Nativa, Cuiabá, v. 14, n. 3, e21062, 2026.
Pesquisas Agrárias e Ambientais
DOI: https://doi.org/10.31413/nat.v14i3.21062
ISSN: 2318-7670

Quality of pine shavings for the microenvironment and behavioral preference of Syrian hamsters (*Mesocricetus auratus*)

José Guilherme dos Santos MOREIRA [1], Juarez Benigno PAES *[1], Leonardo Oliveira TRIVILIN [2], Antônio Thiago Soares de ALMEIDA [1], Yonny Martínez LÓPEZ [3], Arlon Bastos da ROSA [1], Marcos Alves NICACIO [1]

[1] Federal University of Espírito Santo, Jerônimo Monteiro, ES, Brazil.
[2] Federal University of Espírito Santo, Alegre, ES, Brazil.
[3] Federal University of Southern and Southeastern Pará, São Félix do Xingu, PA, Brazil.
*E-mail: jbp2@uol.com.br

Submitted on: 02/19/2026; Accepted on: 07/13/2026; Published on: 08/05/2026.

ABSTRACT: This study analyzed the quality of commercial-brand pine wood shavings and proposed the development of a differentiated product for hamster (*Mesocricetus auratus*) breeding. Thus, the quality of commercial wood shavings was evaluated based on granulometry, moisture content and sanitary conditions. Due to the lack of standardization, sterilized, colored, and scented wood shavings are being developed. To verify the effect of sterilization, two methods were compared (autoclave and oven). Powdered food dyes were used to color the wood shavings. After dyeing, the material was air-dried and sterilized in an oven. Color and aroma preference tests were carried out using 20 sexed hamsters, and changes in food and water consumption were observed in the aroma test (characteristic of stress in the animals). Then, two systems of five coupled cages were set up for the color preference test; each system contained 10 sexed hamsters for 7 days, accompanied by video monitoring. There was growth of fungi in all evaluated commercial brands. It was found that there was no change in the behavior of the hamsters, in terms of particle size and color of shavings, which could be a market differentiator. Scenting alters animal behavior and is not recommended.
Keywords: wood waste; coloring; aroma; small rodents; animal behavior.

Qualidade de maravalhas de pinus para microambiente e preferência comportamental de hamster sírio (*Mesocricetus auratus*)

RESUMO: Este estudo analisou a qualidade da maravalha de pinus de marcas comerciais e propôs o desenvolvimento de um produto diferenciado para a criação de hamsters (*Mesocricetus auratus*). Assim, a qualidade da maravalha comercial foi avaliada com base na granulometria, teor de umidade e condições sanitárias. Devido à falta de padronização, foram desenvolvidas maravalhas esterilizadas, coloridas e aromatizadas. Para verificar o efeito da esterilização, compararam-se dois métodos: autoclave e estufa. Corantes alimentícios em pó foram utilizados para colorir a maravalha. Após a coloração, o material foi seco ao ar e esterilizado em estufa. Testes de preferência de cor e de aroma foram realizados com 20 hamsters sexados. Observaram-se alterações no consumo de ração e de água durante o teste de aroma (indicativas de estresse nos animais). Em seguida, montaram-se dois sistemas de cinco gaiolas interligadas para o teste de preferência de cor; cada sistema abrigou 10 hamsters sexados por 7 dias, com monitoramento por vídeo. Houve crescimento de fungos em todas as marcas comerciais avaliadas. Não houve alteração no comportamento dos hamsters em relação ao tamanho das partículas e à cor da maravalha, o que poderia constituir um diferencial de mercado. A aromatização alterou o comportamento dos animais e não é recomendada.
Palavras-chave: resíduos de madeira; corantes; aroma; pequenos roedores; comportamento animal.

1. INTRODUCTION

Wood has been used for years for energy production, the furniture industry, civil construction, shipbuilding and pulp and paper. In some cases, it is necessary to machine it for its use, and this leads to residue generation, which can have different applications to increase the monetary income of the enterprise, since 30 to 50% of the log volume is used in the process of converting wood into manufactured products (STRAGLIOTTO et al., 2023). Wood from native forests has been replaced by reforestation in order to meet the demand for different uses (*Eucalyptus* and *Pinus*).

Trees of the *Pinus* genus predominate in large forests in the Northern Hemisphere and are representative in the world economic scenario with 50% of wood consumption (LANTSCHNER et al., 2020). There are conditions in Brazil that favor their development, especially *Pinus elliottii*, *Pinus taeda*, and their hybrids. Their appropriate physical and mechanical characteristics make it possible to use them in

producing furniture, wood frame houses, components for civil and rural construction, wooden boxes, sawn products and panels (LIMA et al., 2023). These activities generate appreciable waste volumes in the form of trimmings, sawdust and wood shavings.

Wood residue generation with low utilization causes environmental damage, in addition to a waste and opportunity for the timber industry, local communities, governments and society in general (RIBASKI; BELINI, 2019). Use of the waste is an important form in the market for the timber industry, taking into account that the proper allocation of these resources can stop being an environmental problem and start generating income, which is advantageous for the company.

Pine wood shavings have several uses, such as to poultry production systems, cattle, other type of animal production (rabbits, sheep, goats, and pigs) (Munir et al., 2019), and horse bedding (Ahn et al., 2020), lining for cages for small animals of the Rodentia order (rats, hamsters, mice, chinchillas, and guinea pigs) (Hudda et al., 2019) and pets with the function of absorbing spilled water and the animals' urine, in addition to serving as a thermal insulator to reduce heat conduction from the animals' bodies through the bottom of the cage. It serves as material for building nests for the females to accommodate their young (HUDDA et al., 2019).

It is recommended that *Pinus* spp. be sterilized by steam under pressure in an autoclave to be used in animal husbandry. It is essential that the quality is guaranteed so that it does not negatively interfere with breeding, as a proliferation of pathogens may cause damage to the health of animals (REIS; FRANCO, 2012).

Some organizations and researchers recommend that the wood shavings for rodents be made from coniferous wood and not from hardwoods such as cedar, which emits aromatic hydrocarbons that are harmful to animal health. This is an important subject that has been studied by Sivamaruthi et al. (2024), who carried out research on the importance of using aromatic products that are not harmful to animals.

In addition to rodents being widely used for research, they also serve as human companions as pets due to their ease of handling, intelligence and docility (TANNO; COUTO, 2015). They need bedding on the floor of the cages for both domestic use and for research, which allows urine to be absorbed, provides thermal insulation, is comfortable, devoid of an unpleasant smell, and is easily disposed of and transported (BURN; MASON, 2005). However, the natural color of wood shavings makes it difficult to locate chips and other animal identifications in case of detachment.

