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The symbiotic relationship between Tenebrio molitor and coffee grounds presents an innovative perspective on addressing global challenges

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Abstract: This study unveils a novel approach toward sustainable protein production and waste management by investigating the utilization of coffee grounds as a nutrient source for *Tenebrio molitor* larvae. This research is a pioneering exploration in response to pressing protein shortages, food waste, and food security challenges. The study employs an entirely randomized experimental design, comprising five treatment groups and one control group; each replicated thrice. Notably, the study amalgamates cutting-edge data mining techniques through the Scopus database to elucidate the existing research landscape around *Tenebrio molitor*. This exploration underscores the insect's pivotal role as a prospective alternative protein source. The experimental findings, a core contribution, discern that T1, a blend of 5% coffee grounds and 95% substrate, yields the most significant mass gain in larvae over the initial 7-day phase, surpassing the control group. However, an intriguing revelation follows: specific treatments experience subsequent weight loss, emphasizing the significance of strategic larval harvesting and processing. These collective results underscore *Tenebrio Molitor*'s potential as an innovative protein source while spotlighting coffee grounds as a valuable larval growth catalyst. This study forges a path towards sustainable protein solutions, elucidating the dynamic interplay between *Tenebrio molitor*, coffee grounds, and their promising role in addressing food security concerns, particularly in socioeconomically challenged regions.

Keywords: *Tenebrio molitor*, coffee grounds, protein.

Adherence to the BJEDIS' scope: The article presented here has relevant results regarding the use and application of the RIS files were analyzed using VOSviewer with predefined and utilized automated settings. Additionally, ANOVA statistical analysis and Tukey's method, which made it possible to identify the mortality of the individuals was also evaluated for each treatment.

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1. INTRODUCTION

In the dynamic tapestry of contemporary challenges, science is a paramount ally poised to unveil innovative solutions. The pressing imperatives surrounding sustainable protein production and adept waste management have attained paramount importance due to the escalating global populace and the intensifying ecological strains. To navigate this complex milieu, the amalgamation of inventive viewpoints and interdisciplinary endeavors becomes practical and imperative (1–4).

In this intricate landscape, the synergistic potential inherent in Tenebrio molitor larvae and coffee grounds illuminates as a harbinger of innovation. The astute harnessing of residual streams for protein biosynthesis and the reduction of food waste seamlessly aligns with the fundamental tenets of green chemistry and sustainable frameworks. As is discerned from the literature citations, which collectively unveil pioneering applications spanning diverse spheres, our study sets sail on a voyage to delineate hitherto uncharted dimensions within the expansive realm of sustainability.

In a profound exploration into the intricacies of symbiotic interactions meticulously choreographed between insect larvae and organic residues, our inquiry fundamentally redefines the contours that encapsulate the domain of protein acquisition and waste valorization. Through this scientific odyssey, we not only furnish a responsive strategy to contemporary predicaments but also embark upon a transformative journey characterized by pioneering methodologies that carry the potential to redefine the trajectory of sustainable food systems in the future.

The pertinence of our study is accentuated by the backdrop of Brazil's commanding stature as the world's premier coffee producer and exporter. The United States and Germany stand as principal destinations for this globally coveted commodity, closely followed by Italy, Belgium, and Japan. In 2022, Brazil's coffee exports reached an impressive 2.2 million tons, equivalent to 39.4 million coffee bags. The expansive canvas of coffee cultivation spans over 2.25 million hectares, enveloping around 1900 municipalities across Brazil. Mirroring this expansive production, global coffee consumption has burgeoned to approximately 175.6 million bags within 2021/22, with projected growth to 178.5 million bags in the following year, 2022/23. This growth, representing a remarkable 51.41% increase since 1990, when consumption stood at 90.28 million bags, underscores coffee's indomitable global allure (5).

However, beneath the veneer of this thriving industry, a significant volume of coffee residues, such as leaves, pulp, and husks, is generated at each stage, encompassing harvesting, processing, and consumption. Among these by-products, the husk and pulp materialize as predominant residues yielded during coffee processing, contributing to an appreciable 30% to 50% of the coffee volume (6).

Against the backdrop of this copious waste generation, the study's focal point lies in assessing the capacity of coffee grounds to serve as a substrate for Tenebrio molitor larvae. Coffee grounds, an insoluble waste generated through hot water extraction during coffee preparation, are currently grappling with the absence of commercial value, relegating them to commonplace disposal practices. Nevertheless, contemporary endeavors driven by environmental consciousness have bestowed newfound significance on coffee grounds, positioning them as potential reservoirs of industrially pertinent compounds. This awakening to their latent value underscores the necessity to examine their potential for degradation through Tenebrio molitor larvae (7).

