

# Nutritional responses to different diet quality in the subterranean rodent *Ctenomys talarum* (tuco-tucos)

Natalia S. Martino<sup>a,b</sup>, Roxana R. Zenuto<sup>a,b,\*</sup>, Cristina Busch<sup>a</sup>

<sup>a</sup> *Laboratorio de Ecofisiología, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CC 1245, Mar del Plata 7600, Argentina*

<sup>b</sup> *CONICET, Argentina*

Received 30 November 2006; received in revised form 2 March 2007; accepted 2 March 2007

Available online 12 March 2007

## Abstract

Nutritional response to different diet quality was examined in the subterranean rodent *Ctenomys talarum* (tuco-tuco). Animals maintained in captive conditions were fed with three plant species that differed in their fibre content. Tuco-tucos showed the ability to perform adjusts in short time lapse in response to diet quality; food ingestion, egestion and feces ingestion changed in animals under different plant species diets. Time budget, mainly time devoted to feeding and activity accompanied such changes. Coprophagy was practiced along the day and night following the arrhythmic activity pattern found for this species. Feces reingestion was not associated to resting. Furthermore, it was observed during fresh food ingestion, being pellets chewed. Soft and hard feces differed in morphological and nutritional characteristics.

© 2007 Elsevier Inc. All rights reserved.

**Keywords:** Coprophagy; *Ctenomys talarum*; Herbivory; Nutritional physiology; Subterranean rodent

## 1. Introduction

Herbivorous rodents rely on a food source that is considered abundant. However, one of the most remarkable characteristics of plant-herbivore systems is the increased importance for herbivores of changes in food quality since their survival rate and fecundity may be diminished because of food quality despite an apparent abundance of suitable food (Crawley, 1983). As was proposed by modeling fibre digestion and energy utilization, extremely high fibre diet may not provide energy requirements for reproduction and growth, only permitting survival to the next breeding season (Justice and Smith, 1992). Furthermore, some dietary components including protein, fibre content, minerals, secondary compounds and toxins fluctuate with growing season (Cengel et al., 1978; Cole and Batzli, 1979; Provenza et al., 1998).

When digestibility or nutritional value of diet items decline, an increase in the amount of ingested food was the more com-

mon reported response (Batzli and Cole, 1979; Cranford and Johnson, 1989). In addition to adjustments in food intake, changes in turnover rate, gut morphology and digestibility were reported in order to increase efficiency to obtain energy (Cork and Foley, 1991; Bozinovic, 1993, 1995; Young Owl and Batzli, 1998). Frequency of feces ingestion could be affected similarly by changes in food resources: it may remain constant to supply nutrients at a sufficient level, increase to allow longer retention of selected forages or decrease to allow greater food intake and passage rate (Cranford and Johnson, 1989).

Food reingestion results important in small herbivorous rodents with diets characterized with high fibre contents (Kenagy and Hoyt, 1980). They show anatomical adaptations including an extensive lower digestive tract composed of a large caecum and a large intestine (Golley, 1960). Occurrence of coprophagy varies considerably among different taxa and may relate to specific diets (Batzli and Pitelka, 1969; Kenagy and Hoyt, 1980; Oullette and Heisinger, 1980); it was frequent in strictly herbivorous species while granivorous rarely practice this behavior (Kenagy and Hoyt, 1980). As food ingestion is limited to discrete times of day, coprophagy in rodents was described as a rhythmic activity and complementary to feeding,

\* Corresponding author. Tel./fax: +54 223 4753150.

E-mail address: [rzenuto@mdp.edu.ar](mailto:rzenuto@mdp.edu.ar) (R.R. Zenuto).

mainly occurring during the rest phase of daily cycle (Kenagy and Hoyt, 1980).

Subterranean rodents are largely herbivorous, and because food is costly to locate most species are less selective than surface-dwelling rodents (Buffenstein, 2000; Busch et al., 2000). Since subterranean rodents feed primarily on the fibrous portions of the plants, they consume large quantities of cellulose, hemicellulose and lignin. Nonetheless, they are able to obtain adequate energy supply on low quality diets by long retention time in the digestive tract until the fermentation by the microorganisms located in a well-developed hindgut have liberated available energy (Buffenstein and Yahav, 1994; Buffenstein, 2000). Additionally, coprophagy, an effective manner to ingest extra protein (Takahashi and Sakaguchi, 1998) and essential for the utilization of B-complex and K vitamins and essential fatty acids (Barnes, 1962), was reported in geomyids (Wilks, 1962), bathyergids (Jarvis and Bennet, 1991), ctenomyids (Altuna et al., 1998) and also in the fossorial octodontid *Octodon degus* (Kenagy et al., 1999).

*Ctenomys talarum* (Rodentia: Ctenomyidae), namely tuco-tucos, are herbivorous subterranean rodents that inhabit coastal natural grasslands. Most of their activities are concentrated into permanently sealed burrow systems although animals venture away making brief surface excursions from foraging tunnels to gather vegetation. Both sexes and all ages (except pre-weaned young that share their mother's burrow system) are sedentary and maintain exclusive territories (Busch et al., 1989). Captive individuals showed an asynchronous and arrhythmic activity pattern (Luna et al., 2000). Although *C. talarum* forage within their tunnels for roots and subterranean stems, most gathering occurs aboveground cutting plants near the soil level and carrying them into their burrow by running backwards. The aerial vegetative portion of grasses contributed nearly 80% of the annual diet (Comparatore et al., 1995; Busch et al., 2000; del Valle et al., 2001). *C. talarum* was characterized as a generalist and opportunistic herbivore since it consumes the greatest part of the plant species present in the grassland, changing its diet in relation to food availability (del Valle et al., 2001). Cafeteria test confirmed the fact that this species is a generalist rodent with a marked preference for the above ground fraction of grasses although other food items were also ingested, and a varied diet was maintained (del Valle et al., 2001). Preference for stems and leaves of grasses represent an important fibre intake. Hence, coprophagous behavior would play a key role as a mechanism enhancing the efficiency of food utilization in this species. As was reported (Reig et al., 1990), tucos-tucos present a well-developed caecum that represent 11% of total digestive tube length. Nonetheless, coprophagy was not evaluated as part of the feeding strategy in any member of genus *Ctenomys*. Here we tested the hypothesis that nutritional response to diet quality will occur in *C. talarum* due to their high dependence to local food resources characterized by high fibre content. The aims of the present study were (1) to evaluate the nutritional response, in a short-term basis, to different diet quality in the subterranean herbivorous rodent *C. talarum*. The nature of response was determined by measuring body mass dynamics, food consumption, fecal production, diet digestibility and incidence of feces

ingestion in animals fed with three different plant species that differ in fibre content. (2) To quantify time devoted to activity, resting, feeding and coprophagy in a 24-h cycle in animals under the experimental diets; (3) to characterize the coprophagous behavior; and (4) to analyze and compare the composition of feces ingested and non-ingested.