The golden Syrian hamster (*Mesocricetus auratus*) differs from other rodents, as it has a very short tail and a compact body. Its length as an adult is around 14-19 cm, and it weighs between 110 - 140 g (GAD, 2019). These animals are often used in laboratory tests with immunological, chronobiological and behavioral assays. They are territorial and therefore usually kept in individual cages (ELIDIO et al., 2021).

Due to the close relationship between animal health and welfare, the quality inside the cages does not differ from that applied to the macroenvironment, such as poor aeration and contaminants. The most common contaminants in rodent cages are carbon dioxide and ammonia, so the exchange of wood shaving "beds" must be constant so that it does not reach concentrations that cause adverse effects to animals (ESKANDARANI et al., 2023).

Given the above, there was a need to develop a product (shavings) with differentiated characteristics, greater attractiveness on the part of animals and consumers. Therefore, this study analyzed the quality of commercial-brand pine wood shavings and proposed the development of a differentiated product for hamster (*Mesocricetus auratus*) breeding. Thus, the quality of commercial wood shavings was evaluated based on granulometry, moisture content and sanitary conditions. Due to the lack of standardization, sterilized, colored, and scented wood shavings are being developed.

2. MATERIAL AND METHODS

2.1. Quality assessment of commercial pine wood shavings

The quality of pine wood shavings intended for lining cages for raising small animals existing in the Brazilian market (five manufacturers sampled) was evaluated based on granulometry, moisture content and sanitary conditions. Thus, first, classifying sieves with meshes of 10, 5 and 1 mm were used for the granulometric classification in order to quantify the passing proportions in each one of them. Then, the percentage of fines capable of causing respiratory problems to the animals (particles smaller than 0.250 mm) was quantified from the material passing through the 1 mm mesh.

Next, five representative samples were taken (10% of the total existing in each manufacturer's packaging) to evaluate the moisture content (dry basis) of the material; these were composed of three packages for each of them, which were homogenized, quartered, and the samples were taken. They were transferred to a 1,000 mL glass beaker, weighed (accuracy of 0.01g) and dried in an oven maintained at 103 ± 2 °C until constant mass and weighed again. The moisture content was evaluated on a dry basis, as in American Wood Protection Association - AWPA E10-22 (2024).

As mentioned, five representative samples of each manufacturer were taken to evaluate the sanitary conditions of the particles. Thus, the proportion of particles from the sampled material stained by fungi was evaluated. This material was homogenized and samples were taken and submitted to tests to evaluate fungal growth, according to the methodology described by Alfenas; Mafia (2016). The first pathogenic organisms that begin the biodeterioration of wood are fungi, which colonize newly cut trees (called mold and stain), due to the high concentration of reserve substances that nourish the organisms, and humid conditions for fungal growth (ARPANAEI et al., 2024).

The fungi were grown in culture medium and prepared according to the specification of the AWPA E10-22 (2024). Next, they were identified and classified by genus, and the existence of any pathological damage by them to small domesticated animals was verified in the literature.

2.2. Development of wood shavings with differentiated characteristics

Pinus elliottii and *Pinus taeda* (in unknown proportions) wood shavings were used for the material with different characteristics intended for raising small animals, obtained from machined wood (Figure 1) from existing plantations in Venda Nova do Imigrante, in the southern region of the State of Espírito Santo (latitude 20°23'37.1" S, longitude 41°08'29.6" W and altitude of 730 m), Brazil, belonging to the Complexo Agroindustrial Pindobas Ltda.

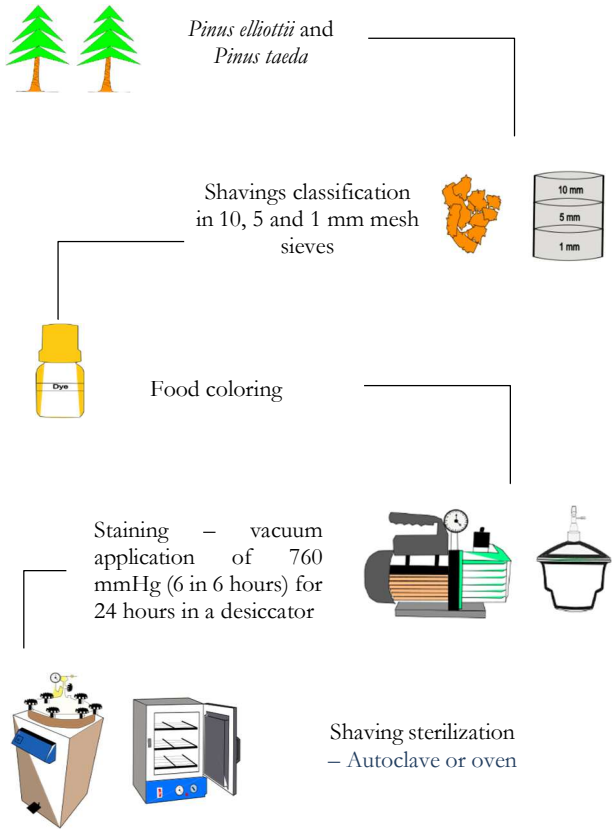

Figure 1. Schematic representation of the classification, coloring and sterilization of shavings.
Figura 1. Representação esquemática da classificação, coloração e esterilização de aparas de madeira.

Shavings were classified into 10, 5 and 1 mm-mesh sieves to remove the fines (< 1 mm). After being classified, they were mixed in equal proportions based on the dry mass and dyed with food coloring (according to the information on the package label), without gluten or allergic products, into red, blue, green and pink colors; to do so, 5 g of dye per liter of distilled water was used to color the wood shavings. This proportion was defined through tests to observe the color enhancement in the particles.

Staining was performed with and without the use of a vacuum of 760 mmHg (6 in 6 h) for 24 hours in a desiccator. To do so, 100 g of shavings were arranged in a non-woven fabric (NWF) mesh composed of 100% polypropylene with a weight of 80 g m^{-1} of 30 × 30 cm, and white in color.

The shavings to be used were sterilized in an autoclave and in an oven. First, 10 g of wood shavings were taken and placed in 600 mL bottles with a screw-on metal lid and exposed to a temperature of 121°C, pressure of 103 kPa, for 15 and 30 minutes for sterilization in an autoclave. The same amount of material and container were used under the same conditions as those used in the autoclave for the oven method, with four times of 6, 12, 24 and 48 hours and three temperatures of 60, 80 and 100 °C being applied.

