wire, equipped with feeders and individual drinkers with water at will. The animals were from the Sorting Center for Wild Animals of the Brazilian Institute of the Environment and Natural Resources (IBAMA) in Belo Horizonte.

Throughout the experimental period the Callitrichids were housed in a closed metabolism room heated with air conditioning to maintain a comfortable temperature – the maximum temperature recorded was 29.8°C and minimum 22.4°C. Each Callitrichid was sheltered individually in a metabolic cage, taking the precaution of keeping an empty cage between the animals to prevent food exchange between them. The metabolic cages used were made according to the models used for testing digestibility in rabbits, with galvanized sheet metal on the sides. A tray was placed under each cage for collection of leftover food. A wooden perch was placed in each cage for use by the animals.

The animals were randomly divided into two treatment groups: those which did not receive behavioral enrichment ('no enrichment') and those which did ('with enrichment'). The enrichment techniques included structural aspects, such as the placement of fixed (nets and trunks) and mobile (toys, rings, mirror, a branch with leaves) objects, and dry foods were supplied in an enriched form (using plastic bags, colored bags, sponges and putting the food above the cage), in order to stimulate cognitive aspects (Figs 1 and 2). There were eight animals per treatment, housed individually to calculate the dry food consumption of each animal. Daily animal care procedures consisted of collecting the remains of food, renewing the drinking water and cleaning the cage. The diet was offered through an adjustment period of seven days followed by five days of collecting remains. The animals were weighed at the beginning of the trial period for the determination of energy maintenance requirements and were provided with 70 g of dry food per day in the form of extrudated pellets. The average daily intake was determined by the difference between what was provided and what was collected. The composition of the dry food given to the animals is represented in Table I.

To calculate the energy requirement for maintenance (ERM), we used the equation suggested by National Research Council (2003), assuming an animal of moderate activity and under thermal comfort: ERM = 145 Kcal ME x $BW^{0,75}$; where (ERM) Daily Energy Requirement for Maintenance, (ME) Metabolizable Energy, and (BW) Body Weight.

Taking the average metabolic weight of the animals, the average estimated consumption of metabolizable energy for maintenance (ERM) was determined. From the intake, through the metabolizable energy predicted by the manufacturer, the actual consumption of metabolizable energy per animal was calculated.

After the experiment, all animals were sent to Fazenda Vale Verde, municipality of Betim, Minas Gerais (19°58'04"S,

Figures 1-2. (1) A black tufted-ear marmoset interacting with environmental enrichment; (2) interaction of the marmoset with food given inside a bag to stimulate cognitive aspects.

Table I. Composition of the dry food given to the animals.

	Guarantee levels
Metabolizable energy (Mín.)	3,200 kcal
Humidity (Max.)	12.0%
Crude Protein (Min.)	25.0%
Ether extract (Min.)	8.0%
Fibrous matter (Max.)	3.0%
Mineral matter (Max.)	10.0%
Calcium (Max.)	1.5%
Phosphorus (Min.)	0.75%
Bacillus subtilis	6.4*105 UFC/g
Bacillus licheniformis	6.4*105 UFC/g
Linoleic Acid	2.5%
Linolenic Acid	0.5%
Mannanoligosaccharides	0.15%
Fructoligosaccharides	0.8%

44°11'54"W), an educational park and conservationist farm for wild animals that cannot be reintroduced to the wild.

All procedures were reviewed and approved by the IBAMA, license number 14931-1, and by the Ethics Committee and Animal Experimentation at the Federal University of Minas Gerais, under protocols 28/2008 and 63/2008.

The evaluated parameter was subjected to analysis of variance by the SAS General Linear Model (SAS 1990); Fisher's test was chosen to compare means. Data were expressed as mean ± SD (standard deviation), and values of $p < 0.05$ were considered statistically significant.

RESULTS

The animals which did not receive behavioral enrichment showed an appropriate adjustment in their consumption of dry food; that is, the values found by calculating the ERM were very close to the real value of consumption. In the second group, with behavioral enrichment, the animals increased ($p < 0.05$) consumption by almost 36% compared to the first group (Tab. II).

The animals that received no enrichment consumed an average of 19.57g of dry food, and the estimated predicted consumption was 21.71g, so there was a drop of 9.85% from estimates. The opposite occurred with the animals that received behavioral enrichment, which consumed an average of 27.67g of food, while the estimated predicted consumption was 22.14 g, generating an increase of 24.97% in consumption.

DISCUSSION

Many articles mention the importance of behavioral enrichment for the welfare of various species (Vitale & Manciocco 2004, Boere 2001, Newberry 1995, Day *et al.* 2002, Siloto *et al.* 2009), but this is the first study regarding the intake of food linked to behavioral enrichment in primates.