## 2. Materials and methods

### 2.1. Animal maintenance

Adult *C. talarum* individuals were trapped during their non-reproductive season (February–June) in Mar de Cobo locality (37° 45' S, 57° 56' W), Buenos Aires Province, Argentina, using PVC live traps. All animals were housed individually in plastic cages of 45 cm × 32 cm × 25 cm provided with wood shavings. During an adaptation phase (7 days) to captive conditions, they were fed *ad libitum* with a diet composed by a mixed of plant species later used in each experimental condition. Animal room was maintained at 23 ± 1 °C and a 12L:12D photoperiod.

### 2.2. Experimental procedure

Since tuco-tucos do not accept pellets for food, three plant species (grasses) present in the area where animals were captured, were used as experimental diets: (a) *Bromus unioloides* (59.7% Neutral Detergent Fibre/ 10.3% Brute Protein); (b) *Panicum racemosum* (68.4% NDF/ 10.7% BP); and (c) *Cortaderia selloana* (74.4% NDF/ 8.7 BP). Because of *C. talarum* individuals do not drink free water, lettuce was added to experimental diets to ensure water provision.

Once the adaptation phase concluded, 18 individuals were randomly assigned to three experimental groups, each one composed by 3 males and 3 females. Each group was fed *ad libitum* with one of the experimental diets for 5 days. On the sixth day, animals were transferred to “metabolic cages” for the determination of food consumption and feces deposition. These cages had the same characteristics than the housing cages but were provided with an additional wire mesh floor. Since daily ingested fresh food was reported as nearly 200 g for *C. talarum* (del Valle et al., 2001); a surplus of pre-weighted food was provided each 4-h period, and the remaining food collected from the cage at the end of the period, during a complete 24-h cycle. Food consumption (available food – remaining food) was corrected for water content. The dry matter mass of each diet was determined after weighting 12 replicate 50-g samples oven-dried at 70 °C for 48 h. The determination of apparent dry matter digestibility of the three diets was obtained by the calculation: (total dry mass of food consumed – dry mass of feces produced)/total dry mass of food consumption (Kenagy et al., 1999). Feces and plants were also oven-dried at 70 °C to constant mass during 48 h. Additionally, apparent fibre digestibility was calculated by the relation between fibre content of ingested food and fibre content in feces (NDF). Fibre content in plant species and feces were obtained on a dry matter basis at the Laboratory of Animal Nutrition and Forage Quality

Table 1  
Nutritional parameters recorded in *C. talarum* fed with three plant species used as experimental diets

	<i>B. uniolooides</i> (n=6)	<i>P. racemosum</i> (n=6)	<i>C. selloana</i> (n=3)
<i>Body mass change</i>			
g	3.17±6.11	-2.25±4.01	-22.31±6.30*
%	3.17±5.39 <sup>a</sup>	-1.49±2.89 <sup>a</sup>	-16.42±3.42 <sup>b,*</sup>
<i>Food consumption</i>			
g/day	38.66±7.38	24.37±3.93	11.67±2.19
g×day <sup>-1</sup> ×body mass <sup>-1</sup>	0.30±0.09 <sup>a</sup>	0.19±0.04 <sup>a,b</sup>	0.12±0.03 <sup>b</sup>
<i>Fibre consumption</i>			
g/day	23.08±4.40	16.67±2.69	8.69±1.63
g×day <sup>-1</sup> ×body mass <sup>-1</sup>	0.18±0.06 <sup>a</sup>	0.13±0.03 <sup>a</sup>	0.09±0.02 <sup>a</sup>
<i>Feces production</i>			
g/day	6.29±2.47	12.61±1.84	3.89±0.71
g×day <sup>-1</sup> ×body mass <sup>-1</sup>	0.05±0.01 <sup>a</sup>	0.10±0.01 <sup>b</sup>	0.04±0.01 <sup>a</sup>
<i>Diet digestibility</i>			
Apparent dry mass digestibility	0.82±0.05 <sup>a</sup>	0.48±0.08 <sup>b</sup>	0.67±0.01 <sup>a,b</sup>
Apparent fibre digestibility (NDF)	0.81±0.04 <sup>a</sup>	0.41±0.10 <sup>b</sup>	0.68±0.01 <sup>a,b</sup>
<i>Reingestion</i>			
Feces production (number of pellets)	360±42.3 <sup>a</sup>	323.8±70.39 <sup>a,b</sup>	210±56.93 <sup>b</sup>
Feces ingestion (number of pellets)	139±7.9 <sup>a</sup>	61±19.27 <sup>b</sup>	97.6±6.42 <sup>a,b</sup>
%	38.56±0.65 <sup>a,b</sup>	19.78±7.73 <sup>a</sup>	48.14±9.13 <sup>b</sup>

Values are given as mean±SD. Different letters denote statistical significant differences between diets.

\*n=8.

Evaluation, INTA, Balcarce. Body mass of individuals were registered to the nearest 0.1 g each day (at the same hour) during 1 week since experiments started.

For the determination of incidence of coprophagy (number of feces consumed/ number of feces produced), all animals from the three treatments were video-taped during a 24-h cycle. Each tuco-tuco was placed in a 40 cm×30 cm×30 cm acrylic box mounted on a rack, underneath which a mirror was fixed at a 45° angle (as was described in Kenagy and Hoyt, 1980). The corresponding diet was provided *ad libitum* and the animal room where the observations were made was maintained at 23±1 °C and a 12L:12D photoperiod. These continuous visual

observations allowed both to record timing of resting, activity, feeding and coprophagy for a complete 24-h period and a complete description of coprophagous behavior.

Animals under *B. uniolooides* diet were observed during a daily cycle (as described above), and percentages of occurrence of different types of behavior (activity, resting and feeding—fresh food ingestion) before and after coprophagy were calculated. Each kind of behavior was recorded as a discrete event independently of time elapsed.