After being sterilized, the flasks were kept under laboratory conditions (25 ± 2 °C and 65 ± 5% relative humidity - RH), placed in a laminar flow hood, and fungal-stained particles were removed in order to evaluate fungal growth. The material was sterilized with 1% sodium hypochlorite, washed in distilled water and placed in a Petri dish with potato, dextrose and agar (PDA) culture medium, according to Alfenas; Mafia (2016). Three replicates were performed per sample, totaling 42 dishes.

The sterilized shavings were exposed to the same temperature and humidity conditions for 48 hours to evaluate the moisture content. Thus, 10g samples were taken, which were submitted to the procedure already described. In addition, natural aromas were sprayed on part of the remaining material (citronella and eucalypts) in order to evaluate aroma fixation.

The fixation test was carried out with two aromas (eucalypts and citronella) and one solvent (prepared with 90% alcohol and 10% Tween 20), with a solution in a volume of 50 mL containing a concentration of 10% aroma. It was directly applied using a manual sprayer with a capacity of 50 mL onto the shavings arranged at the bottom of the cages intended for breeding/raising laboratory animals, with dimensions of 49 × 16 × 34 cm (width × height × length), and containing 300 g of wood shavings.

The cages remained in a laboratory environment (25 ± 2 °C and 65 ± 5% RH), and exhalation of the aroma was verified at intervals of 12 hours by smelling in the sensory analysis paired comparison test according to the Brazilian Association of Technical Standards - ABNT, Brazilian Standard - NBR, International Organization for Standardization - ISO 13299 (2021). This procedure was carried out for 48 hours.

2.3. Wood shavings used in carrying out the experiment

Wood shavings were used *in natura*, colored and aromatic. For coloring, 5 kg of shavings (in the proportions mentioned) were added to a raffia bag (polypropylene). The wood shavings were immersed for 24 hours in a 50-liter plastic (polyethylene) container containing 40 L of the coloring solution (5 g of powdered dye per liter of water).

After this time, the shavings were left to drain the excess solution for 12 hours, and then placed on a black plastic sheet (polyethylene) to air dry (≈ 20% humidity, dry basis). After drying, the material was placed in a 100-liter non-woven fabric (NWF) bag for drying and sterilization in an oven at a temperature of 103 ± 2 °C for 6 hours. After sterilization, the shavings were kept in laboratory conditions for cooling (24 hours) and stored in transparent polystyrene bags (25 cm × 35 cm) with a volumetric capacity of 5 liters. Uncolored wood shavings were used to evaluate only the effect of aromas, which could be a factor in changing the behavior of hamsters, as assessed by water and food consumption.

Adult Syrian hamsters (*Mesocricetus auratus* Waterhouse) (120-150 g and a total of 20 animals) from the Central Animal Facility - UT/Imbio, Biosciences Institute of the Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil were used to experiment the Experimentation Laboratory of the Veterinary Hospital, Center for Agricultural Sciences and Engineering, Federal University of Espírito Santo, Alegre, Espírito Santo, Brazil. They were kept in groups of five animals of different sexes, at a temperature of 24 ± 2 °C with a light-dark cycle of 12 × 12 hours, and with free access to food and water. Each group (10 animals) was separately subjected to color and aroma tests.

Water and feed were replaced in the cages every two or three days, depending on the need. The quantities were made available according to their consumption, taking care not to run out of food, which could influence the result of the study. Initial and final weighing of water and feed available in the

cages was performed to verify the amounts consumed in each group.

Hamsters have the habit of eating other hamsters that die in the same cage, and so surveillance was intensified, and the animals were checked daily to check their physical condition to prevent this from happening during the experimental period. If a hamster showed any sign of stress, weakness or severe diarrhea, it was placed in a cage separate from the others to prevent the other members from eating it after its death, as this would compromise the experiment since it was based on the specific diets of each group of animals.

2.4. Aroma and color tolerance tests (Pilot evaluation)

Uncolored (test with aromas) and colored wood shavings (test with colors) were used in the preliminary tests to verify the tolerance of the animals. In turn, the existence of some adverse behavior of the hamsters was observed in the tests, such as excessive water and feed consumption, which indicated a reaction of some effect caused by the altered microenvironment, taking into account that the only element altered was wood shavings with an aroma.

A total of 300 g of wood shavings were used for the aroma tolerance test, sprayed with 50 mL of solution at concentrations of 1, 5 and 10% of aroma. The material was placed in the cages after being homogenized. In the test, 10 hamsters (5 males and 5 females) were placed in the cage with food and water, as well as 500 g of pelleted feed intended for rearing rats, mice and hamsters and two drinkers with a capacity of 500 mL, filled with filtered water through an activated carbon filter, were used.

Water and feed consumption were measured (500 mL test tube and scale with a precision of 1.0 g, respectively), changing them every 48 hours and repeating the aromatization process three times over a period of 7 days. A similar procedure was adopted for the shavings color tolerance test. In this case, a color was chosen at random, and the hamsters (with the same characteristics and conditions) were placed in the cages for the same period, and the food and water consumption were evaluated in the same way.

2.5. Hamster color preference tests

The preference test was only performed for color, since water and food consumption behavior was observed above the standards analyzed in the daily lives of the hamsters submitted to the preliminary aroma test. We used five polyethylene (PE) cages measuring 49 × 16 × 34 cm (width × height × length) in this test, connected to each other by 50 mm threaded polyvinyl chloride (PVC) flange-type connectors (Figure 2), containing colored shavings. The colors were randomly distributed, and 10 hamsters (5 males/5 females) were released into a certain cage defined by drawing lots, and their preference for a certain color was evaluated. The animals were nourished similarly during the test as adopted in the preliminary test.

The preference of the animals was evaluated based on the environment where they were (cage - color) at the pre-established time for observation (every 30 min) for 7 days, totaling 2,500 observations, in which the number of animals per environment was counted using a video surveillance camera.

2.6. Statistical evaluation of the data

Percentages and descriptive analysis (means and standard deviation) were used to evaluate moisture content (dry basis), granulometry and fungal growth, in which brands obtained on the market were compared with the proposed improved shavings, and five samples were used for each product.

Figure 2. Assembly and arrangement of cages (color preference test).
Figura 2. Montagem e disposição das gaiolas (teste de preferência de cor).