In nature, callithricids are known to feed on fruits, flowers, plant exudates, insects, spiders, slugs, lizards, frogs and

Table II. Values of the predicted and actual food intake.

Treatment	Body weight (g)	Mean of ERM (kcal)	Dry food consumption predicted according to ERM (g)	Observed dry food consumption (g)	Consumption difference (%)
No enrichment	375 ± 65	69.48	21.71	19.57[b]	-10.93
With enrichment	385 ± 50	70.87	22.15	27.67[a]	24.92

ERM = Daily Energy Requirement for Maintenance. Coefficient of variation of 18.29%. Means followed by different letters in the same column differ significantly by Fisher's test at $p < 0.05$.

birds' eggs (Stevenson & Rylands 1988, Crissey *et al.* 2003), and there is a foraging technique for each item (Sussman & Kinsey 1984). Captive Callitrichids that are given a limited variety of succulent foods may not select food by its nutritional content; instead, they are likely to select those that are high in sugar, high in fat, or simply novel. Thus, it is important to offer foods that complement each other nutritionally (Price 1992). Another important factor for the well-being of non-human primates is the social aspect. The presence of a compatible conspecific in the enclosure is considered the greatest enrichment measure for primates (Reinhardt & Reinhardt 2000). However, in this study, the animals had to be kept in individual cages and given only dry foods in order to calculate the exact amount of food each animal was consuming. This was a temporary condition – after the experiment, the animals were given fruits and vegetables together with the dry food, and were housed with conspecifics at Fazenda Vale Verde.

Institutions that keep animals in captivity, such as zoos, safari parks or research facilities, may not provide the key environmental factors that encourage and preserve species-typical behaviors in the wild (Dawson 2009), such as foraging, finding shelter, intraspecific relationships and anti-predator behavior. In captivity, the environment loses significance and the individual remains in a constant state of boredom. "Environmental poverty", defined as inappropriate social and physical surroundings, compared to their ideal needs, can trigger a series of extreme non-adaptive responses (Boere 2001). Therefore, it is commonly believed that making an enclosure more natural improves the captive animal's wellbeing; it is considered an enrichment that can help to reduce non-adaptive responses besides increasing the frequency of behaviors appropriate for the species (Newberry 1995, Boere 2001, Young 2003). In this context, the significant increase in dry food consumption seen among members of the group that received environmental enrichment can indicate a reduction in the state of boredom and an improvement in physiological responses.

The modulation of food consumption for primates, according to National Research Council (2003), seems to be associated with the energy needs of the animal and with their concentration in the diet. In general, primates ingest sufficient food to meet their energy requirements. However, some primates in captivity unconsciously over-eat and become obese (National Research Council 2003). Animals that go through moderate dietary restriction can adjust their energy consumption to their maintenance needs, through devices such as decreasing the production of muscle tissue and reducing physical activity (Ingram *et al.* 1990). In our study, the animals that did not receive enrichment consumed less dry food than expected. This excludes the possibility of animals over-eating because they are in captivity; thus, results suggest that the increase in intake was due to the increased energy requirements of the animals that received environmental enrichment. Moreover, the fact that the dry food was provided with enrichment may have increased the animals' interest in their food, causing an increase in consumption. The results suggested that behavioral enrichment contributes to stimulating food consumption and reducing stress for primates in captivity.

This was a pilot study conducted to verify if environmental enrichment influences food intake. As such, it was a relatively small experiment designed to test logistics and gather information prior to a larger study, with the aim of improving the latter's quality and efficiency. This type of study is very important, because it can reveal deficiencies in the design of a proposed experiment or procedure and these can then be addressed before time and resources are spent on large-scale studies (NC3Rs, 2006). In this context, results indicated that the use of behavioral enrichment items raised the energy requirements of the black tufted-ear marmoset and, therefore, the consumption of dry food, suggesting that environmental enrichment is important in stimulating food consumption. Environmental enrichment stimulates behavior such as exploration, locomotion and reduction of sleep. So, this conclusion may alert scientists, technicians and primatologists to the importance of controlling body weight of marmosets when introducing environmental enrichment to avoid overfeeding and obesity. However, this conclusion needs to be verified with a study involving a longer time frame. Other parameters that could corroborate the results would be a record of each animal's behaviors and weight, along with measurements of stress-related hormones before and after environmental enrichment, to verify if the environmental enrichment actually reduced stress for the marmosets or if it was only an adjustment of energy balance to maintain homeostasis.

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