The amount of residual coffee biomass generated in coffee shops, restaurants, households, and other locations needs to be better documented due to the difficulty of quantifying this type of waste in different places (8, 9). This study evaluates coffee grounds' degradation capacity by *Tenebrio molitor* larvae.

The composition of coffee (10, 11) pulp and husk can vary depending on the processing method, efficiency, coffee variety, and cultivation conditions, including soil type (Pandey et al., 2000). The constituents of coffee pulp and husk, expressed as a percentage of dry weight, include approximately 50% carbohydrates, 10% proteins, 18% fiber, 2.5% caffeine, 1.3% tannins, and 1.8% polyphenols (12). These values serve as a general reference, but it's essential to consider that the actual composition percentage may differ based on specific circumstances and coffee production changes.

The significant amount of waste generated in coffee production necessitates a waste management plan. *Tenebrio molitor*, an insect from the Coleoptera order and commonly known as the mealworm, infests stored grains and their by-products, making it a pest of grains (13). Its nutritional diet is based on low-density cereal bran with soft consistencies and devoid of moisture, requiring the presence of amino acids for the production of structural proteins and enzymes. The value of any protein an insect ingests depends on its amino acid content and its ability to digest it (14, 15).

The standard diet for *Tenebrio molitor* is wheat bran (16), which serves as an excellent protein source for the insect (14). However, it has the potential to feed on organic residues of low commercial value, such as flours,

cereals, leaves, coffee grounds, and decaying vegetables, and convert them into biomass rich in proteins (approximately 50%), essential fatty acids, vitamins, and minerals. For this reason, their larvae are considered an alternative source of protein for human and animal food (17–26), according to the thresholds for the World Health Organization and Food and Agriculture Organization of the United Nations food labels (27).
Therefore, coffee processing residues can be considered as an alternative feed source for *Tenebrio molitor* as they contain various amino acids, such as Alanine, Arginine, Cystine, Aspartic Acid, Leucine, Tyrosine, and Leucine, among others (28).

Several studies indicate that *Tenebrio molitor* can be used as an alternative source of protein for both human and monogastric animal consumption (15, 29–37). The nutritional profile of mealworms has been compared with conventional meats, revealing that mealworms have a significantly higher nutritional value than beef and chicken and are not significantly less nutritionally balanced. They also provide a good source of all essential amino acids (32, 38–45). Considering large-scale insect production for human consumption, the costs associated with *Tenebrio molitor* production must be considered. After labor, the most significant insect production cost is their diet (46). Therefore, in light of this issue, our study resonates as a vital exploration into the uncharted dimensions within the synergy between Tenebrio molitor larvae and coffee grounds. It threads the complex sustainability net, addressing pressing challenges while championing transformative methodologies poised to reshape the contours of sustainable food systems for posterity.

2. MATERIALS AND METHODS

2.1 Data Mining

The Scopus database was queried to investigate the volume of scientific publications on Tenebrio molitor. A comprehensive analysis was conducted using the search term "Tenebrio molitor" AND (LIMIT-TO (DOCTYPE, "ar")). The data from the Scopus database was exported in RIS format. The RIS files were analyzed using VOSviewer with predefined and utilized automated settings. For a more detailed analysis, a Python script was developed to analyze the TXT files generated by VOSviewer. This script utilized various libraries and modules for data analysis and visualization, including NumPy (for numerical computations), Pandas (for data manipulation), Matplotlib (for plotting), Kaleido (for exporting plots), Plotly (for interactive plots), PIL (for image processing), and Seaborn (for statistical graphics). Additionally, IPython.display (for displaying rich media), Plotly.express (for high-level plotting), and Statsmodels.stats.multicomp.pairwise_tukeyhsd (for multiple comparisons) modules were employed. This methodology was presented elsewhere (47).

2.2 Collection of Residual Material

The coffee used for the experiment was Pilão® (composed of approximately 70% Arabica and 30% Robusta beans, with a grain size <9.5 mm). To ensure the accuracy of the experiment, the coffee grounds were collected immediately after the coffee was prepared, ensuring freshness. A comprehensive sampling strategy was employed to ensure the representation of the residual material's chemical composition. Four samples were meticulously collected from the pile/heap of residual material from the top, middle, and base areas surrounding the pile/heap. These samples were transported to the Multidisciplinary Waste Management Laboratory (LMGR) at IFRJ-CDUC for further analysis. An in-depth analysis of the samples was carried out, involving disaggregation and drying. The samples underwent a controlled drying process in an oven maintained at a constant temperature of 80 ± 5ºC until they reached nearly 0% moisture content, ensuring absolute dryness. This rigorous process allowed accurate measurements of the samples' dry matter mass.

