


# Effect of feeding on the body development of newborn opossums (*Didelphis albiventris*)

## *Efeito da alimentação no desenvolvimento corporal de gambás (D. albiventris) filhotes*

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### ABSTRACT

Many orphaned Joey opossums are sent to Wild Animal Screening Centers for parental care. There are no studies on breast milk substitutes. Thus, this study aimed to compare two milk replacer formulations adapted from studies conducted with small animals routinely used for feeding them. For this purpose, 36 newborns of *Didelphis albiventris* weighing 20 and 25 grams were divided into two groups, each fed with a type of milk replacer (commercial or homemade). The groups were monitored for four weeks, and the animals were weighed and had their length measured weekly, in addition to daily individual assessments at each feeding. The stomach volume of seven animals that died during the evaluation period and were not part of the experimental groups was measured. The homemade milk replacer presented higher energy and fat levels than the commercial one, and the animals fed with it presented higher body development.

**Keywords:** Growth. Marsupials. Orphans. Milk replacers.

### RESUMO

Muitos filhotes órfãos de gambás são encaminhados aos Centros de Triagem de Animais Silvestres necessitando de cuidados parentais. Não há estudos sobre substitutos do leite materno, assim, o objetivo deste estudo foi comparar duas formulações de sucedâneo do leite adaptadas de estudos realizados com pequenos animais, rotineiramente utilizadas para a alimentação desses animais. Para tanto, foram utilizados 36 neonatos de *Didelphis albiventris*, com peso entre 20 e 25 gramas, divididos em dois grupos, cada grupo alimentado com um tipo de sucedâneo (comercial ou caseiro). Os grupos foram acompanhados durante quatro semanas, os animais foram pesados e tiveram seu comprimento mensurado semanalmente, além das avaliações individuais diárias a cada alimentação. O volume estomacal foi mensurado em sete animais que morreram durante o período de avaliação e que não faziam parte dos grupos experimentais. Observou-se que o sucedâneo caseiro apresentou maiores níveis de energia e de gordura em comparação ao comercial, e os animais alimentados com ele apresentaram maior desenvolvimento corporal.

**Palavra-chave:** Crescimento. Marsupiais. Órfãos. Substitutos de leite.

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## Introduction

Nutrition is one of the essential factors for proper development in the first weeks of life. Protein and energy intake can determine the growth rate of baby animals. Animals orphaned before the weaning phase require strict monitoring, environmental control (humidity and temperature), and adequate handling and nutrition, which can provide essential nutrients for the proper development of baby animals (Domingos et al., 2008).

Feeding newborns and baby animals using breast milk substitutes is recommended in cases of maternal death and absent, insufficient, or toxic milk production (Vannuchi & Abreu, 2017). Diet is essential for the adequate development and survival of baby animals. Therefore, the adequacy of a specific diet for each species (Domingos et al., 2008) has been the subject of many scientific studies. However, most of the research is directed toward production (Cardoso et al., 2006; Cypriano, 2008; Miranda, 2013) and companion animals (Rocha, 2008; Vannuchi & Abreu, 2017; Araújo et al., 2018).

The literature has described specific cases of wild animals. There is a work carried out by Green et al. (1996) on the composition of opossum milk (*D. virginiana*) and a substitute for Fox Valey® milk produced and marketed in the United States of America. However, there is no work with the species found in South America.

The white-eared opossum (*D. albiventris*) is a marsupial species widely distributed in the American continent, with an extended lactation period that can reach the hundredth day of life (Jansen, 2002). This species is often received in Wild Animal Screening Centers (Cavalcanti et al., 2021), as it is a victim of anthropic actions due to their synanthropic behavior, being run over, hunted, and attacked by domestic animals (Massari et al., 2019; Nascimento & Horta, 2020),

and many females end up dying due to the injuries suffered, leaving their joeys orphaned.

The literature on marsupials approximates their energy requirements using the formula: Kcals required = 2.85 x (49 x body weight in kg<sup>0.75</sup>) (Taylor, 2002). The orphan opossum is often fed with an industrialized or homemade commercial formula developed for small animals (Nascimento & Horta, 2020). Thus, this study compares the development of orphaned opossums (*D. albiventris*) fed with either commercial or homemade substitutes for four weeks.