Feces required for its characterization were collected from one tuco-tuco at a time. Animals fed with *B. uniolooides* for 1 week were carefully observed on the 7th day of treatment and each time a fecal pellet (soft feces) was taken directly from the anus with incisors, coprophagy was prevented using forceps. Hard feces were directly collected from the cage floor. For both kinds of feces, morphology, color pattern, size and texture was recorded. Determination of pH was done from ten soft and hard fecal pellets collected from three individuals. Each fecal pellet was dissolved in 120 µl of distilled water and the pH of the solution was obtained using pH paper (Merck®). Quantification of bacteria present in feces was done using dilution methods. Microorganisms from each kind of feces were developed in Plate Count Nutritive Agar (Hi-media®) by pour plate quantitative plating method (Seeley et al., 1991). A duplicate was done for each individual sample (n=3). Incubations were performed during 24 h at 30 °C in dark conditions. Additionally, for the determination of dry mass and ash, 20 fecal pellets from three individuals were individually oven-dried to constant mass (60 °C) and then incinerated during 5 h at 550 °C. Time elapsed in formation of hard feces (transit time) was obtained recording the time of first ingestion of food and time of first deposition of a colored fecal pellet. *B. uniolooides* plants, previously stained by immersion into a solution of blue edible stain for 24 h, were used for feeding those animals (n=3).

### 2.3. Statistical analysis

Mean values for the variables measured as physiological responses to different diet quality were evaluated using the non-parametric Kruskal–Wallis test. Dunn's test was used as a posteriori multiple comparison method ( $p<0.05$ ). Parameters measured for soft and hard feces characterization are presented as descriptive data due to small sample size. Data were reported as mean±SD. We used the Statistical package Statistica 6.0 (StatSoft, Inc., 2001) for data analysis.

Table 2  
Body mass dynamics of *C. talarum* individuals fed with three plant species used as experimental diets

Diet	Sex	Initial body mass	Final body mass	Body mass change (g)	Body mass change (%)	n
<i>B. uniolooides</i>	Male	166.58±11.01	165.61±9.80	-0.97±1.5	-0.55±0.91	3
	Female	104.61±10.95	111.91±14.38	7.30±6.30	6.89±5.49	3
<i>P. racemosum</i>	Male	174.36±16.45	171.10±13.93	-3.25±3.68	-1.78±1.89	3
	Female	116.65±6.33	115.40±10.87	-1.25±4.87	-1.21±4.13	3
<i>C. selloana</i>	Male	147.14±30.44	121.77±27.26	-25.37±5.61	-17.43±3.37	5
	Female	117.06±3.05	99.84±6.11	-17.22±3.71	-14.75±3.39	3

Values are given as mean±SD.

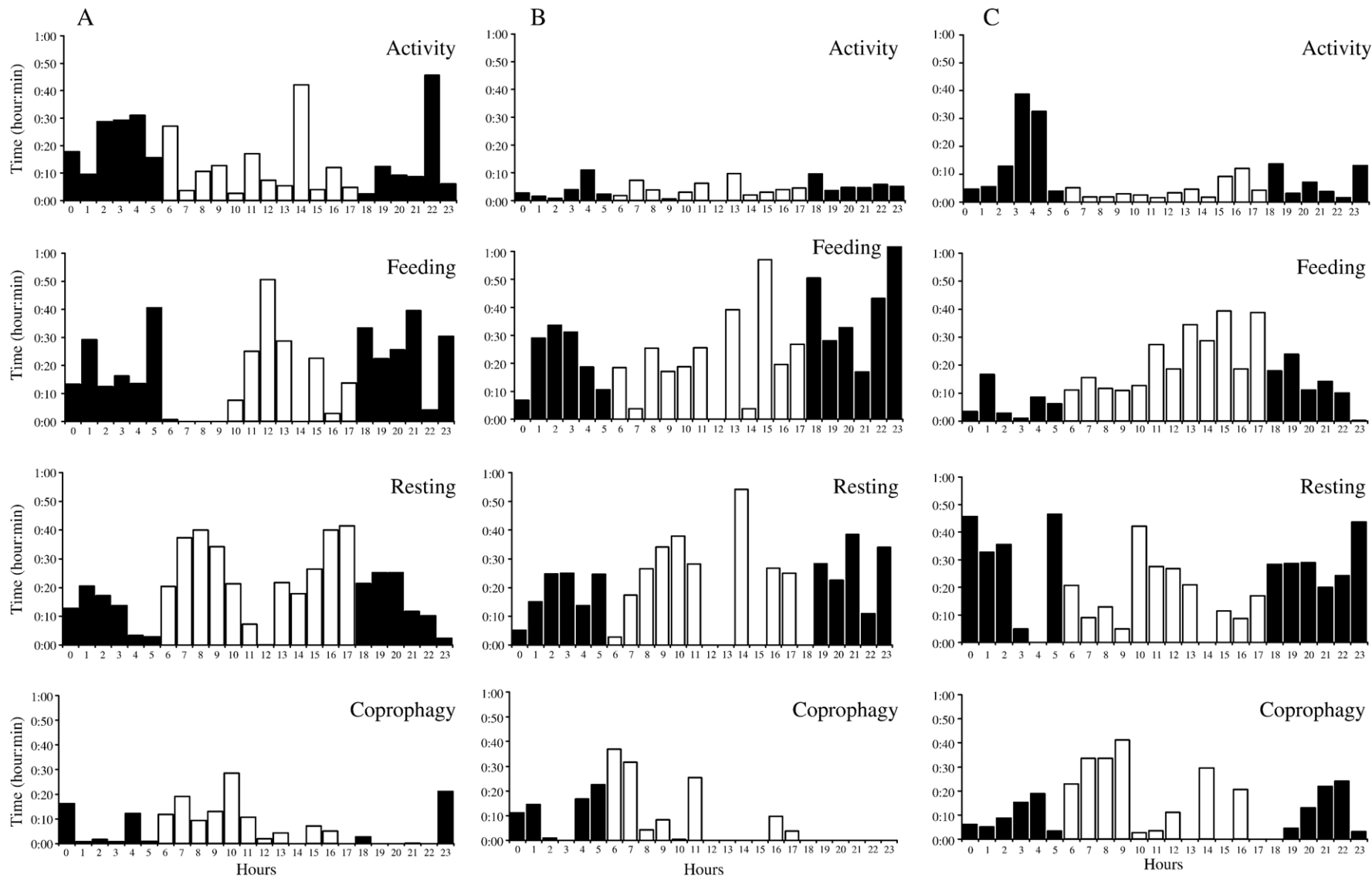


Fig. 1. Time spent in different behaviors during a 24-h cycle by a single tuco-tuco under different diet conditions. (A) *B. unioides*, (B) *P. racemosum*, (C) *C. selloana*. Black and white bars represent the dark and light hours.

