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Research

Effects of food supplementation on litter size and oestradiol concentration during gestation and oestrous cycle of capybaras (*Hydrochaeris hydrochaeris*) in captivity

G. K. Becker, G. C. Pederassi, E. A. Santos and E. P. Colares*

Programa de Pós-graduação em Fisiologia Animal Comparada, Departamento de Ciências Fisiológicas, Fundação Universidade Federal do Rio Grande, Cx. Postal 474 CEP 96201-900, Rio Grande, RS, Brazil

The present study analysed the response of adult female capybaras (*Hydrochaeris hydrochaeris*) to different dietary supplementation in relation to litter size per parturition and oestradiol secretion profile during pregnancy and the oestrous cycle. All four experimental groups received 'camerum' grass (*Pennisetum purpureum*) and water *ad libitum* and three of the groups were also fed a protein, lipid or protein and lipid supplement. Litter size per parturition did not show any significant difference among treatment groups, but was significantly higher ($P < 0.05$) than in the control group. There was no significant difference in oestradiol concentrations among treatments and the control group, during each phase of the oestrous cycle or during gestation. The average oestradiol concentrations in dioestrous, oestrous and metoestrous phases were not significantly different from each other, but were significantly lower ($P < 0.05$) than the average oestradiol concentration in the pro-oestrous phase. In addition, average oestradiol concentrations increased after the second gestation month, but were significantly higher ($P < 0.05$) only after the fourth gestation month, achieving maximum value by the end of gestation. Dietary supplementation had no significant effect on hormonal concentrations during the oestrous cycle and gestational period. However, an increase in litter size per parturition was observed among female capybaras that received dietary supplementation.

Introduction

It has been suggested that food acts on the endocrine control of reproduction by changing the hypothalamus–hypophysis–gonad system (Bronson, 1989; Bronson and Heideman, 1990, 1994; Blache *et al*., 2000). Food supplementation causes an increase in reproductive capacity in several rodents (Watts, 1970; Smith, 1971; Cole and Batzli, 1978; Boutin, 1990; Akbar and Gorman, 1993a,b, 1996; Colares, 1997). In mild winters or with the provision of food supplementation there is an increase in the number of pregnant female rodents in fields in Argentina (Mills *et al*., 1992; Cittadino *et al*., 1994).

According to Thomas *et al*. (1997), the consumption of polyunsaturated fatty acids causes a greater rate of ovarian follicular growth in cattle, which can lead to an increase in oestradiol concentrations. The provision of higher protein content forage and vegetable oils results in an increase in ovulation rate and reproductive efficiency in sheep (Luque *et al*., 2000). Protein supplementation increases the release of LH in cattle (Kane *et al*., 2002).

Capybaras (*Hydrochaeris hydrochaeris*) are found in Central and South America, from Panama to Northern Argentina (Alho and Rondon, 1987; Emmons, 1990). They are herbivorous animals and are found close to water courses with abundant vegetation, where they form family groups (Emmons, 1990). Capybaras are the largest rodent, weighing up to 70 kg. They are annual polyestrous animals, with spontaneous ovulation and an oestrous cycle of 8 days, and a receptivity period of at least 8 h (López, 1982). The gestation period of capybaras is 150.6 ± 2.8 days (López, 1987); average litter size is four per parturition and lactation lasts 4 months (Zara, 1973). In the Pantanal (Brazil), reproduction takes place all year round, but peaks among young animals from July to August, when plant production is greatest (Alho and Rondon, 1987). However, in captivity, the reproductive capacity of capybaras is related to the size of paddocks and the quantity and quality of the diet (Andrade *et al*., 1998).

From evidence linking nutritional aspects to reproductive processes in different rodent species and other mammals, the present study analysed the response of adult female capybaras to different dietary supplementation in relation to litter size per parturition and the oestradiol hormone secretion profile during pregnancy and the oestrous cycle.

