



How can low germination rates in Amazon chicory seeds be overcome?

Isabelle Caroline Bailosa do Rosário^{1*}  Renata da Silva Arruda²
Andressa de Oliveira Silva³  Rafaelle Fazzi Gomes³ 

¹Departamento de Ciências da Produção Agrícola, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista “Júlio de Mesquita Filho” (Unesp), 14884-900, Jaboticabal, SP, Brasil. E-mail: carolinebailosa@gmail.com. *Corresponding author.

²Departamento de Produção e Melhoramento Vegetal, Faculdade de Ciências Agrônômicas, Universidade Estadual Paulista “Júlio de Mesquita Filho” (Unesp), Botucatu, SP, Brasil.

³Universidade Federal Rural da Amazônia (UFRA), Capanema, PA, Brasil.

ABSTRACT: *Eryngium foetidum* exhibits low germination rates and heterogeneous seed maturation. Thus, in order to reduce problems during seedling production, a number of treatments can be used. The aim of this study was to assess the physiological quality of Amazon chicory seeds submitted to water immersion treatments. Two experiments were conducted, under controlled temperature (25 °C) and nursery conditions, with six treatments (0, 12, 24, 36 and 48 hours) in each. A completely randomized design was used in the controlled condition and random blocks in the nursery. The following were assessed: germination/emergence speed index (GSI/ESI), mean germination/emergence time (MGT/MET), germination/emergence percentage, normal seedling development, fresh weight and dry weight. The data were submitted to linear and second-order polynomial regression analysis in the R statistical program. Under controlled conditions, Maximum GSI was 4.65 after 18.03 hours of immersion, and for MGT, 12 h of immersion provided a shorter average time (13.76 days) and obtained 39.26% of germinated seeds after 26.27 h of immersion, as well as 19.34% of normal seedlings in 27.97 h. In the nursery, a lower MET was observed after 48 h and mean fresh and dry weight were higher after 12 h (18.47 and 1.44 g, respectively). Thus, water immersion for 12 hours resulted in a shorter germination time and higher fresh and dry weight, and the treatment may have helped reactivate embryo metabolism and growth.

Key words: *Eryngium foetidum*, physiological seed quality, germination.

Como superar as baixas taxas germinativas em sementes de chicória da Amazônia?

RESUMO: A *Eryngium foetidum* apresenta desuniformidade e baixas taxas de germinação. Assim, visando reduzir as problemáticas durante a produção de mudas, alguns tratamentos podem ser realizados. Por isso, essa pesquisa teve como objetivo avaliar a qualidade fisiológica de sementes de chicória da Amazônia submetidas a tratamentos de imersão em água. Foram realizados dois experimentos, em condição controlada de temperatura (25 °C) e em condição de viveiro, testados seis tratamentos (0, 12, 24, 36 e 48 horas) em ambos. Na condição controlada, utilizou-se delineamento inteiramente casualizado e no viveiro, blocos ao acaso. Sendo avaliado: índice de velocidade de germinação/emergência (IVG/IVE), tempo médio de germinação/emergência (TMG/TME), porcentagem de germinação/emergência, desenvolvimento de plântulas normais, massa fresca e massa seca. Os dados foram submetidos à análise de regressão linear e polinomial de 2ª ordem no programa estatístico computacional “R”. Na condição controlada, o IVG apresentou valor máximo de 4,65 após estimativa de 18,03 horas de imersão, para o TMG 12 h de imersão proporcionou tempo médio menor (13,76 dias) e obteve 39,26% de sementes germinadas após estimativa de 26,27 h de imersão, assim como 19,34% de plântulas normais em 27,97 h. Enquanto no viveiro, para o TME observou menor valor após 48 h e massa fresca e seca se obteve maiores médias após 12 h, 18,47 g e 1,44 g, respectivamente. Assim, a imersão em água por 12 horas proporcionou menor tempo de germinação e maior massa fresca e seca, possivelmente o tratamento auxiliou na reativação do metabolismo e crescimento do embrião.

Palavras-chave: *Eryngium foetidum*, qualidade fisiológica de sementes, germinação.