### 3. Results

#### 3.1. Nutritional parameters

*C. talarum* showed body mass changes related to different diets (Kruskal–Wallis;  $H=14.9$ ,  $df=2$ ,  $p=0.0006$ ). Animals fed with *C. selloana* suffered significant body mass decrease (as percentage of initial body size) than animals under *P. racemosum* and *B. uniolooides* diets (Dunn test  $p<0.05$ ; Table 1). Males seem to be the more labile to sex than to food quality; they lost body mass (although in different degree) under the three diet conditions, while females increased body mass (*B. uniolooides* diet) or suffered a lesser decrease (*P. racemosum* and *C. selloana* diets; Table 2), but this tendency should be confirmed with larger sample sizes. Both sexes lost body mass during the day 1 to day 3 under *C. selloana* as exclusive diet. However, female body mass stabilized since the fourth day while males continued their body mass decrease, also accompanied by a clear activity decline. Then, males in that condition were removed from the experiments and a diet composed by mixed vegetables and grasses allowed them to recover their initial condition in few days. Response of males to *C. selloana* treatment was confirmed in two additional individuals. As consequence, food consumption, feces production, feces ingestion, diet digestibility and continuous observation of animals during 24 h cycle could not be performed in males under *C. selloana* diet.

Mass-specific rates of food consumption by tuco-tucos were affected significantly by diet quality (Kruskal–Wallis,  $H=7.55$ ,  $df=2$ ,  $p=0.023$ ). Mean consumption of high quality diet (*B. uniolooides*) was greater than consumption of lower quality diet (*C. selloana*; Table 1). Such differences in food consumption and fibre content of diets resulted in no differences in mass-specific rate of fibre consumption among treatments (Kruskal–Wallis;  $H=5.54$ ,  $df=2$ ,  $p=0.063$ , Table 1). Feces production was also associated with differences in diet quality (Kruskal–Wallis;  $H=10.8$ ,  $df=2$ ,  $p=0.0046$ ). Collection of feces in animals housed in metabolic cages may have resulted in an underestimation of actual fecal production. Tuco-tucos

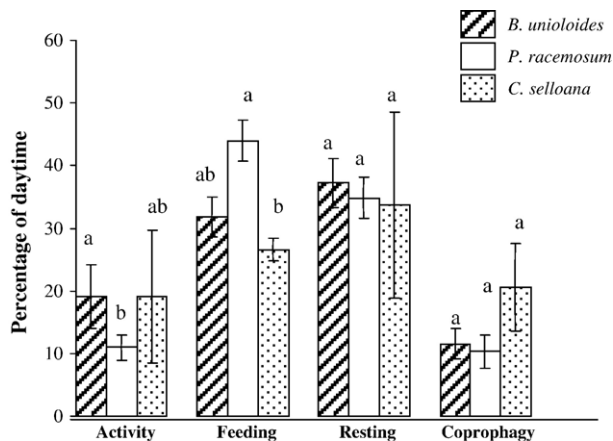


Fig. 2. Percentage of daytime spent in different behaviors during a 24-h cycle. Values are given as mean±SD. Different letters mean significant differences between diets.

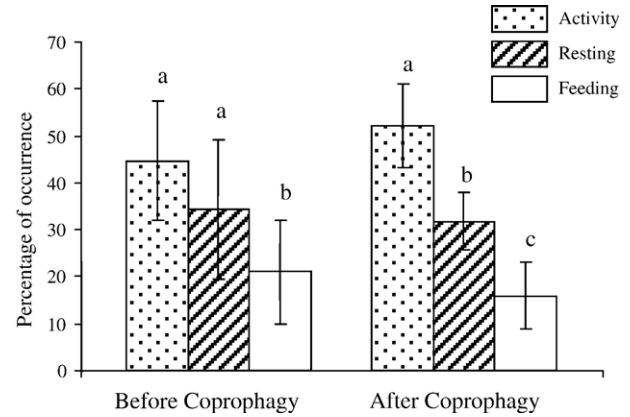


Fig. 3. Percentage of occurrence of different behaviors in relation to coprophagy in animals fed with *B. uniolooides* diet. Values are given as mean±SD. Different letters mean significant differences between diets.

showed two modalities of coprophagy: picking up pellets from the floor and, more frequently, obtaining feces directly from the anus. Only the first modality was prevented by the methodological procedure used in this study. Digestibility of diets were significantly different (Table 1) both in dry matter and fibre basis (Kruskal–Wallis;  $H=12.2$ ,  $df=2$ ,  $p=0.023$  and  $H=12.2$ ,  $df=2$ ,  $p=0.023$ , respectively). *B. uniolooides* diet presented the higher digestibility index and differed significantly from *P. racemosum* diet (Dunn test  $p<0.05$ ). Percentage of reingestion was affected by diet quality (Kruskal–Wallis;  $H=96$ ,  $df=2$ ,  $p=0.008$ ); individuals fed with *C. selloana* showed the higher proportion of feces ingestion and differed significantly from those fed with *P. racemosum* (Dunn test  $p<0.05$ ). Feces production (sum of feces ingested and no ingested) did not differ between *B. uniolooides* and *P. racemosum* diets whereas verified reingestion was higher for *B. uniolooides* than *P. racemosum*. Reingestion rate was not different between animals fed with *B. uniolooides* and *C. selloana* diets, but feces production was higher for the first one.

#### 3.2. Twenty-four-hour rhythms of activity, feeding, resting and coprophagy

Continuously observed *C. talarum* over a 24-h cycle showed an arrhythmic pattern of activity, feeding, resting and coprophagy irrespective of diet quality (Fig. 1). Coprophagy occurred during both day and night and an association between that activity and others such as feeding or resting was not clear. Percentage of time devoted to different kind of behaviors was related to diet quality (Fig. 2). Time spent in activity during a daily cycle differed significantly between individuals fed with *B. uniolooides* and *P. racemosum* (Kruskal–Wallis,  $H=6.26$ ,  $df=2$ ,  $p=0.033$ ; Dunn test,  $p<0.05$ ). Also time spent feeding differed between diets, as occurred in the case of *P. racemosum* and *C. selloana* (Kruskal–Wallis,  $H=10.5$ ,  $df=2$ ,  $p=0.0001$ ; Dunn test,  $p<0.05$ ). A different pattern was verified for coprophagous and resting behaviors, for which no differences were detected between individuals fed with different quality diets.