*Correspondence
Email: ecolares@octopus.furg.br

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Table 1. Composition of supplementation diet offered to capybaras (*Hydrochaeris hydrochaeris*) during the experimental period

	Protein (%)	Lipids (%)	Carbohydrates (%)	Ash (%)	Moisture (%)	Crude energy (Kcal kg^{-1})
Grass	8.0	1.8	68.6	10.5	11.1	3763.9
Treatment 1	12.1	4.5	51.5	8.3	23.6	2929.8
Treatment 2	8.2	22.5	54.5	6.5	8.3	3848.2
Treatment 3	10.9	24.1	47.4	6.6	10.9	3877.6

The four experimental groups each received camerum grass and water *ad libitum* and three of the groups also received a 500 g supplement of protein (treatment 1), lipids (treatment 2), or protein and lipids (treatment 3).

Materials and Methods

Animals

Forty-eight healthy, non-pregnant female capybaras (*Hydrochaeris hydrochaeris*), aged 3–6 years, average 67.6 ± 0.72 kg body weight, were obtained from a breeding facility located close to the city of Santo Antônio da Patrulha, Rio Grande do Sul State, Brazil. These animals were fed with elephant grass of the camerum variety (*Pennisetum purpureum*) and water *ad libitum*. The experiment began in spring (November). The animals were divided into four treatment groups, two families of capybaras per treatment, containing one male and six females, and were kept in 0.8 hectare paddocks with natural lighting. Each paddock had a 0.1 hectare pool and a covered area for food administration. All animal procedures were approved by the collegiate of the Department of Physiological Sciences, Fundação Universidade Federal do Rio Grande.

Food supplementation

Barley roots (14% protein) and corn oil were used as dietary supplements. The choice of the supplement and the calculation of the amount of food supplied was based on the scheme used by the owner of the breeding facility. Camerum grass was chosen as the main forage as it was already available at the breeding facility and because of results from previous studies (Parra *et al*., 1978; Baldizan *et al.,* 1986; Max *et al*., 1993).

The animals were fed camerum grass *ad libitum* each morning. Of the eight groups two were fed grass only, and the other six (two groups per treatment) were given 500 g of food supplement per animal (Table 1). The animals ate all supplementation before grass was administered.

Determining pregnancy and litter size per parturition

The animals were weighed every 2 months. Changes in body weight that followed a growing and gradual trend were taken as signs of pregnancy and sudden falls in weight were taken as the occurrence of parturition. In addition, paddocks were inspected on a daily basis for parturition and the number of offspring was noted.

Blood collection

After being weighed, blood was collected from the saphenous vein on the cranial branch with a disposable syringe and 'scalp' needle G19. The blood was placed in a test tube without anticoagulant and centrifuged for 20 min at 2000 ***g***. The serum was then transferred to a freezing tube and stored at –20°C for later hormone analysis.

Colpocytology

Samples of vaginal mucosa sloughing were collected with a swab moisturized with saline. Smears were made on microscope slides, fixed in absolute alcohol and then stained by the Shorr method (Arruda *et al*., 1976). The smear was examined using a × 100 magnification microscope for the presence and number of cells (Lópes, 1982) to determine the phase of the oestrous cycle.

Oestradiol assay

Oestradiol assay measurement was made in duplicate using reagent kits (ICN® Biomedicals Inc., Costa Mesa, CA) of radioimmunoassay in solid phase. Readings were made for 1 min in a gamma counter (Count-rate Computer P-30A, GAMMA TOM®). Measurement accuracy was assessed using the formula of Cook and Beastall (1987).

Standard curves to determine serum concentrations of oestradiol were constructed using the 'Immunoassay Calibration Curve and Sample Calculation' program written in Basic by R. Roberts and K. Warren (University of Florida).

Statistical analysis

Data on oestradiol serum concentrations in capybaras during the oestrous cycle were analysed by two-way ANOVA and data on litter size per parturition were analysed by one-way ANOVA. Oestradiol serum concentrations during gestation were analysed by repeated measure ANOVA. Analyses were followed by Tukey's multiple range test (Statistica for Windows 4.3, StatSoft Inc., 1993). The level of significance used was 5%. ANOVA assumptions (homocedasticity and normality) had been tested previously.