INTRODUCTION

According to TAIZ (2017), germination starts with water absorption and ends with emergence of the embryonal axis, while BRASIL (2009) affirms that germination is not only related to the development of essential embryonal structures, but also has the capacity to establish a normal plant.

However, some obstacles may interfere in germination, such as inhibition caused by the action of a number of substances contained in the inner

and outer part of the seed, blocking metabolism or hindering the passage of oxygen to the embryo (MARCOS FILHO, 2005). Some substances, such as coumarin, which is present in plants from the families Apiaceae, Asteraceae and Fabaceae, may affect germination and interfere in other processes such as cell elongation, root growth and respiration (HUSSAIN et al., 2018).

Eryngium foetidum L. belongs to the family Apiaceae, popularly known as Amazon chicory, coentrão, nhambi, Paráchicory or wild coriander, and cilantro in other

countries (GOMES et al., 2011). In addition, it belongs to the group of non-conventional food plants (PANC in Portuguese), which are a source of nutrients, vitamins, and mineral salts, with antioxidant and anti-inflammatory properties (GOMES et al., 2011; PASCHOAL & SOUZA, 2015). Its seed maturation is heterogeneous because flowering follows an indeterminate pattern, common in species of the family Apiaceae, characterized initially by primary umbels, then secondary, tertiary, and so on (EKPONG; SUKPRAKARN, 2006; EKPONG; SUKPRAKARN, 2008).

The seeds of this herb have low germination rates (6-10%) and germination potential, precluding synchronization and uniformity (MOZUMDER et al., 2010; FERRAZ et al., 2019). Thus, heterogeneous maturation and the likely presence of germination-inhibiting substances in seeds may contribute to this low germination. For this reason, some treatments are applied to promote an increase in germination rate and seedling development. MOZUMDER et al. (2017b) observed germination inhibition when seeds were not submitted to water immersion.

Given the existence of different semi-domesticated species with significant socioeconomic potential, such as *E. foetidum*, and the need to understand the dynamics of PANC propagation, the aim of this study was to assess the physiological quality of Amazon chicory seeds submitted to water immersion treatments, providing alternatives for this and other crops with germination problems.

MATERIALS AND METHODS

The study was conducted at the Rural Federal University of Amazônia, Capanema Campus, Pará state. *E. foetidum* seeds were collected in an experimental area of the university, stored in paper bags and refrigerated for 180 days. After storage, they exhibited a water content of 13%.

In order to test the six treatments proposed, at water immersion times of 0 h, 12 h, 24 h, 36 h, and 48 h, two experiments were carried out, one under controlled temperature and humidity (biochemical oxygen demand (BOD) incubator) and the other in a nursery. It is important to note the importance of testing in two environments, given the influence of climate conditions on seedling germination and development.

A completely randomized design was used for the controlled condition and random blocks for the nursery experiment. Four repetitions were used for both experiments, with 100 seeds per treatment.

In order to apply the treatments in the two experiments, first the number of seeds to be used per

repetition was separated. Next, the seeds were placed in 50 mL plastic cups containing distilled water. The cups remained on a bench at a temperature of 27.05 °C and humidity of 72.33% in a laboratory, according to the immersion times proposed (12 h, 24 h, 36 h and 48 h), based on the methodology of MOZUMDER & HOSSAIN (2013). The 0h treatment was not immersed.

After the immersion times were applied, the seeds were removed with a sieve and washed under running water to conduct the standard germination and emergence tests. For the germination test, four repetitions of 100 seeds were used for each treatment. Seeds submitted to different immersion times were placed equidistant on two sheets of germitest paper, saturated with distilled water, at 2.5-fold the dry paper weight, in Gerbox boxes. Next, the boxes were stored in a BOD incubator, at a temperature of 25 °C and relative humidity of 80 % (BRASIL, 2009).

The seedling emergence test was conducted with four repetitions of 100 seeds for each treatment, but under nursery conditions. Seeds submitted to different immersion times were sown in 200-cell polystyrene trays, filled with Tropstrato® commercial substrate, with one seed per cell.