Next, a completely randomized design (CRD) in a factorial arrangement was used to evaluate the efficiency of the shavings sterilization method (autoclave and oven), in which the effects of the sterilization mode (two factors) and sterilization time (two and four factors, autoclave and oven, respectively) were tested. In this case, ten replicates were used for each situation evaluated. The effect of the treatments (temperatures and times) was verified by applying the analysis of variance (ANOVA) and the F-test ($p < 0.05$), and the Tukey test was applied ($p < 0.05$) when significant to discriminate the means (MAGALHÃES; LIMA, 2002).

A Latin square design was adopted (two sexes and two aromas) for the aroma tolerance test to verify whether there was a difference between sex and the aroma concentration applied, and if the lowest concentration approached the control (no aroma). This was verified by applying the ANOVA and the F-test ($p < 0.05$), and Tukey's test was again applied ($p < 0.05$) when significant to discriminate the means (MAGALHÃES; LIMA, 2002). The CRD was also used for tolerance to the coloring product, and the occurrence of a significant difference between feed and water consumption for the tested dyes was verified by applying the ANOVA and F-test ($p < 0.05$) (MAGALHÃES; LIMA, 2002). For these cases, ten replicates (cages) per treatment were used, and the behavior (water and feed consumption) of the group of animals was evaluated.

Finally, a descriptive analysis was used to assess the preference of the animals for the colored material, in which the preference of the hamsters for the wood shavings used was compared. Ten repetitions were evaluated for each sex, which were changed three times during the experiment. In this case, the frequency of the animals in each cage containing colored wood shavings was evaluated. The test involved analyzing the number of animals grouped in the same place, whether or not they formed groups, and their feed and water

consumption. In this case, the behavior in relation to wood shavings was measured by applying the ANOVA and the F-test ($p < 0.05$), and the Scott-Knott test was applied ($p < 0.05$) when significant to discriminate average water and feed consumption (MAGALHÃES; LIMA, 2002). In all cases, prior to performing the ANOVA, the assumptions regarding normality (Lilliefors test, $p < 0.05$) and homogeneity of variances (Cochran test, $p < 0.05$) were checked.

The behavioral analyses (frequency of animals per cage) were purely descriptive, without tracking the specific animals present in each environment during the evaluated period. At the end of each trial, the animals returned to their original cages and were not used in further trials.

3. RESULTS

3.1. Particle size evaluation of pine wood shavings

Brand A had 6.70% of particles retained on the 10 mm-mesh sieve; 34.9% in the 5 mm; 49.52% in the 1 mm; and 8.88% of particles smaller than 1 mm, having the highest proportion of smaller particles. Next, brand B had 0.56% of material retained in the 10 mm-mesh sieve; 28.64% in the 5 mm; 69.92% in the 1 mm; with 0.88% of particles smaller than 1 mm.

For brand C, 0.63% of the material was retained in the 10 mm-mesh sieve; 43.67% in the 5 mm; 49.27% in the 1 mm; and 6.43% of the material was smaller than 1 mm. Brand D had 9.78% of particles retained in the 10 mm-mesh sieve; 66.60% in the 5 mm; 23.21% in the 1mm; and 0.41% of particles smaller than 1 mm. Lastly, brand E had 5.22% retained in the 10 mm-mesh sieve; 68.60% in the 5 mm; 25.62% in the 1 mm; and 0.56% of material smaller than 1 mm.

3.2. Evaluation of the pine wood shaving quality

The shavings brands analyzed showed moisture content below 12% (Table 1), which is a condition that inhibits fungal growth. Brands B, C and E had the highest moisture content, above 9%, while brands A and D had the lowest values, below 9%.

Regarding the fungal growth in the stained particles of the five commercial brands tested, it was observed that fungal growth occurred in more than 40% of the 11 Petri dishes inoculated (Table 1). This allows us to state that the shavings were not sterilized or that the sterilization process was not efficient. Growth was observed in all brands, with brand E having the highest proportion, but there was fungal growth in all samples.

Trichoderma sp. growth was observed on nine dishes for brand A, it was not possible to identify one of the fungi present due to the absence of spores. There were three dishes with *Trichoderma* sp. in brand B, and one with *Aspergillus flavus*; it was not possible to identify the pathogen in three dishes. *Trichoderma* sp. was found on three dishes for brand C; and in two of them, there was the growth of many spores, but it was not possible to identify the conidiophore.

Trichoderma sp. growth was observed in five dishes for brand D, but in another five, it was not possible to identify because there was no growth of spores. Next, one dish with *Guilmaniella* sp. and five with *Trichoderma* sp. growth were observed for brand E, but in another five, it was not possible to identify, as there was no occurrence of spores.

The proportion of stained particles by granulometric class in relation to the total mass existing in the packaging of each brand (Table 2) indicated that the largest amount of material with stains is found in the smallest granulometries (1-5 mm, brands A, D and E; and 1 mm, B and C).

Table 1. Moisture content and fungal contamination rate of shavings by trademarks were evaluated.
Tabela 1. Teor de umidade e taxa de contaminação fúngica das aparas, de acordo com as marcas avaliadas.

Trademarks	Moisture content (%)	Petri dishes	
		Units	Contamination percentage (%)
A	8.5	10	99.90
B	11.2	7	63.64
C	9.3	5	45.45
D	8.8	10	90.90
E	9.2	11	100.00
Control*	8.8	0	0.00

* Wood shavings were elaborated for the research.
* Aparas de madeira elaboradas para a pesquisa.

Table 2. Proportion of stained particles by particle size class (granulometry) and trademarks (manufacturer) in shavings.
Tabela 2. Proporção de partículas coradas por classe de tamanho de partícula (granulometria) e por marca comercial (fabricante) em aparas.

Trademark	Proportion of stained particles/Granulometry (mm)					
	10		5		1	
	Mass (g)	(%)	Mass (g)	(%)	Mass (g)	(%)
A	1.10	0.16	15.02	2.23	8.16	1.21
B	1.28	0.41	30.01	9.65	55.20	17.69
C	3.62	0.53	24.45	3.35	75.23	10.91
D	17.71	5.91	50.40	16.83	10.11	3.38
E	14.01	1.98	84.65	11.97	25.50	3.61
Control*	16.50	3.30	16.50	3.30	17.00	3.40

* Shavings prepared for research.
* Aparas preparadas para pesquisa.