### 3.3. Coprophagous behavior

Two different modalities of coprophagy were distinguished in *C. talarum*:

- (1) Direct coprophagy: an animal sitting on its hindquarters bent the head in direction to the anus. Contractions of body flanks preceded each act of coprophagy. Fecal pellets were taken using the incisors although in few opportunities the forepaws are also used to manipulate feces. Olfaction of feces extruding from the anus was frequently detected. Each ingested fecal pellet was always chewed before swallowing. Reingestion was detected also during ingestion of fresh food.
- (2) Indirect coprophagy: tuco-tucos also ingested feces collected from floor cages although in a lesser degree, representing only a 6% of total reingestion. Feces were also tested by olfaction and individuals used the incisors and forepaws to manipulate them. In several cases, feces were not eaten completely and half-feces with a characteristic chisel-cut were frequently abandoned on floor cages used for housing animals.

Percentages of occurrence of different behaviors previously to direct coprophagy were not similar (Kruskal–Wallis,  $H=6.26$ ,  $df=2$ ,  $p=0.046$ ). Reingestion was preceded mainly by activity or resting and in a lesser degree, feeding (Fig. 3). Similarly, co-

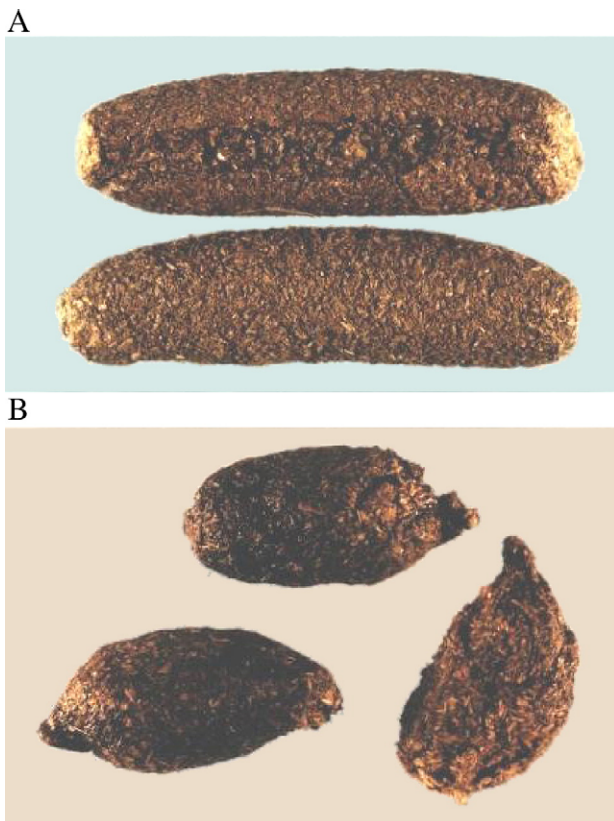


Fig. 4. Fecal pellets produced by *Ctenomys talarum*. (A) Pellets not ingested (hard feces); (B) pellets ingested (soft feces).

Table 3

Comparison of soft and hard feces collected in *C. talarum*

	Soft feces	Hard feces
% Dry matter	37.15±3.34	51.20±4.52
% Ash	9.62±1.36	12.67±2.77
pH	6.4±0.1	7.4±0.4
Transit time	no data	290±22 min
Bacteria content ( $\times 10^7$ )	85246±59672	465±286
Length (mm)	7±0.04	11.52±0.71

Values are means ( $\pm$ SD) for three adult animals fed with *B. unioides*.

prophagy was not followed by different behaviors in the same proportion (Kruskal–Wallis,  $H=12.5$ ,  $df=2$ ,  $p=0.002$ ); activity, feeding and resting resulted in importance order.

### 3.4. Feces characterization

Feces under direct coprophagy (soft feces) differed from feces found in the bedding (hard feces). Soft feces were drop-shaped and presented a dark color while hard feces were enlarged, with two distinguishable parts according to their color: a dark and a green one (Fig. 4). Such differences in color were related to particle size; it was fine in the dark zone and coarse in the green zone of hard feces while in soft feces particles were mostly fine and intermediate in size. The two kind of feces differed in percentage of dry mass, percentage of ash, pH, bacteria content and size (Table 3).

## 4. Discussion

The study of nutritional parameters allows the connection between the physiological process occurring in the digestive tract and the behavioral ecology perspective of foraging. Digestive efficiency could influence food intake, time budgets and at last the resultant energetic and nutritional rates (Mangione and Bozovic, 2002). In this sense, nutritional response found in *C. talarum* individuals under different diet quality comprised both physiological and behavioral adjustments, being reingestion an important component in the short-term basis.

*C. talarum* under the more favorable diet (*B. unioides*) gained or maintained their body weight whereas a considerable body weight loss was recorded with the lower quality diet (*C. selloana*). Contrary to that expected, higher food consumption was found in animals with the best diet quality. Feces production and digestibility were quite similar for this two extreme diet conditions. Digestibility found for the most favorable condition was high, nearly 80% and was obtained performing 40% reingestion of produced feces. Animals under the lowest quality diet consumed less food and produced a small quantity of feces, but reached a good digestibility record (nearly 70%) reingesting 48% of their feces production. The intermediate condition (*P. racemosum* diet) allowed animals to maintain body weight with an intermediate level of food intake but a significantly higher egestion. Digestibility was lower than that found in animals under the most fibrous diet (48%) and reingestion represented only 20% of produced feces. Lower

retention times were related to low digestibility (Batzli, 1985). Transit time for *B. uniolooides* (Table 3) was higher than that obtained for *P. racemosum* ( $157.2 \pm 31.45$  min; Perissinotti, 2003) and could explain the observed differences in digestibility levels found both in dry mass and fibre basis. Nonetheless, both lower digestibility and retention times were related to high fibre content (Woodall, 1989), and then lower digestibility would be expected for the most fibrous *C. selloana* diet. Presence of trichoma structures, a physical antiherbivory component found in *P. racemosum* may be responsible for both the high production of feces in animals fed with this plant and the lower retention times of this food item, determining lower digestibility efficiency. Unfortunately, transit time for *C. selloana* is not available yet.