In order to observe the dynamics of embryonic growth, five Gerbox boxes containing 100 seeds each were set up. Twenty seeds per day were desiccated over 14 days, according to the adapted methodology of WALCK et al. (2002). After desiccation, the embryo was photographed to measure the length using ImageJ software.

Water content was determined based on a sample of seeds from the lot that was tested in the present study. To that end, the oven method was applied at 105 ± 3 °C for 24 hours (BRASIL, 2009), using two repetitions of 100 seeds. The results were expressed in mean percentage based on seed fresh and dry weight.

Under the controlled condition, the germination speed index (GSI) was calculated from the number of seeds germinated per day, divided by the number of days from sowing to germination (MAGUIRE, 1962); mean germination time (MGT) from the daily germinated seed counts until 30 days after sowing, according to the methodology of LABOURIAU (1983); primary root immersion, obtained from the number of seeds that the primary root emitted at the end of the test, divided by the total number of seeds (100); and the percentage of normal seedlings, based on the number of normal seedlings at the end of the test divided by the total number of seeds (100).

For the nursery, the emergence speed index (ESI) was assessed, following the methodology of

MAGUIRE (1962); mean emergence time (MET) according to LABOURIAU (1983); emergence percentage and normal seedling percentage, based on the number of emerged seeds, observing the hypocotyl above the substrate; and the number of normal seedlings at the end of the test, divided by the total number of seeds (100).

In addition, in the nursery, after normal seedling selection, seed fresh weight was measured on an analytical precision balance. Later, based on the methodology of NAKAGAWA (1999) to obtain dry weight, the seedlings were dried in a forced air oven at 65 °C, until constant weight.

Germination and emergence data were previously submitted to determine the ANOVA assumptions. Residual normality was tested by the Shapiro-Wilk test, using the “RVAIdeMemoire” package with the “byf.shapiro” function, and homoscedasticity between residual variances by Levene’s test, with the “car” package and “leveneTest” function. They were then submitted to linear and 2nd order polynomial regression with the best fit equation for each parameter based on the coefficient of determination (R^2), using the “ExpDes. pt” package. The germination speed index was transformed using Box Cox methodology (BOX; COX, 1964) and the “MASS” package, in the R statistical program (version 4.1.0). The regression graphs were constructed in Office Excel®.

RESULTS AND DISCUSSION

In relation to embryonic growth (Figure 1), the embryonic axis increased, stabilizing between

the 7th and 14th day, with initial and final length of 0.042 and 0.077 mm, respectively. The embryo may have been physiologically immature, since growth stabilized from the seventh day, with no radicle protrusion. This result may be due to the collection of different umbels, deteriorated and/or immature seeds or the difference in developmental stage.

According to MARCOS FILHO (2005), embryo physiological immaturity may be associated with the absence of uniform maturation, and some processes may have been interrupted by the harvest. As such, some seeds may not have reached physiological maturity, which may explain the low germination rates in this species.

Under controlled conditions, GSI (Figure 2A), showed a quadratic fit as a function of water immersion time, with an estimated maximum value of 4.65 for 18.03 hours of water immersion. For MGT, when the seeds were immersed in water for longer, germination took more time to occur (approximately 17 days) (Figure 2B). The 12h treatment resulted in shorter average germination time (13.76 days) (Figure 2B), which may have guaranteed enough water absorption for germination, without causing deterioration.

Thus, water immersion may be favorable up to a certain period, one of the possible benefits being anticipating the first stage of the triphasic pattern. MARCOS FILHO (2005) reported that phase I is characterized by rapid water absorption, and metabolic reactivation due to the different potential. However, according to MOZUMDER et al. (2017a), although immersion helps trigger germination, prolonged direct contact with water may decrease