3.3. Preliminary tests carried out on the improved shavings

The quantity in grams of liquid absorbed by the particles was measured in the improved shavings to evaluate the absorption of the dye solution, which had similar moisture contents (dry basis) (≈ 8%). No significant difference was observed by the F-test ($p > 0.05$) in the absorption of the solution, whose values ranged from 142.59 to 151.21 g. In the evaluation of the sterilization process of the particles in an oven (Table 3), it was observed that there was fungal growth at a temperature of 60 ºC, even for the time periods of 24 and 48 hours, demonstrating the inefficiency of the temperature and time used to cause the death of fungi existing in stained shavings. Sterilization was effective for a

temperature of 80 ºC with only 48 hours of exposure. However, no fungal growth was observed in shavings subjected to a temperature of 100 ºC, regardless of the time spent in the tested situation.

Table 3. Number of plates on which fungal growth occurred after sterilization in an oven, for the time and temperature tested.
Tabela 3. Número de placas com crescimento fúngico após esterilização em estufa, para o tempo e a temperatura testados.

Time (Hours)	Sterilization temperature (ºC)		
	60	80	100
6	5	5	0
12	5	4	0
24	4	3	0
48	3	0	0

3.4. Preliminary tests performed with the animals

For tolerance tests to the aromas and dyes used, it was observed that there was no significant difference by the F-test ($p > 0.05$) in terms of aroma tolerance between the animals' behavior for both males and females and for the type of aroma tested. This indicates that the hamsters reacted in the same way, regardless of sex. However, there was a difference in the applied concentration (F, $p < 0.05$), which caused an increase in feed and water intake (Table 4).

Table 4. Total food consumption (ration and water) daily by the two groups of 10 males and 10 females for aroma concentrations.
Tabela 4. Consumo total diário de alimentos (ração e água) dos dois grupos (10 machos e 10 fêmeas) em função das concentrações de aroma.

Consumption	Aroma concentrations (%)			
	0	1	5	10
Feed (g)	172.16 b	310.08 a	314.33 a	312.00 a
Water (mL)	419.58 b	575.00 a	589.08 a	595.00 a

Means followed by the same letter, on the line, do not differ (Tukey test, $p > 0.05$).
Médias seguidas pela mesma letra na mesma linha não diferem (teste de Tukey, $p > 0,05$).

Next, there was no significant difference for food consumption (water and feed) between the hamsters in the tolerance test to the colored product by the F-test ($p > 0.05$) and regardless of sex, with water consumption ranging from 407.0 to 422.0 mL (females) and from 397.0 to 419.0 mL (males), and feed from 122.6 to 124.4 g (females) and from 120.8 to 123.0 g (males). However, the color × sex interaction was significant for water consumption. The interaction was dismembered, with higher consumption by females of uncolored wood shavings (397 mL for males and 419 mL for females). Based on these results, a color preference test for wood shavings was performed.

3.5. Color preference test

In the analysis of the permanence of the hamsters (females and males), it was observed that the females stayed more frequently (number of observations) in cages with natural shavings, or colored with pink, red, green (637) and blue dye (641) (Table 5). Note that the values had a maximum variation of 57 between the observations in the established period of every 30 minutes of the hamsters in the cages with the tested colors.

It was observed that the male hamsters remained longer (frequency) in cages with shavings colored with green, red, blue, pink and natural dye (Table 5). Regardless of the color of the material, the animals more frequently manipulated larger granulometric particles to compose the place chosen for resting or nesting (Figure 3).

With regard to food consumption by the animals, there was a statistical difference by the F-test ($p < 0.05$) between treatments (colors). It was observed that water and feed were consumed in all cages, with differences between the colors and sex of the animals (Table 6).

Table 5. Permanence of animals (females and males) in each cage with colored shavings.
Tabela 5. Permanência de animais (fêmeas e machos) em cada gaiola com serragem colorida.

Evaluated Parameters	Females/Colors of wood shavings				
	Red	Pink	Green	Natural	Blue
Frequency	664	693	637	694	641
Mean	1.98	2.06	1.90	2.07	1.91
SD	0.92	0.89	0.89	0.86	0.96
	Males/Colors of wood shavings				
Frequency	709	610	735	560	635
Mean	2.11	1.82	2.19	1.67	1.89
SD	1.01	0.99	1.07	1.01	1.10

SD: Standard deviation.
SD: Desvio padrão.

Figure 3. Cages with colored and natural wood shavings, where the accumulation of material of greater granulometry can be seen (arrow) at the site of the nest.
Figura 3. Gaiolas com maravalhas coloridas e ao natural, nas quais se observa (seta) o acúmulo de material de maior granulometria no local do ninho.

Table 6. Consumption of water and feed by the animals (females and males) in each cage with colored shavings.
Tabela 6. Consumo de água e de ração pelos animais (fêmeas e machos) em cada gaiola com maravalha colorida.

Animals	Water consumption (mL)/ Shaving colors				
	Blue	Natural	Pink	Green	Red
Female	41.0Ab	62.5Bb	90.0Aa	43.5Bb	53.0Ab
Male	28.5Ba	70.5Aa	46.5Ba	61.0Aa	53.5Aa
	Feed consumption (g)/ Shaving colors				
Female	25.1Ab	51.8Aa	27.0Bb	19.7Bb	37.3Aa
Male	23.3Aa	26.3Ba	35.2Aa	43.3Aa	35.4Aa

Means followed by the same uppercase letter (in the column) or lowercase letter (in the row) did not differ (Scott-Knott test, $p > 0.05$).
Médias seguidas pela mesma letra maiúscula (na coluna) ou minúscula (na linha) não diferiram (teste de Scott-Knott, $p > 0,05$).

4. DISCUSSION

4.1. Particle size evaluation of pine wood shavings

Brands A and C had the highest proportion of smaller particles (particles ≤ 1 mm) and similar proportions of particles with granulometry between 5 mm and 1 mm. The largest proportion of particles from brand B was retained in the 1 mm sieve, and, similar to brand C, the smallest amount of material was greater than 10 mm. In brands B, D and E, the lowest proportions of fine particles were found. The highest concentration of material for D and E manufacturers was retained in the 5 mm mesh. This could have been caused by the type of plane used on the wood.

Like ultrafine and dine particles can be harmful to the respiratory system of animals (Zaręba et al., 2024), the proportions of 33% for the 10 mm-mesh sieve, 33% for the 5 mm and 34% for the 1 mm were selected to compare the proportions used with the improved shavings (control), with particles smaller than 1 mm being removed so as not to be harmful to hamsters.

The largest amounts of particles in the evaluated commercial brands have particle sizes between 1mm and 5 mm. These values may be related to the fragility of the particles, which is a product of mechanically processing wood, with a thickness ranging from 0.2 to 0.5 mm. This can cause breakage during material handling and transport. In research carried out in the laboratory with rats, Kawakami et al. (2012) observed the preference of animals for materials with larger granulometry. A similar result occurred in relation to the hamsters tested in this study.