## Materials and Methods

Thirty-six newborn orphan opossums (*D. albiventris*), 19 males and 17 females, approximately 15 to 20 days old, weighing between 20 and 25 grams, arrived for veterinary monitoring at the Rehabilitation Nucleus of Wild Fauna and Wild Animal Screening Center (NURFS/CETAS) of the Federal University of Pelotas (UFPEL), were used for this experiment. The animals were considered healthy after general clinical examination. The animals' development was followed by biometrics, with total length (cm) and weight (g) measurements over four weeks. The newborns (n=36) were maintained in Animal Care Units (ACU) with temperatures between 30 and 35 °C and controlled humidity (60-70%).

The animals were randomly distributed into two groups, 18 animals each, respecting the litter, and submitted to two types of treatments, consisting of two milk replacers already recognized in the literature as substitutes for breast milk. The first group was submitted to a homemade formulation with the following ingredients: 200 mL lactose-free whole milk; 1 large raw egg yolk hen (20 g); 2 coffee spoons of lactose-free milk cream (8 g); and 2 coffee spoons of crushed cat food (10 g) (Golden Gatos®), and 0.5 mL vitamin supplements (Glicopan Pet®) (adapted from Viana, 2019). The second group was subjected to a commercial formulation (Pet Milk®).

The newborns of both groups were fed enterally five times a day, between 8 am and 6 pm, using a 3-mL syringe. The volume administered per feeding was 1 mL in the first week, 1.5 mL in the second and third weeks, and 1.8 mL in the fourth week. Genito-anal stimulation was performed at the end of each feeding. The general appearance of the urine and feces was evaluated, in addition to the presence of milk in the nose and abdominal distention.

The composition of the formulations was extracted from tables provided by the manufacturers and calculated for the production of 1 mL of the milk substitute. Thus, the homemade formulation was estimated according to the

nutritional tables provided for each component (lactose-free milk cream, lactose-free milk, egg yolk, and cat food).

Seven opossums (*D. albiventris*) newborns that were not part of the study and died during the follow-up period at NURFS were used as the basis for the assessment of stomach capacity (mL). Scammon & Doyle (1920) measured the stomach capacity of newborn opossums as proposed.

The statistical analysis of the data consisted of evaluating the distribution using the Shapiro-Wilk normality test. Once the parametric distribution was determined, analysis of variance (ANOVA) was adopted, and the means were compared using Tukey's test (5%). Subsequently, a linear correlation was performed to compare groups.

## Results

The data followed a parametric distribution, and the means analysis showed no significant differences between the sexes.

The homemade milk replacer contained higher levels of protein and fat, and the commercial milk replacer contained higher levels of fiber, iron, phosphorus, taurine, and potassium (Table 1). In addition, the commercial milk replacer's manufacturer-provided composition does not contain sodium or carbohydrates. The milk replacer used in this study had approximately 1.78 Kcal/mL (homemade milk replacer) and 1.24 Kcal/mL (commercial milk replacer).

The stomach volume of the seven evaluated animals had a mean of 1.5 mL/25 g of weight. The smallest volume was 1.5 mL/14 g, while the largest was 1.6 mL/28 g. The mean weight was 22 g ( $\pm 5.54$ ), and the mean volume was 1.5 mL ( $\pm 0.05$ ).

The difference in the growth rate of the animals submitted to each milk replacer over the four weeks was significant ( $p < 0.05$ ). The animals submitted to the homemade milk replacer presented length (Figure 1) and weight (Figure 2) higher than those fed commercial milk replacer. Animals

Table 1 – Nutritional composition comparison between homemade formula (consisting of lactose-free whole milk, large raw egg yolk from hens, lactose-free milk cream, crushed cat food, and vitamin supplements) and commercial formulas for puppies and kittens, provided to opossums (*D. albiventris*) over four weeks