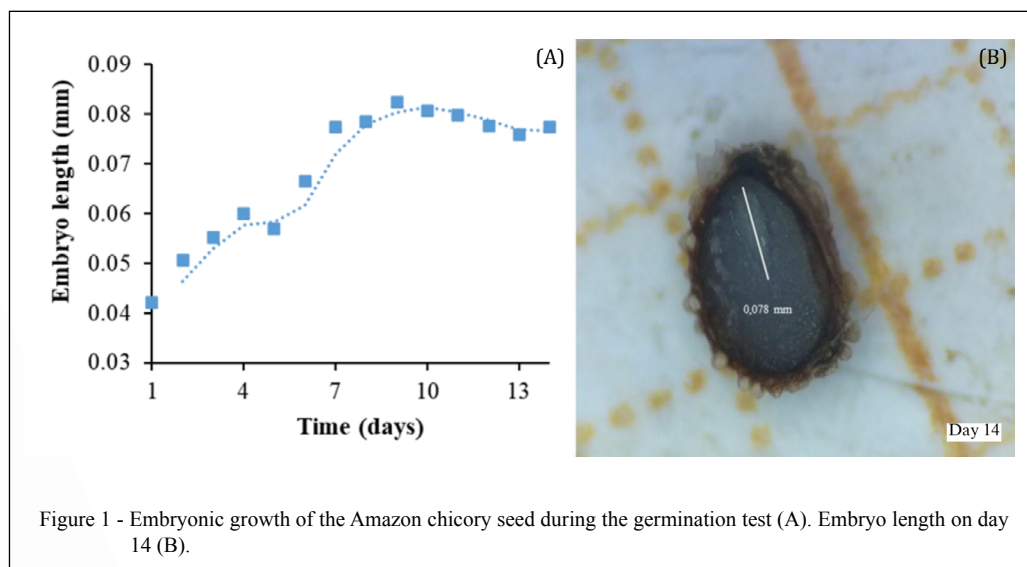
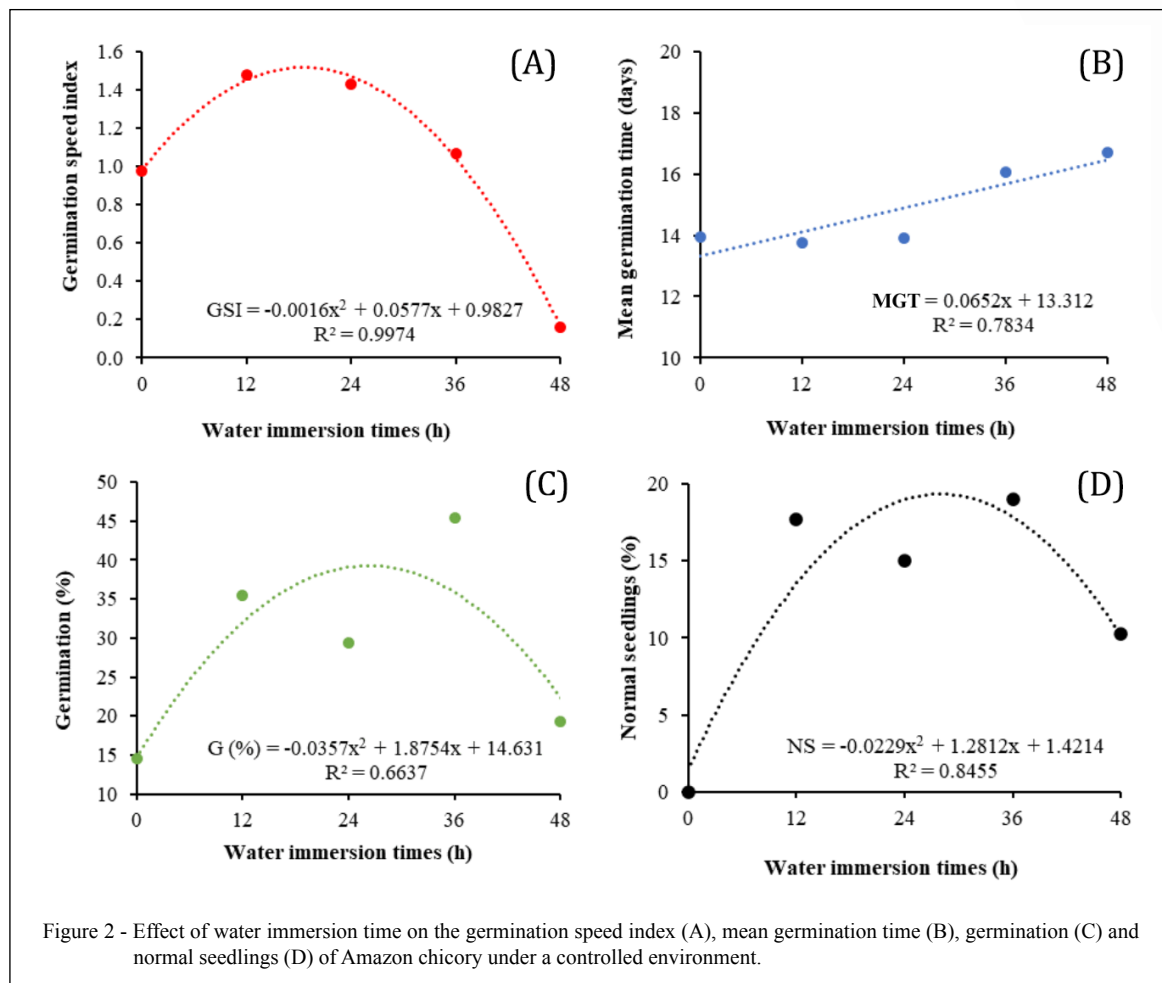