The results regarding animal welfare are similar to those of the aforementioned authors, proving that hamsters have a preference for shavings with larger particle sizes where their nests are built for both resting and for females to take care of their young. This probably happened because larger particles are easier to bite.

4.2. Evaluation of the pine wood shaving quality

All brands of shavings analyzed showed moisture content below 12% (Table 1). It is very important for the health of the animals that the shavings are dry (≤ 20%) for urine absorption, and that contaminants such as carbon dioxide and ammonia are absorbed and do not harm the rodents. However, these values are not a guarantee of product quality, considering that fungi (such as some species of the genus *Aspergillus*) can proliferate if they are in a latent state when exposed to favorable conditions and, in turn, cause damage to animals, such as to the respiratory system (LI et al., 2019). In addition, they must be properly sterilized to prevent the growth of pathogens.

The proliferation of fungi may occur when there is water spillage (during the watering process), increased humidity caused by contact with saliva (during the manipulation of the particles), or when in contact with the urine of the animals themselves. Regarding the fungal growth in the stained particles of the five commercial brands tested, it was observed that fungal growth occurred. This allows us to state that the shavings were not sterilized or that the sterilization process was not efficient.

Trichoderma sp., *Aspergillus flavus* and *Guilmaniella* sp. was observed in the evaluated commercial brands (Figure 3), but it was not possible to identify all the fungi that grew on the plates, as there was no occurrence of spores, since identification is not possible, according to the methodology described by Barnett; Hunter (1998), adopted in the identification of fungi. It is noteworthy that only one type of fungus grew per dish. *Trichoderma* spp. can cause skin inflammation and hyperplasia in small animals such as mice (Zhang; Li, 2022) and *Aspergillus* spp. can cause damage to the respiratory system of animals (SEYEDMOUSAVI et al., 2015).

In addition to damage to the respiratory system of animals, the mentioned fungi can cause diseases in rodents, such as aflatoxicosis, which is caused by the ingestion of aflatoxins released by fungi. These fungi have also been described as having immunosuppressive, mutagenic, teratogenic and hepatocarcinogenic activities. Thus, they can cause health problems to humans and to animals exposed to them (SEYEDMOUSAVI et al., 2018).

The proportion of stained particles by granulometric class in relation to the total mass existing in the packaging of each brand (Table 2) indicates that the largest amount of material with stains is found in the smallest granulometries. This may be related to the fragility of the material, which generates breaks and fines during handling.

Brands A and C had the highest proportion of fines (particles < 1 mm), which the animals could aspirate, and as they could contain hyphae of the pathogen (fungus), they could cause harm to the animals, especially to the respiratory system (ZARĘBA et al., 2024). Even if the shavings have gone through a sterilization process, which guarantees their quality in terms of the non-growth of pathogens, the occurrence of stained particles in the packaging causes an unpleasant aesthetic effect, which can devalue the product.

4.3. Preliminary tests carried out on the improved shavings

The quantity in grams of liquid absorbed by the particles was measured in the improved shavings to evaluate the absorption of the dye solution, which had similar moisture contents (dry basis) (≈ 8%). It was decided to use the method at ambient atmospheric pressure (without application of a vacuum) in the coloring of shavings to be submitted to animal tolerance and preference tests.

In the evaluation of the sterilization process of the particles in an oven (Table 3), it was observed that there was fungal growth at a temperature of 60 ºC, even for the time periods of 24 and 48 hours, demonstrating the inefficiency of the temperature and time used to cause the death of fungi existing in stained shavings. Sterilization was effective at a temperature of 80 ºC with only 48 hours of exposure. However, no fungal growth was observed in shavings subjected to a temperature of 100 ºC, regardless of the time spent in the tested situation.

In turn, no fungal growth occurred for any of the situations tested for autoclave sterilization. However, setting up a sterilization system using this type of equipment on a commercial scale would cost more when compared to one consisting of drying ovens, considering the costs of the equipment, process and maintenance in relation to the amounts to be invested in sterilization.

Thus, the shavings to be used in the study were sterilized in a drying oven, despite the recommendation by Dremova et al. (2023) regarding the need to use steam and pressure for the effective sterilization of material to be used in raising rodents. This is because no fungal growth was observed in shavings submitted to a temperature of 100 °C in the oven, even when a time of 6 hours was adopted.

The use of inadequately sterilized wood shavings can lead to fungal growth, potentially compromising the health of livestock, laboratory animals, and pets. Therefore, the trade in wood shavings requires quality standardization regarding particle size and, above all, proper sterilization.

4.4. Aroma and color preference tests

For the aroma test, the maximum increment of feed consumption in relation to the absence of aroma was 82.14% (5% concentration), and 42.34% for water (10% concentration) (Table 4). These values indicate the behavior of the hamsters' food and water consumption in response to the tested aromas and concentrations. Based on this result, it was decided not to apply aromas to shavings due to the atypical consumption of food and water. However, no hamsters died during the test period.

Regarding the behavior of hamsters in response to the color test, in the analysis of the permanence of the hamsters (females and males), it was observed that the females stayed more frequently (number of observations) in cages with natural shavings or colored with pink, red, green and blue dye. Meanwhile, male hamsters remained longer (more frequently) in cages with colored sawdust dyed green, red, blue, pink, and natural colors (Table 5). It is also noted that the values had a greater variation than that observed for females (149) between the frequencies of the animals in the cages with the colors tested. The average number of individuals (female and male) per environment was around two. This explains a characteristic behavior of forming small groups of hamsters. As the animals were similarly distributed in the cages, it can be stated that color did not influence the exploratory behavior of the animals.

Regardless of the color of the material, the animals more frequently manipulated larger granulometric particles to compose the place chosen for resting or nesting (Figure 3). This observation is in line with that obtained by Robinson-Junker et al. (2016) for laboratory rats and mice with a preference for bedding with larger particle shavings, and they even mixed the colored materials.

Water consumption by females to hydrate was not influenced by blue, green, and red shaving colors (Table 6). It has been observed that they consumed more water at the ends in relation to the position of the cages, which is a typical behavior of the species (KAWAKAMI et al., 2007). Hydration for males occurred at the same intensity, regardless of shavings color and cage position. It was also observed that females consumed more water than males, but only in cages with blue and pink colored particles.