Nutritional composition	Homemade formulation (mL)	%	Commercial formulation (mL)	%
<b>Calories</b>	1.78 Kcal		1.24 Kcal	
<b>Ether extract</b>	0.16 g	49.18	0.07 g	21.51
<b>Protein</b>	0.09 g	27.66	0.07 g	21.51
<b>Carbohydrate</b>	0.06 g	18.44	–	–
<b>Fiber</b>	0.01 g	3.07	0.2 g	6.14
<b>Calcium</b>	2.6 mg	0.8	2.6 mg	0.8
<b>Potassium</b>	0.9 mg	0.28	1.6 mg	0.48
<b>Sodium</b>	0.8 mg	0.24	–	–
<b>Phosphorus</b>	0.8 mg	0.24	1.6 mg	0.48
<b>Zinc</b>	0.1 mg	0.03	0.2 mg	0.06
<b>Taurine</b>	0.1 mg	0.03	0.6 mg	0.18
<b>Iron</b>	0.01 mg	0.003	0.02 mg	0.006
<b>Magnesium</b>	0.01 mg	0.003	0.02 mg	0.006

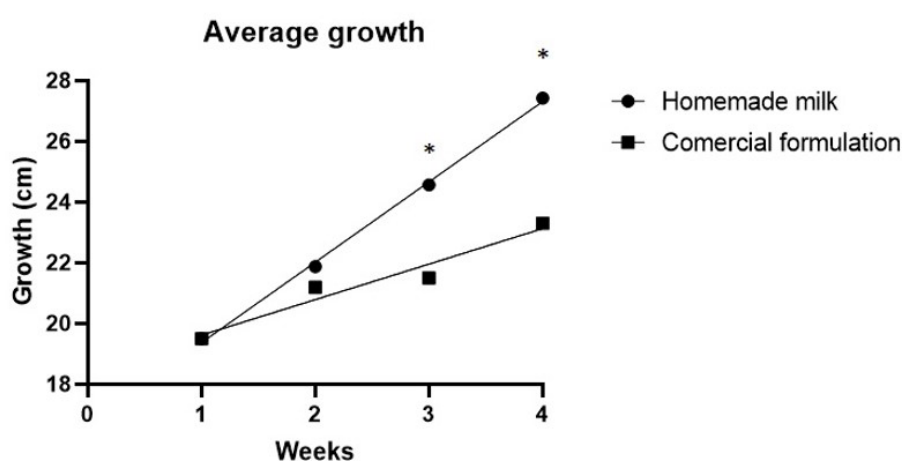


Figure 1 – Mean growth (cm) of orphaned joey opossums (*D. albiventris*) fed homemade formula (consisting of lactose-free whole milk, large raw egg yolk from hens, lactose-free milk cream, crushed cat food, and vitamin supplements) and commercial formula for puppies and kittens. Mean comparison by Tukey's test ( $*P < 0.05$ ).

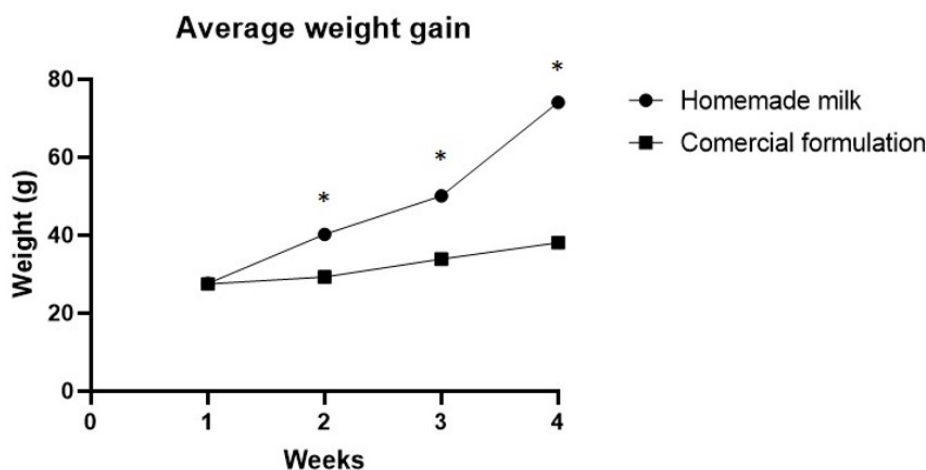


Figure 2 – Mean weight gain of orphaned joey opossums (*D. albiventris*) fed homemade formula (consisting of lactose-free whole milk, large raw egg yolk from hens, lactose-free milk cream, crushed cat food, and vitamin supplements) and commercial formula for puppies and kittens. Mean comparison by Tukey's test (\* $P < 0.05$ ).

fed homemade milk replacers obtained approximately 7% weight gain per day, while those fed commercial milk replacers presented approximately 1% weight gain per day.