Figure 1 - Embryonic growth of the Amazon chicory seed during the germination test (A). Embryo length on day 14 (B).



seed vigor and viability due to deterioration of the cell membrane and organelles. This behavior was observed in the 48h treatment, evidenced by the decline in germination speed.

A quadratic fit was also observed for germination percentage (Figure 2C), with 39.26%, obtained after the maximum estimated time of 26.27 h. Pre-germination treatment likely helped in embryonic development, given the hypothesis of being physiologically immature. In addition, the absence of immersion resulted in a lower germination percentage, since according to TAIZ (2017), the embryo cannot germinate before reaching physiological maturity, requiring more time after the harvest. In this case, contact with water reactivates metabolism, causing nutrient mobilization.

For normal seedlings (Figure 2D), second-order polynomial regression was observed, with 19.34% of seedlings deemed normal when Amazon chicory seeds were immersed in water

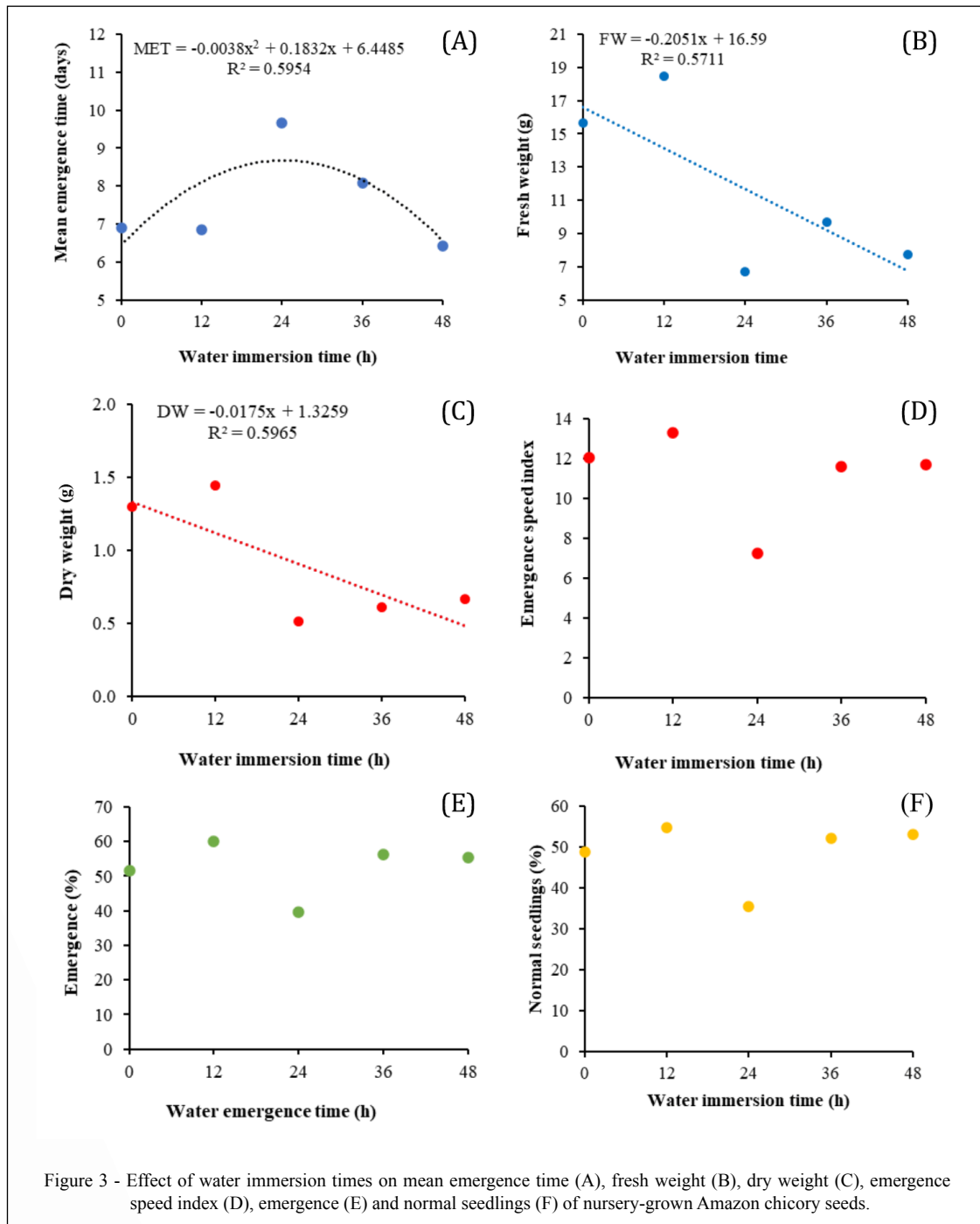
for 27.97 h. The low percentage is due to fungal infection, hard or dormant seeds and abnormal seedling production.