Regarding feed consumption, the females ate more in the cages with natural particles and in the one colored in red. In contrast, the males ate at the same intensity regardless of the color of the wood shavings. Females consumed more food than males on uncolored particles. Consumption by males was higher in cages with green and pink colored material, while there was no difference in feed intake for the other cases.

Even with the formation of small groups at times, the animals preferred one environment to another. This was more frequently observed in the cages that were at the ends. This occurred regardless of color, with no formation of large groups that could define a preference for a particular color. Veillette; Reebs (2010) observed that Syrian hamsters have an aversion to old bedding, and the aroma of urine from the material in the end cages may have been lower, caused by the ventilation system of the laboratory where the experiment was conducted. This may explain their preference for these cages.

Since no adverse behaviors related to the color of the wood shavings were observed, nor was there any preference among the animals for a certain color, the use of color as a commercial differentiator is suggested, mainly for aesthetic reasons (commerce) and practical laboratory purposes (location of chips and other animal identifications in case of detachment). However, research is needed to determine the optimal concentrations and methods for incorporating aromas into the particles (without compromising the animals' behavior or health) in order to add value to the wood shavings.

5. CONCLUSIONS

The analysis conducted to verify the sterilization quality of the commercialized shavings indicated the growth of fungi, and it was possible to identify three of them, which were all capable of producing microtoxins, which can cause damage to the animals, such as *Trichoderma* sp., *Aspergillus flavus* and *Guilmaniella* sp.

There was no standardization of the amount of grains regarding the granulometry, taking into account the fragility of the particles, which are residues from mechanical processing when planing and straightening the wooden pieces. Moreover, all evaluated brands met the desired moisture content.

The sterilization process in an oven at a temperature of 100 °C for 6 hours was effective in causing the death of pathogens in stained shavings particles. The coloring process by immersion in the coloring solution, without the use of a vacuum, was efficient for absorption and fixing the color in the wood shavings.

No atypical behavior was identified in relation to water and feed intake for the preliminary tolerance tests of non-scented colored wood shavings. However, there was an atypical event of increased water and feed consumption in the aromatic shavings tolerance test, regardless of the aroma and concentration used, with values of 82.14% (feed) and 42.34% (water).

Lastly, addressing deficiencies identified in commercial wood shavings, such as fungal growth and stained particles, which indicate ineffective or absent sterilization, the proposed improved product resolves these issues while also incorporating color as an aesthetic and market differentiator.

6. REFERENCES

AHN, G. C.; JANG, S. S.; LEE, K. Y.; BAEK, Y. C.; OH, Y. K.; PARK, K. K. Characteristics of sawdust, wood shavings and their mixture from different pine species as bedding materials for Hanwoo cattle. **Asian-Australasian Journal of Animal Sciences**, v. 33, p. 856-865, 2020. https://doi.org/10.5713/ajas.19.0519

ALFENAS, A. C.; MAFIA, R. G. Isolamento de fungos fitopatogênicos. In: ALFENAS, A. C.; MAFIA, R. G. (Eds.). **Métodos em fitopatologia.** 2. ed. Viçosa: UFV, 2016. p. 55-93.

AWPA_American Wood Protection Association. **AWPA E10-22:** Laboratory method for evaluating the decay resistance of wood-based materials against pure basidiomycete cultures: soil/block test. AWPA Book of Standards. Birmingham, 2024, p. 406-417.

ARPANAEI, A.; FU, Q.; SINGH, T. Nanotechnology approaches towards biodeterioration-resistant wood: A review. **Journal of Bioresources and Bioproducts**, v. 9, p. 3-26, 2024. https://doi.org/10.1016/j.jobab.2023.09.001.

ABNT_Associação Brasileira de Normas Técnicas. **NBR ISO 13299:** Análise sensorial-metodologia-orientação geral para o estabelecimento de um perfil sensorial. Rio de Janeiro: ABNT, 2021. 32p.

BARNETT, H. L.; HUNTER, B. B. **Illustrated genera of imperfect fungi**. 4. ed. Minneapolis: American Phytopathological Society, 1998. 240p.

BURN, C. C.; MASON, G. J. Absorbencies of six different rodent beddings: commercially advertised absorbencies are potentially misleading. **Laboratory Animals**, v. 39, p. 68-74, 2005.

DREMOVA, O.; MIMMLER, M.; PAESLACK, N.; KHUU, M.P.; GAO, Z.; BOSMANN, M.; GARO, L.P.; SCHÖN, N.; MECHLER, A.; BENEICH, Y.; REBLING, V.; MANN, A.; PONTAROLLO, G.; KIOUPTSI, K.; REINHARDT, C. Sterility testing of germ-free mouse colonies. **Frontiers in Immunology**, v. 14, e1275109, 2023. https://doi.org/10.3389/fimmu.2023.1275109

ELIDIO, H. S. M.; COELHO, J. W. R.; SILVA, L. C. C. P.; SANTOS, I. B. Housing density and aggression in Syrian hamsters. **Journal of the American Association for Laboratory Animal Science**, v. 60, p. 506-509, 2021. https://doi.org/10.30802/AALAS-JAALAS-21-000020

ESKANDARANI, M. A.; HAU, J.; KALLIOKOSKI, O. Rapid ammonia build-up in small individually ventilated mouse cages cannot be overcome by adjusting the amount of bedding. **Laboratory Animals**, v. 52, p. 130-135, 2023. https://doi.org/10.1038/s41684-023-01179-0

GAD, S. C. Rodent models for toxicity testing and biomarkers. In: GUPTA, R. C. (Ed.). **Biomarkers in toxicology**. 2. ed. Cambridge: Academic Press, 2019. p. 7-73. https://doi.org/10.1016/B978-0-12-814655-2.00002-5

HUDDA, N.; DURANT, J.; NEMETH, A.; MANN, P.; PETITTO, J.; BRUGGE, D.; NEPHEW, B.C. Bedding-generated particulate matter: implications for rodent studies. **Inhalation Toxicology**, v. 31, p. 368-375, 2019. https://doi.org/10.1080/08958378.2019.1694109

KAWAKAMI, K.; SHIMOSAKI, S.; TONGU, M.; KOBAYASHI, Y.; NABIKA, T.; NOMURA, M.; YAMADA, T. Evaluation of bedding and nesting materials for laboratory mice by preference tests. **Experimental Animals**, v. 56, p. 363-368, 2007. https://doi.org/10.1538/expanim.56.363