2.3 Test Organism

The test organism for the degradation experiment was adult larvae of Tenebrio molitor. These larvae were sourced from a reputable producer and maintained under controlled laboratory conditions. The larvae were approximately 50 days old, measuring around 48 mm long, with an average weight of 0.89 g. To ensure the integrity of the study, the larvae were used directly without any pre-treatments. The cultivation substrate utilized in the study was a blend of food-grade components, including wheat bran, cornmeal, breadcrumbs, and oats. These components were employed in a precise weight ratio of 3:1:1:1. Importantly. They were sourced in their food-grade form and used as received without any alterations. The choice of food-grade materials maintains the experiment's integrity and underscores the findings' applicability to real-world scenarios. This attention to detail in the material selection and handling ensures the experimental results' accuracy and reliability.

2.4 Degradation Test

In the study, four *Tenebrio molitor* larvae were placed in plastic containers (95 mm in diameter and 50 mm in height) along with the cultivation substrate's mass and coffee grounds' mass. The containers were then sealed. The environmental conditions for the test were a temperature of 26 ± 2ºC and a photoperiod of 12 hours light/12 hours dark. The experimental period lasted for 14 days.

A completely randomized experimental design was adopted, consisting of five treatments for the test group and one control group. Three replicates were used for all treatments (6x3). The tested treatments in the test group were: T1 (1 g (5%) coffee grounds + 19 g substrate), T2 (3 g (15%) coffee grounds + 17 g substrate), T3 (5 g (25%) coffee grounds + 15 g substrate), T4 (10 g (50%) coffee grounds + 10 g substrate), T5 (only 20 g (100%) coffee grounds). The control group (TC) consisted of 20 g substrate only.

2.5 Evaluation of Test Organism

The average weight was assessed at seven and fourteen days. At the end of the experimental period, the mortality of the individuals was also evaluated for each treatment.

2.6 Statistical Analysis

The data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test at a 5% significance level (48, 49), using the STATISTICA 10 software, and the graph was created using SigmaPlot 12.5 software.

3. RESULTS AND DISCUSSION

Correlation between Total Link Strength (TLS) and Occurrences:

Analyzing Total Link Strength (TLS) and Occurrences in Vosviewer provides valuable insights into the significance of individual elements or terms within the network. TLS represents the total number of links between two items, while Occurrences indicate the frequency of appearance of a specific term or item in the dataset. Understanding the relationship between TLS and occurrences is crucial for comprehending the relationships and importance of elements or terms in the research area.

The calculated R^2 value of 0.928 indicates a high correlation between Total Link Strength and Occurrences. This suggests that items with high occurrence counts are closely associated with other items in the network, indicating their central importance in the research area.