In addition to assessment in the controlled environment (constant temperature of 25 °C), characteristics under nursery conditions were also evaluated. In this case, for MET, fresh and dry weight were fit to the regression models. However, for ESI, emergence and normal seedlings, no significant effect was found for the regression models (Figure 3).

For MET (Figure 3A), the best fit was for the second-order polynomial regression, estimating an optimal value at a water immersion time of 24.11 h, resulting in MET of approximately nine days. Water capture during the pre-germination period was not sufficient to reduce germination time in the 24h treatment. In addition, climate conditions and harvest time may have contributed to the higher MET, given that the seeds used were from the 4th order inflorescence (mixture) onwards.

Thus, the lot used contained seeds in different stages of maturation, which may explain the quadratic fit, resulting in lower values for the control and 48 h treatments. FIGUEIREDO et al. (2017) reported that depending on harvest time, the physiological potential of seeds may decline due to deterioration, long residence time in the field or na immature embryo.

Figures 3B and 3C demonstrate the decrease in fresh (FW) and dry weight (DW) of *Eryngium foetidum* L. seedlings, exhibiting a better fit of the equation to the linear model. During immersion, seedling FW and DW declined, with the control and 12h treatments obtaining the highest means.



Periods of more than 24 hours likely contributed to destroying the cell structure, influencing seedling development and causing greater solute leaching, which may be essential for germination. In a study with the “Preta Comprida” eggplant cultivar, ALVES et al. (2012) observed the inferior performance of lots with a larger amount of solutes leached by the seeds.

It is important to note that the characteristics of non-germinated seed and abnormal seedling data did not fit any regression model. This may be due to the different developmental levels of the seeds used, in addition to the climate of the region and microclimate formed from the shade cloth of the nursery, thereby interfering in the results obtained.

CONCLUSION

Twelve-hour water immersion resulted in a shorter germination time and higher fresh and dry weight, and the treatment may have helped reactivate embryonic metabolism and growth.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

ICBR and RFG conceived and designed experiments. ICBR, RSA and AOS performed the experiments. ICBR performed statistical analyses of experimental data. ICBR and RFG prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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