Even though increased food intakes are expected as compensation response to low quality diet (Cranford and Johnson, 1989), this strategy was not found in *C. talarum*. As explained above, trichomas presence in *P. racemosum* and silica deposits in *C. selloana* (Cabrera, 1970) would act as additive components to fibre content in determining the mechanism used by animals to compensate food challenges. In the case of *C. selloana*, high reingestion level contributed to obtained high digestibility. However, fat reserves were used for animals under that diet so that an important body weight loss was recorded. Sexual differences in body fat reserves between sexes need to be studied to understand the better tolerance of females to *C. selloana* diet condition.

Digestibility values found in *C. talarum* are between the higher levels reported for herbivorous species under different diet qualities. Furthermore, estimated digestibility in dry matter and fibre basis were similar, showing the high capacity of this species for fibre digestion. Apparent dry mass digestibility reported in animals fed with alfalfa (*Medicago sativa*, 38.2% NDF) was 45%, 17%, 27%, 26% and 21% for Guinea Pigs, rats, hamsters, voles and lagomorphs, respectively (Hume and Sakaguchi, 1991). For the hystricomorph *O. degus*, 50–80% digestibility was found using diets with 35–57% NDF (Velo and Bozinovic, 1993; Bozinovic, 1995; Bozinovic et al., 1997). Also, for *Myocastor coypus*, 71% digestibility in dry matter basis and 48% in NDF basis was reported for alfalfa diet (Sakaguchi and Nabata, 1992).

Reingestion proved to be an important component in nutritional adjustments to quality diets in *C. talarum*. Number of feces ingested was higher for animals fed with the most favorable diet, *B. uniolooides*, even though fresh food was always available. For *C. selloana* diet a high quantity of feces was reingested, representing nearly half of produced feces. The lower level of reingestion was found with *P. racemosum* diet; it is possible that lower transit time would determine that less time is available for soft feces formation.

One important point in reingestion characterization includes the timing of this behavior in relation to feeding and resting. *C. talarum* showed an arrhythmic pattern of reingestion, both during day and night hours which agree with the observed arrhythmic activity pattern reported for this species (Luna et al., 2000). Reingestion behavior does not appear associated with resting or alternated with feeding but also was performed during

fresh food ingestion. In subterranean rodents *Geomys bursarius*, *G. personatus* and *Thomomys monticola*, reingestion was found associated to resting but also traveling and while ingesting fresh food (Wilks, 1962). On the other hand, coprophagy pattern in *M. coypus* showed an inverse relation to feeding and drinking patterns (Takahashi and Sakaguchi, 1998), in a similar manner as reingestion is presented in lagomorphs. Kenagy et al. (1999) found for *O. degus* that coprophagy is practiced when food is not available; furthermore, timing of feces ingestion suffer changes according to seasonal changes in daily foraging. Under conditions of such segregation in feeding and reingestion behavior, feces do not compete with food for space and time in the digestive tract (Kenagy et al., 1999).

Diet quality affected time budget in *C. talarum*. Even though food consumed was higher for *B. uniolooides* diet than *C. selloana*, time assigned to feeding did not differ between treatments. Tuco-tucos usually spent time cutting *C. selloana* sheaths in small pieces previously to eat them; this food processing may allow them to eat a drier and also brittle food. In a similar manner, food consumption was rather similar for *P. racemosum* and *C. selloana* diets but tuco-tucos spent also a considerable time lapse cleaning food plants from external sheaths, which contain more trichoma structures, and selecting the inner ones. Time used in this activity implied less time devoted to activity. Resting and coprophagy were not modulated in relation to different diet quality.

Reported coprophagous behavior is quite similar in the studied rodents; first bending the body to allow the mouth reach the anus, then use the incisors – sometimes with the aid of the forelegs – to obtain the feces, and finally ingest and masticate them (Hirakawa, 2001). Feces suffer a phase of selection before ingestion, demonstrating that differences between them determine if each one will be ingested or rejected. Feces examination occurs both into the mouth (Kenagy and Hoyt, 1980; Oullette and Heisinger, 1980) or smelling feces before ingesting them, like in *C. talarum*. As was indicated (Jason and Van Wieren, 1999), short chain fatty acids are products of microorganism fermentation and may be used as a cue in feces examination.

Feces under reingestion in *C. talarum* represented properties including higher organic matter, water content, bacteria concentration and lower pH. Lower pH values would favor digestion process whereas high bacteria contents provide an important source of extra protein and vitamins from bacterial origin (Stevens and Hume, 1998). Such nutritional feces characteristics reinforce the idea that reingestion does not represent a merely recycling strategy for poor digested food but has an important digestive and nutritional function. Prevention of reingestion was proved to provoke body weight and growth decrease and even death in rodents (Barnes, 1962).

Two distinctive characteristics differentiate coprophagy in lagomorphs and rodents: in the former, soft feces never suffer mastication and are regularly found whole in the stomach and also, ingested and non-ingested feces can be differentiated by their morphology (Schmidt-Nielsen, 1994). Contrary to that expected for rodent species, two distinctive types of feces can be recognized in *M. coypus* (Takahashi and Sakaguchi, 1998), *Hydrochaeris hydrochaeris* (Mendes et al., 2000) and in

*C. talarum* (present study). Furthermore, the coypu and tucos also share the particular bicolor pattern for hard feces. As was reported (Takahashi and Sakaguchi, 1998) for *M. coypus*, the dark component of hard feces is similar to the soft feces in their concentrating bacteria and then would represent a waste of protein. That extra protein supply could be used in *C. talarum* in occurrence of hard feces consumption.