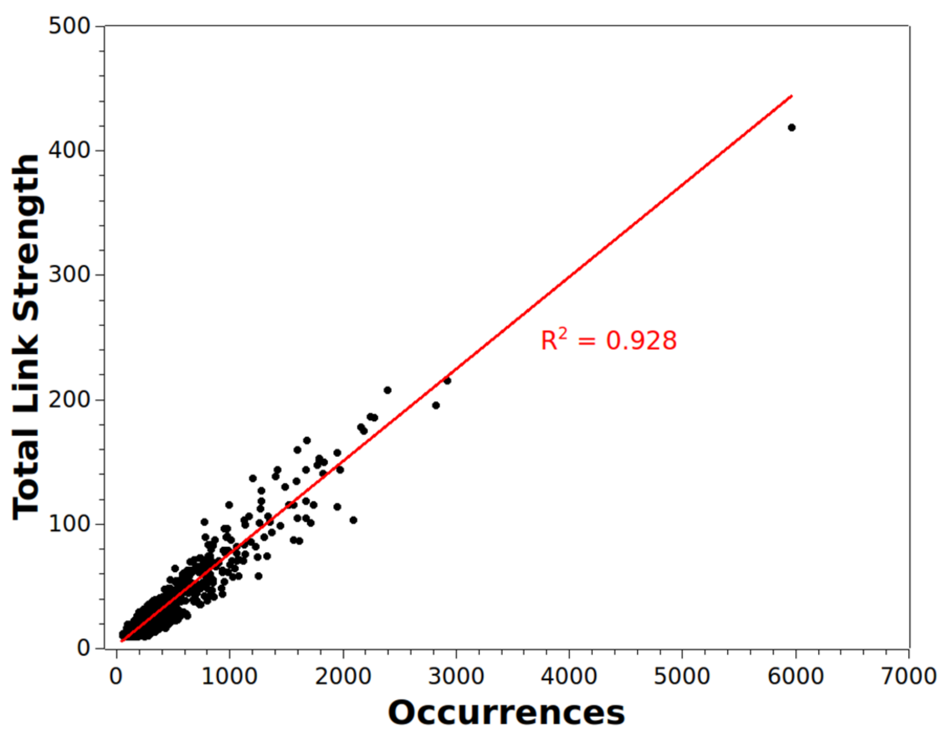

Figure 1. Scatter plot: Total Link Strength vs Occurrences.

Data from Main Terms per Cluster:

Figure 2 shows the result of the VOSviewer analysis.

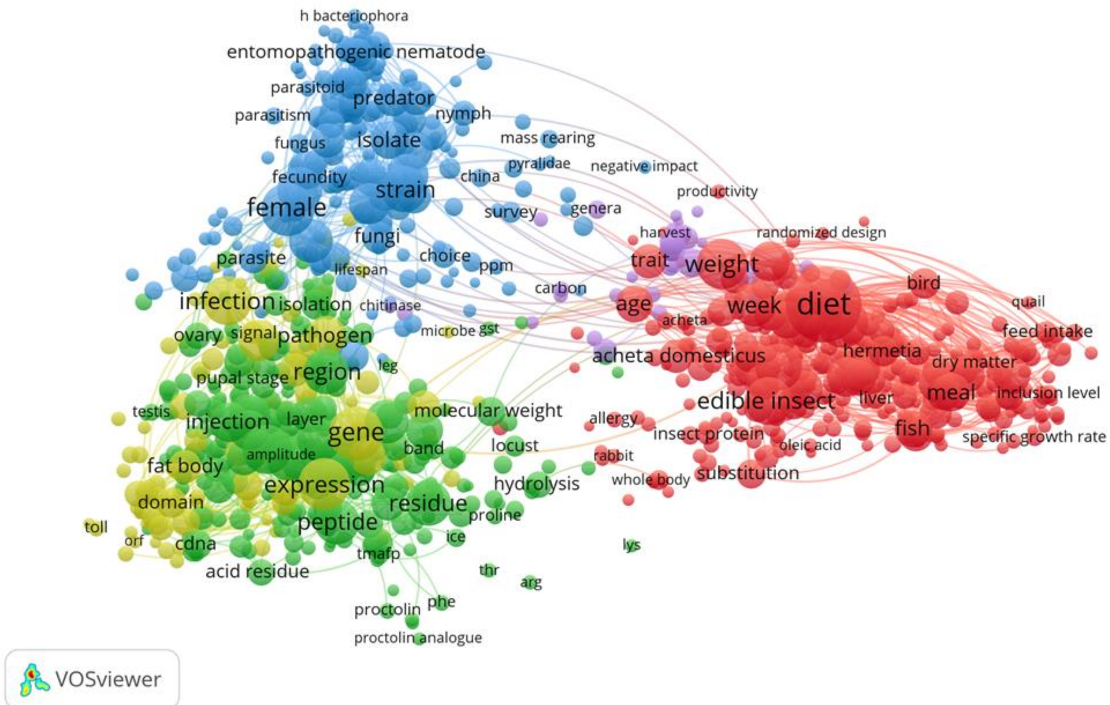

Figure 2. Clusters extracted from Scopus database regarding *Tenebrio molitor*

In Cluster 1, the prominent terms are ratio, edible insect, feed, weight, and diet. These terms occur 158, 168, 186, 187, and 419 times, respectively. The average publication years for these terms are 2013.7, 2019.5, 2018.8, 2008.9, and 2014.1, respectively. This cluster focuses on topics related to the ratio of edible insects in feed and their impact on weight and diet. The research in this cluster has been more recent, with publications spanning from 2008 to 2019.

In Cluster 2, the main terms include vitro, residue, region, peptide, and sequence. These terms occur 144, 148, 151, 153, and 175 times, respectively. The average publication years for these terms are 2000.6, 2008.8, 2002.4, 2008.4, and 2004.8, respectively. This cluster revolves around in vitro studies, residues, regional aspects, peptides, and sequences. The research in this cluster has a longer timeline, with publications ranging from 2000 to 2008.