KAWAKAMI, K.; XIAO, B.; OHNO, R. I.; FERDAUS, M. Z.; TONGU, M.; YAMADA, K.; YAMADA, T.; NOMURA, M.; KOBAYASHI, Y.; NABIKA, T. Color preferences of laboratory mice for bedding materials: evaluation using radiotelemetry. **Experimental Animals**, v. 61, p. 109-117, 2012. https://doi.org/10.1538/expanim. 61.109

LANTSCHNER, M. V.; CORLEY, J. C.; LIEBHOLD, A. M. Drivers of global Scolytinae invasion patterns. **Ecological Applications**, v. 30, e02103, 2020. https://doi.org/10.1002/eap.2103

LI, Z.; LU, G.; MENG, G. Pathogenic fungal infection in the lung. **Frontiers in Immunology**, v. 10, e1524, 2019. https://doi.org/10.3389/fimmu.2019.01524

LIMA, I. L.; RANZINI, M.; FIORUCI, W. J.; BUCCI, L. A.; LONGUI, E. L.; ZANATA, M.; GARCIA, J. N. Physical and mechanical wood properties of two varieties of *Pinus caribaea*. **Research, Society and Development**, v. 12, e7712943146, 2023. https://doi.org/10.33448/rsd-v12i9.43146

MAGALHÃES, M. N.; LIMA, A .C. **Noções de probabilidade e estatística.** 7. ed. São Paulo: Edusp, 2002. 440p.

MUNIR, M. T.; BELLONCLE, C.; IRLE, M.; FEDERIGHI, M. Wood-based bedding material in animal production: A minireview. **Approaches in Poultry, Dairy & Veterinary Sciences**, v. 6, eAPDV.000644, 2019. https://doi.org/10.31031/APDV.2019.06.000644

REIS, S. R.; FRANCO, A. M. R. **Manual básico de bioterismo**. Manaus: Projeto Fronteira, 2012. 47p.

RIBASKI, N. G.; BELINI, U. L. Aproveitamento de resíduos sólidos urbano madeireiro. **Brazilian Journal of Technology**, v. 2, p.742-757, 2019.

ROBINSON-JUNKER, A.; MORIN, A.; PRITCHETT-CORNING, K.; GASKILL, B. N. Sorting it out: bedding particle size and nesting material processing method affect nest complexity. **Laboratory Animals**, v. 51, p. 170-180, 2016. https://doi.org/10.1177/0023677216652384

SEYEDMOUSAVI, S.; BOSCO. S. M. G.; DE HOOG, S.; EBEL, F.; ELAD, D.; GOMES, R. R.; JACOBSEN, I. D.; JENSEN, H. E.; MARTEL, A.; MIGNON, B.; PASMANS, F.; PIECKOVÁ, E.; RODRIGUES, A. M.; SINGH, K.; VICENTE, V. A.; WIBBELT, G.; WIEDERHOLD, N. P.; GUILLOT, J. Fungal infections in animals: a patchwork of different situations. **Medical Mycology**, v. 56, p. S165-S187, 2018. https://doi.org/10.1093/mmy/myx104

SEYEDMOUSAVI, S.; GUILLOT, J.; ARNÉ, P.; DE HOOG, G. S.; MOUTON, J. W.; MELCHERS, W. J. G.; VERWEIJ, P. E. *Aspergillus* and aspergilloses in wild and domestic animals: a global health concern with parallels to human disease. **Medical Mycology**, v. 53, p. 765-797, 2015. https://doi.org/10.1093/mmy/myv067

SIVAMARUTHI, B. S.; KESIKA, P.; DAUNGCHANA, N.; SISUBALAN, N.; CHAIYASUT, C. Composition, bioactivities, microbiome, safety concerns, and impact of essential oils on the health status of domestic animals. **Applied Sciences**, v. 14, e6882, 2024. https://doi.org/10.3390/app14166882

STRAGLIOTTO, M. C.; OLIVEIRA, A. C.; LENGOWSKI, E. C.; ARRUDA, T. P. M.; SILVA, J. R. M.; PEREIRA, B. L. C. Log quality and lumber yield for export of five species from the Brazilian Amazon. **Environment, Development and Sustainability**, v. 27, p. 10059-10082, 2023. https://doi.org/10.1007/s10668-023-04298-3

TANNO, D. R.; COUTO, E. P. Estudo retrospectivo das principais afecções gastrointestinais em roedores e logomorfos em uma clínica veterinária de São Paulo, Brasil. **Nosso Clínico**, v. 18, p. 50-60, 2015.

VEILLETTE, M.; REEBS, S. G. Preference of Syrian hamsters to nest in old versus new bedding. **Applied Animal Behaviour Science**, v. 125, p. 189-194, 2010. https://doi.org/10.1016/j.applanim.2010.04.001

ZARĘBA, Ł.; PISZCZATOWSKA, K.; DŻAMAN, K.; SOROCZYNSKA, K.; MOTAMEDI, P.;

SZCZEPAŃSKI, M. J.; LUDWIG, N. The relationship between fine particle matter (PM2.5) exposure and upper respiratory tract diseases. **Journal of Personalized Medicine**, v. 14, e98, 2024. https://doi.org/10.3390/jpm14010098

ZHANG, G.; LI, D. *Trichoderma longibrachiatum* - Associated skin inflammation and atypical hyperplasia in mouse. **Frontiers in Medicine**, v. 9, e865722, 2022. https://doi.org/10.3389/fmed.2022.865722

Acknowledgments: The authors thank the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001, Research and Innovation Support Foundation of Espírito Santo - Brazil (FAPES), and the National Council for Scientific and Technological Development - Brazil (CNPq) for the scholarships and support to the research (respectively), Central Animal Facility - UT/Imbio, Institute of Biosciences at the Federal University of Mato do Sul, Brazil, for donating Syrian hamsters for the experiment, and to the Complexo Agroindustrial Pindobas Ltda., Brazil, for donating the pine shavings used in the research.

Notes: All experiments were performed in accordance with the Ethics Committee on Animal Use - CEUA of the Federal University of Espírito Santo, Vitória, Espírito Santo, Brazil, under number 05/2017.

Author contributions: The article was written through the contributions of all authors, and all of them have approved the final version of the article. JGSM. Data curation; formal analysis; investigation; methodology; and writing - original draft. JBP and LOT. Conceptualization; supervision; validation; visualization. ATSA, YML, ABR, and MAN. Formal analysis; validation; visualization. JBP. Writing - review & editing, and correcting both spelling and grammar errors.

Research funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Only scholarships and research support. All are duly cited in the acknowledgments.

Data availability: All results are presented in the publication. Any additional results will be made available upon request.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.