Hirakawa (2001) proposed four developmental stages for reingestion habit: (1) the primitive stage does not present a clear developed separation mechanism and a fermentation chamber, feces are ingested only in starvation. Food habits are not strictly herbivorous. (2) Herbivory is stronger, fermentation chamber and separation mechanisms are developed but feces are not regularly used and are not differentiated. (3) The development of the separation mechanism allows feces to be nutritionally but not morphologically differentiated. Reingestion is an important component in low quality diets. (4) A more developed separation mechanism determines that morphologically differentiated feces are produced. Feces ingestion is imperative and is practiced in a period when feeding is suppressed. Reingestion behavior in *C. talarum* shows characteristics from the last two stages since feces are nutritionally and morphologically differentiated, but timing of this behavior was found collated with other activities, including feeding. Nutritional differentiation in feces were reported for herbivorous caecal fermenters under 1 kg, including voles, hamsters, pocket gophers, rats, chinchilla and Guinea pigs, while differences in feces morphology were reported for species weighting between 0.1 and 100 kg, including pikas, sportive lemur, common ringtail possum, mountain beaver, leporids, coypu and capibara (Hirakawa, 2002). Then, tuco-tucos appear as the smallest species suffering reingestion of feces with a distinctive morphology, although it was not practiced exclusively associated to the resting phase of the day. Activity pattern in the wild was characterized as mainly diurnal in *C. talarum*, although some crepuscular and nocturnal activity was also recorded (Cutrera et al., 2006). Moreover, vegetation collected below ground was found into the burrow system (Antinuchi CD, personal communication) allowing us to consider that even when low or no food would be collected at night, food availability is continuous for tuco-tucos, as used in the present study.

Finally, *C. talarum* showed the ability to perform adjusts in short time lapse in response to diet quality. Food ingestion, egestion and feces ingestion changed in animals under different plant species diets. Time budget, mainly feeding and activity accompanied such changes. Coprophagy was practiced along the day and night following the arrhythmic activity pattern found for this species. Reingestion of feces was not associated to resting. Feces under reingestion and rejected ones differed in morphological and nutritional characteristics. Fibre content represents an important component determining behavioral and physiological adjustments but also other factors such as anti herbivory structures may be considered. Changes in food availability as well as increased energetic demands associated to thermal conditions and reproductive activity are expected to represent challenges in energetic and nutritional food utilization (Antinuchi et al., in press). As was shown in this study, co-

prophagy contribution in such ecological and physiological situations must be considered.

## Acknowledgments

Financial support for this study was provided by Universidad Nacional de Mar del Plata 15/E175 granted to C. Busch.

## References

- Altuna, C.A., Bacigalupe, L.D., Corte, S., 1998. Food handling and feces reingestion in *Ctenomys pearsoni* (Rodentia: Ctenomyidae). *Acta Theriol.* 43, 433–437.
- Antinuchi, C.D., Zenuto, R., Luna, F., Cutrera, A.P., Perisinotti, P.P., Busch, C., in press. Energy budget in subterranean rodents: insights from the tuco-tuco *Ctenomys talarum* (Rodentia: Ctenomyidae). In: Kelt, D.A., Lessa, E., Salazar-Bravo, J.A., Patton, J.L. (Eds.), *The quintessential naturalist: honoring the life and legacy of Oliver P. Pearson*. University of California Publications in Zoology, pp. xx-xx.
- Barnes, R.H., 1962. Nutritional implications of coprophagy. *Nutr. Rev.* 20, 289–291.
- Batzli, G.O., 1985. Nutrition. In: Tamarin, R.H. (Ed.), *Biology of New World Microtus*. The American Society of Mammalogists Special Publication 8, Shippensburg, PA, pp. 779–811.
- Batzli, G.O., Cole, F.R., 1979. Nutritional ecology of microtine rodents: digestibility of forage. *J. Mammal.* 60, 740–750.
- Batzli, G.O., Pitelka, F.A., 1969. Condition and diet of cycling populations of the California vole, *Microtus californicus*. *J. Mammal.* 52, 141–163.
- Bozinovic, F., 1993. Nutritional ecophysiology of the Andean mouse *Abrothrix andinus*: energy requirements, food quality and turnover time. *Comp. Biochem. Physiol. A* 104, 601–604.
- Bozinovic, F., 1995. Nutritional energetics and digestive responses of an herbivorous rodent (*Octodon degus*) to different levels of dietary fibre. *J. Mammal.* 76, 627–637.
- Bozinovic, F., Novoa, F.F., Sabat, P., 1997. Feeding and digesting fibre and tannins by an herbivorous rodent, *Octodon degus* (Rodentia: Caviomorpha). *Comp. Biochem. Physiol. A* 118, 625–630.
- Buffenstein, R., 2000. Ecophysiological responses of subterranean rodents to underground habitats. In: Lacey, E.A., Patton, J.L., Cameron, G.N. (Eds.), *Life Underground: The Biology of Subterranean Rodents*. University of Chicago Press, Chicago, pp. 62–110.
- Buffenstein, R., Yahav, R., 1994. Fibre utilization by Kalahari dwelling subterranean Damara mole-rats (*Cryptomys damarensis*) when fed their natural diet of gembok cucumber tubers (*Acanthosicyos naudinianus*). *Comp. Biochem. Physiol. A* 109, 431–436.
- Busch, C., Malizia, A.I., Scaglia, O.A., Reig, O.A., 1989. Spatial distribution and attributes of a population of *Ctenomys talarum* (Rodentia: Octodontidae). *J. Mammal.* 70, 204–208.
- Busch, C., Antinuchi, C.D., del Valle, J.C., Kittlein, M.J., Malizia, A.I., Vasallo, A.I., Zenuto, R.R., 2000. Population ecology of subterranean rodents. In: Lacey, E.A., Patton, J.L., Cameron, G.N. (Eds.), *Life Underground: The Biology of Subterranean Rodents*. University of Chicago Press, Chicago, pp. 183–226.
- Cabrera, A.L. Flora de la provincia de Buenos Aires. Parte 2: Gramíneas. Colección científica del INTA, 1970 Bs. As. 540 pp.
- Cengel, D.J., Estep, J.E., Kirkpatrick, R.L., 1978. Pine vole reproduction in relation to food habits and body fat. *J. Wildl. Manage.* 42, 822–833.
- Cole, F.R., Batzli, G.O., 1979. Nutrition and population dynamics of the prairie vole, *Microtus ochrogaster*, in central Illinois. *J. Anim. Ecol.* 48, 455–470.
- Comparatore, V.M., Cid, M.S., Busch, C., 1995. Dietary preferences of two sympatric subterranean rodent populations in Argentina. *Rev. Chil. Hist. Nat.* 68, 197–206.
- Cork, S.J., Foley, W.J., 1991. Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperate and tropical forest. In: Palo, R.T., Robbins, C.T. (Eds.), *Plant Defenses Against Mammalian Herbivory*. CRC Press, Boca Ratón, Florida, pp. 133–166.