Results

Litter size per parturition did not show any significant difference among the groups fed dietary supplementation,

Table 2. Litter size per parturition in each treatment group of capybaras (*Hydrochaeris hydrochaeris*)

Group	Number of parturitions	Litter size per parturition
Control	5	2.20 ± 0.37[a]
Treatment 1	8	4.36 ± 0.48[b]
Treatment 2	12	4.29 ± 0.42[b]
Treatment 3	9	4.00 ± 0.22[b]

The four experimental groups each received camerum grass and water *ad libitum* and three of the groups also received a 500 g supplement of protein (treatment 1), lipids (treatment 2), or protein and lipids (treatment 3).
Same letters in the same column indicate homogeneous groups ($P > 0.05$).

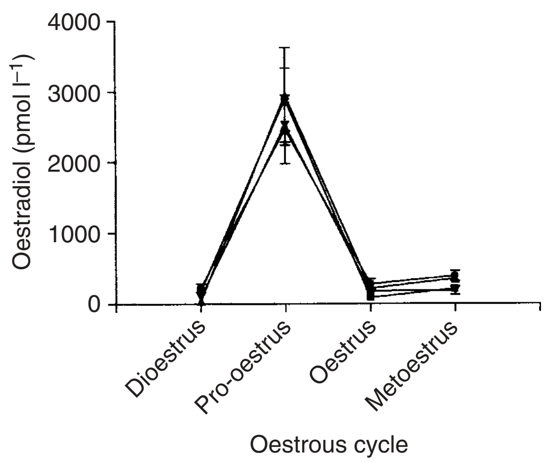

Fig. 1. Means and standard error of oestradiol concentration during the oestrous cycle of capybaras (*Hydrochaeris hydrochaeris*), subject to food supplementation. The four experimental groups each received camerum grass and water *ad libitum* and three of the groups also received a supplement. ●: control; ■: treatment 1, 500 g protein supplement; ▲: treatment 2, 500 g lipid supplement; ▼: treatment 3, 500 g protein and lipid supplement.

but was significantly higher ($P < 0.05$) than in the control group (Table 2).

No significant difference was detected in oestradiol concentrations among the treatment and control groups during each phase of the oestrous cycle (Fig. 1). In addition, average oestradiol concentrations in the dioestrous, oestrous and metoestrous phases did not show any significant difference from each other, but were significantly lower ($P < 0.05$) than the average oestradiol concentration in the pro-oestrous phase.

There was no significant difference in average oestradiol concentrations among the treatment and control groups during gestation (Fig. 2). However, average oestradiol concentrations increased during gestation and can be expressed either as a linear ($F = 55.57$; $P = 0.0000$) or quadratic equation ($F = 7.35$; $P = 0.0168$).

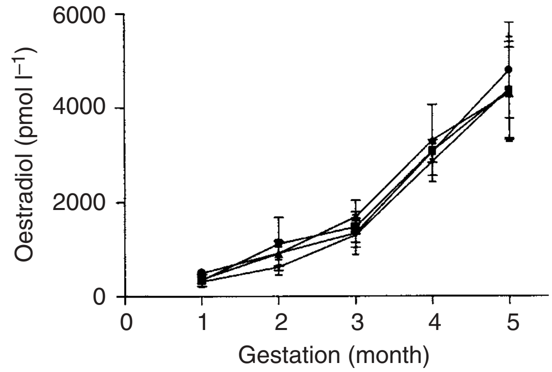

Fig. 2. Means and standard error of oestradiol concentration during gestation of capybaras (*Hydrochaeris hydrochaeris*), subject to food supplementation. The four experimental groups each received camerum grass and water *ad libitum* and three of the groups also received a supplement. ●: control; ■: treatment 1, 500 g protein supplement; ▲: treatment 2, 500 g lipid supplement; ▼: treatment 3, 500 g protein and lipid supplement.

Discussion

Variations in reproduction in many mammals are caused by changes in the quantity and quality of food the animals find in their environment (Flowerdew, 1987; Bronson, 1989). Food restriction can result in delay of the onset of sexual maturity, suppression of the oestrous cycle, uterus atrophy, resorption of embryos during gestation, decrease in milk production and changes in reproductive hormone concentrations (Long *et al*., 1999; Blache *et al*., 2000; Angel-Meza *et al*., 2001; Kusina *et al*., 2001). However, food supplementation can improve reproductive capacity in animals, promoting earlier onset of sexual maturity, oestrous cycle activation, increase in milk production, increase in reproductive hormone concentrations and increase in litter size per parturition (Colares, 1997; Long *et al*., 1999; Kusina *et al*., 2001).