Animals fed commercial milk replacers ( $n=18$ ) showed changes in the appearance of feces, abdominal distension, and gases, unlike animals fed homemade milk replacers, for which these changes were not frequent.

## Discussion

The mean stomach capacity of the opossums evaluated in this study was 1.5 mL/25 g of weight. In addition, the stomach volume associated with the amount of energy assists in determining the frequency of food offered. In this study, the feeding frequency was five times. It would be sufficient for animals fed homemade milk replacers but inadequate for those fed commercial milk replacers, as seven daily feedings would be necessary.

Success in neonatology care is based on the animal's physiology, but this information is scarce for wild animals or inaccessible in our reality, requiring adaptations from domestic species (Nascimento & Horta, 2020). In the United States, there is a commercial substitute for milk produced for opossums (*D. virginiana*), squirrels, and rabbits, Fox Valley®. The composition of this substitute is approximately 1.83 Kcal per mL, 15% carbohydrates, 32% protein, and 40% fat, similar to the homemade substitute used in this study.

A significant difference ( $p > 0.05$ ) was observed between groups, probably due to the amount of energy and fat in the homemade milk replacer. A study by Green et al. (1996) presents milk composition from North American opossums (*D. virginiana*) at different stages of lactation. The maximum values obtained for calories were 2.31 Kcal, from 8.8-20% of carbohydrates, 10-29% of proteins, and

23-50% of fat. In comparison with the formulation of homemade and commercial milk replacers used in this study, it is noted that the percentage of fats and carbohydrates present in the homemade substitute would be the closest to the composition of natural milk and that the amount of calories present in the commercial milk replacer is much lower than the needs of the baby *D. virginiana*.

Weight is considered a suitable parameter for monitoring the development of newborns and opossums, also allowing the estimation of body size in non-adult opossums (Iskjaer et al., 1989; Cáceres & Monteiro-Filho, 1999; Domingos et al., 2008). A healthy newborn should increase its weight by 5 to 10% daily. Thus, the diet provided with the homemade milk replacer reached the objective, unlike the diet based on the commercial milk replacer, which was below what is considered ideal (Domingos et al., 2008). This difference may be due to the amount of calories and fat in the milk replacers. Moreover, baby opossums fed commercial milk replacer presented abdominal distension and diarrhea, which may be an indication of an inadequate diet (Lawler, 2008; Münnich & Kuchenmeister, 2014; Vannuchi & Abreu, 2017).

In general, milk from dogs and cats has a high percentage of fat, as fat is the source of energy for the baby animal (Adkins et al., 2001), unlike cow's milk, which contains a large amount of lactose and is considered low in fat and other proteins (Dobenecker et al., 1998). Lactose is an essential source of metabolizable energy in cow's milk, but the amount present in traditional milk exceeds the digestive capacity of some species, causing diarrhea (Vannuchi & Abreu, 2017). For this reason, lactose-free milk and milk cream were used for the homemade formulation. The use of yolk is recommended as a source of fat (52%), protein (16%), lipids (34%), fat-soluble vitamins (A, D, E, and

K), glucose, lecithin, and mineral salts. Cat food is an additional source of essential nutrients such as vitamins and amino acids.

## Conclusion

Our study reveals that opossums (*D. albiventris*) fed with the homemade formulation exhibited superior growth and weight gain when contrasted with those nourished by the commercial formulation intended for puppies and kittens. Furthermore, we investigated the stomach capacity of newborn opossums, providing crucial insights into determining optimal feeding amounts and frequencies.

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