- Cranford, J.A., Johnson, E.O., 1989. Effects of coprophagy and diet quality on two microtine rodents (*Microtus pennsylvanicus* and *Microtus pinetorum*). *J. Mammal.* 70, 494–502.
- Crawley, M.J., 1983. Herbivory. The dynamics of animal–plant interactions. *Studies in Ecology*, vol. 10. University of California Press, Berkeley. 437 pp.
- Cutrer, A.P., Antinuchi, C.D., Mora, M.S., Vassallo, A.I., 2006. Home-range and activity patterns of the South American subterranean rodent *Ctenomys talarum*. *J. Mammal.* 87, 1183–1191.
- del Valle, J.C., Lohfeld, M.I., Comparatore, V.M., Cid, M.S., Busch, C., 2001. Feeding selectivity and food preference of *Ctenomys talarum* (tuco-tuco). *Mamm. Biol.* 66, 165–173.
- Golley, F.B., 1960. Anatomy of the digestive tract of *Microtus*. *J. Mammal.* 41, 89–99.
- Hirakawa, H., 2001. Coprophagy in leporids and other mammalian herbivorous. *Mamm. Rev.* 31, 61–80.
- Hirakawa, H., 2002. Supplement: coprophagy in leporids and other mammalian herbivorous. *Mamm. Rev.* 32, 150–152.
- Hume, I.I., Sakaguchi, E., 1991. Patterns of digesta flow and digestion in foregut and hindgut fermenters. Physiological aspects of digestion and metabolism in Ruminants. Proceedings of the Seventh International Symposium on Ruminant Physiology.
- Iason, G., Van Wieren, S.E., 1999. Digestive and ingestive adaptations of mammalian herbivores to low-quality forage. In: Olf, H., Brown, V.K., Drent, R.H. (Eds.), *Herbivores: Between Plants and Predators*. BES Symp., vol. 38. Blackwell Scientific, Oxford, pp. 337–370.
- Jarvis, J.U.M., Bennet, N.C., 1991. Ecology and behavior of the family Bathyergidae. In: Jarvis, P.W., Sherman, J.U.M., Jarvis, R.D., Alexander (Eds.), *The Biology of the Naked Mole-Rat*. Princeton University Press, Princeton, New Jersey, pp. 66–96.
- Justice, K.E., Smith, F.A., 1992. A model of dietary fibre utilization by small mammalian herbivores, with empirical results for *Neotoma*. *Am. Nat.* 139, 398–416.
- Kenagy, G.J., Hoyt, D.F., 1980. Reingestion of faeces in rodents and its daily rhythmicity. *Oecologia* 44, 403–409.
- Kenagy, G.J., Veloso, C., Bozinovic, F., 1999. Daily rhythms of food intake and faeces reingestion in the Degu, an herbivorous Chilean rodent: Optimizing digestion through coprophagy. *Physiol. Biochem. Zool.* 72, 78–86.
- Luna, F., Antinuchi, C.D., Busch, C., 2000. Ritmos de actividad locomotora y uso de cuevas en condiciones seminaturales en *Ctenomys talarum* (Rodentia Octodontidae). *Rev. Chil. Hist. Nat.* 73, 39–46.
- Mangione, A.M., Bozinovic, F., 2002. Ecología nutricional y estrategias de digestión: compromisos entre obtención de energía y eliminación de toxinas. In: Bozinovic, F. (Ed.), *Fisiología Ecológica y Evolutiva, Teoría y casos de estudio en animales*. Ediciones Universidad Católica de Chile, Santiago, pp. 125–150.
- Mendes, A.C., da Nogueira, S.S., Lavoretti, A., Nogueira-Filho, S.L.G., 2000. A note on cecotrophy behavior in capybara (*Hydrochaeris hydrochaeris*). *Appl. Anim. Behav. Sci.* 66, 161–167.
- Oullette, D.E., Heisinger, J.F., 1980. Reingestion of feces of *Microtus pennsylvanicus*. *J. Mammal.* 61, 366–368.
- Perissinotti, P.P., 2003. Efecto de la dureza del suelo y la calidad de la dieta sobre la tasa metabólica de reposo en el roedor subterráneo *Ctenomys Talarum*. Licenciatura thesis Universidad Nacional de Mar del Plata, 45 pp.
- Provenza, F.D., Villalba, J.J., Cheney, C.D., Werner, S.J., 1998. Self-organization of foraging behaviour: from simplicity to complexity without goals. *Nutr. Res. Rev.* 11, 199–222.
- Reig, O.A., Busch, C., Ortells, O., Contreras, J.R., 1990. An overview of evolution, systematics, population biology and speciation in *Ctenomys*. In: Nevo, E., Reig, O.A. (Eds.), *Biology of Subterranean Mammals at Organismal and Molecular Levels*. Alan Liss Inc., New York, pp. 71–96.
- Sakaguchi, E., Nabata, A., 1992. Comparison of fibre digestion and digesta retention time between nutrias (*Myocastor coypus*) and guinea-pigs (*Cavia porcellus*). *Comp. Biochem. Physiol. A* 103, 601–604.
- Schmidt-Nielsen, K., 1994. *Animal Physiology, Adaptation and Environment*, 4th ed. Cambridge University Press. 602 pp.
- Seeley Jr., H.W., Van Demark, P.J., Lee, J.J., 1991. *Microbes in Action, A Laboratory Manual of Microbiology*, 4th ed. Freeman and Company, New York. 450 pp.
- StatSoft, Inc., 2001. STATISTICA 6.0 (Data Analysis Software System). [www.statsoft.com](http://www.statsoft.com).
- Stevens, E.C., Hume, I.D., 1998. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiol. Rev.* 78, 393–427.
- Takahashi, T., Sakaguchi, E., 1998. Behaviors and nutritional importance of coprophagy in captive adult and young nutrias (*Myocastor coypus*). *J. Comp. Physiol. B* 168, 281–288.
- Veloso, C., Bozinovic, F., 1993. Dietary and digestive constraints on basal energy metabolism in a small herbivorous rodent (*Octodon degus*). *Ecology* 74, 2003–2010.
- Wilks, B.J., 1962. Reingestion in geomyid rodents. *J. Mammal.* 43, 267.
- Woodall, P.F., 1989. The effects of increased dietary cellulose on the anatomy, physiology and behaviour of captive water voles, *Arvicola terrestris* (Rodentia: Microtinae). *Comp. Biochem. Physiol. A* 94, 615–621.
- Young Owl, M., Batzli, G.O., 1998. The integrated processing response of voles to fibre content of natural diets. *Funct. Ecol.* 12, 4–13.