Watts (1970) studied the variation in the duration of reproductive period in relation to food availability in *Clethrionomys glareolus* and *Apodemus sylvaticusi*, and found that food supplementation does not alter the reproductive period. However, in optimal environmental conditions and optimal food supply, capybaras breed twice per year (Ojasti, 1972). Mills *et al*. (1992) found that during summer and mild winters there is an increase in the number of pregnant females of *Calomys laucha*, *C. musculinus* and *Akodon azarae* rodents in cereal fields in Argentina, which may be related to an increase in food supply.

Food supplementation using rabbit feed increases the reproductive capacity of the mouse *Microtus ochrogaster* but cannot prevent a decline during the non-reproductive period (Cole and Batzli, 1978). This is also the case for other rodents (Watts, 1970; Smith, 1971; Boutin, 1990; Akbar and Gorman, 1993a,b, 1996). However, the reproductive capacity of the rodent *Calomys laucha* is increased in winter when the diet is supplemented with sunflower seeds, compared with non-supplemented animals (Colares, 1997).

Mammals require a balanced diet for reproduction, and minimum amounts of proteins and fats are necessary (Sadleir, 1969; Flowerdew, 1987; Bronson, 1989; Trujillo and Broughton, 1995). Colares (1997) determined that dietary supplementation with sunflower seeds increases litter size per parturition and the number of parturitions in the rodent *Calomys laucha* and suggested that these increases are due to the content of fatty acids and essential amino acids in sunflower seeds. Studies with laboratory rats have shown that food supplementation with corn oil can increase reproduction (Soares, 1992). Lipids in general cause an increase in reproductive capacity in ruminants by influencing follicular growth and corpus luteum function (Mattos *et al.*, 2000). Increased protein content in the diet (8%, 16% or 24%) results in earlier sexual maturation in young females and an increase in litter size per parturition in the mouse *Mus musculus* (Vandenbergh *et al.*, 1972). A minimum of 7.5% protein per dry weight of food is critical for reproduction in the rat *Sigmodon hispidus* (Randolph *et al.*, 1995). Similar results have been found for other rodents by Donald *et al*. (1981), Batzli (1986) and Angel-Meza *et al*. (2001).

The present study found that food supplementation results in an increase in the reproductive performance of female capybaras. Female *Calomys laucha* also have increased parturitions and significantly smaller litter sizes when subjected to food restriction and larger litter sizes when fed sunflower seeds (Colares, 1997). Colares (1997) has suggested that this decrease in the reproductive capacity of animals subjected to food restriction may be due to a decrease in follicular development or to resorption of embryos early in gestation. Bronson and Marsteller (1985) also found that food restriction can cause resorption of embryos during gestation and that the number of resorptions is directly proportional to food restriction. The authors of the present study believe that the same may occur in capybaras.

A 50% reduction in food causes a decrease in the concentration of LH in the blood in rats (Howland, 1971) and other mammal species (Thong and Graham, 1999). This decrease in LH is due to a failure in the release of this hormone by the hypophysis, which may influence oestradiol concentrations, compromising the ovulation process (Thong and Graham, 1999). In addition, a 48 h fasting period on the first day of the oestrous cycle in hamsters results in a decrease in serum oestradiol concentrations, causing suppression of the oestrous cycle (Morin, 1986).

Despite the smaller litter size in control animals in the present experiment, the absence of food supplementation did not cause any variation in oestradiol concentrations in supplemented or control animals, both during the oestrous cycle and during gestation. It is probable that the group of control capybaras that became pregnant were in good nutritional status, and this enabled an oestradiol production similar to that of supplemented animals. This contention is in agreement with Schneider *et al*. (2000), who found that animals that receive a low quality or quantity of food can breed if their energy storage is high.

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