Cluster 3 comprises the main terms egg, male, host, strain, and female. These terms occur 135, 144, 150, 160, and 208 times, respectively. The average publication years for these terms are 2004.4, 2005.0, 2008.7, 2011.7, and 2003.0, respectively. This cluster focuses on characteristics related to the egg, male and female individuals, hosts, and strains. The research in this cluster spans a period from 2003 to 2011.

In Cluster 4, the main terms are pathogen, mealworm beetle, infection, expression, and gene. These terms occur 116, 127, 178, 196, and 216 times, respectively. The average publication years for these terms are 2015.8, 2005.6, 2010.3, 2011.6, and 2013.2, respectively. This cluster revolves around studying pathogens, mealworm beetles, infections, gene expression, and genes. The research in this cluster spans from 2005 to 2015, focusing on more recent publications.

Cluster 5 consists of the main terms bran, spectroscopy, polystyrene, biodegradation, and frass. These terms occur 39, 46, 55, 55, and 62 times, respectively. The average publication years for these terms are 2013.8, 2014.9, 2020.6, 2021.1, and 2016.8, respectively. This cluster centers around topics such as bran, spectroscopy, polystyrene, biodegradation, and frass. The research in this cluster includes more recent publications, ranging from 2013 to 2021.

Data from Recent Terms per Cluster:

Data from the respective ages of the nodes are gathered in Figure 3. Within Cluster 1, the recent terms of focus include circular economy, total phenolic content, *Dicentrarchus labrax*, final body weight, and chewiness. These terms occur 12, 10, 14, 12, and 11 times, respectively. The average publication years for these terms are 2021.8333, 2021.3, 2021.2857, 2021.25, and 2021.0909, respectively. This cluster focuses on recent topics such as the circular economy, total phenolic content, and their relationship with *Dicentrarchus labrax* (a fish species). It also explores factors like final body weight and chewiness. The average publication years indicate a current interest in these subjects.

In Cluster 2, the recent terms encompass Tenebrio molitor antifreeze protein, *Zophobas atratus*, PCR, target, and z atratus. These terms occur 15, 26, 37, 29, and 13 times, respectively. The average publication years for these terms are 2015.1333, 2014.6923, 2014.4595, 2013.8276, and 2013.0769, respectively. This cluster revolves around recent topics such as Tenebrio molitor antifreeze protein, the species *Zophobas atratus*, PCR (Polymerase Chain Reaction), and target analysis. These terms indicate a focus on molecular studies and specific organisms. The average publication years suggest ongoing research in this field.

Cluster 3 highlights recent terms such as EPF, negative impact, Beauveria, molecular identification, and non-target organism. These terms occur 12, 14, 18, 11, and 13 times, respectively. The average publication years for these terms are 2021.0, 2020.5, 2019.3889, 2019.1818, and 2019.0769, respectively. This cluster highlights recent terms such as EPF (Entomopathogenic Fungi), negative impact, Beauveria (a genus of fungi), molecular identification, and non-target organism. These terms indicate a focus on research's ecological and molecular aspects, including the study of interactions between organisms. The average publication years suggest active research in this area.

Within Cluster 4, the recent terms of interest are AMP gene, antimicrobial activity, expression level, microbe, and fungal infection. These terms occur 11, 17, 23, 15, and 16 times, respectively. The average publication years for these terms are 2020.0, 2018.8824, 2018.8696, 2018.8, and 2018.75, respectively. This cluster revolves around recent terms such as AMP (Antimicrobial Peptide) gene, antimicrobial activity, expression level, microbe, and fungal infection. These terms focus on studying the antimicrobial properties, gene expression, and interactions with microbes and fungal infections. The average publication years suggest research conducted in the past few years.

Cluster 5 presents recent terms, including polyvinyl chloride, plastic degradation, polyethylene, low-density polyethylene, and plastic. These terms occur 10, 12, 24, 10, and 38 times, respectively. The average publication years for these terms are 2022.0, 2021.9167, 2021.6667, 2021.5, and 2021.4211, respectively. This cluster centers around recent terms such as polyvinyl chloride, plastic degradation, and different types of polyethylene. These terms indicate a focus on studying plastics' characteristics, degradation, and environmental impact. The average publication years suggest ongoing research in this field, with recent contributions.

These primary characteristics provide insights into the current focus areas and trends within each cluster, highlighting the specific topics and publication patterns associated with each one.

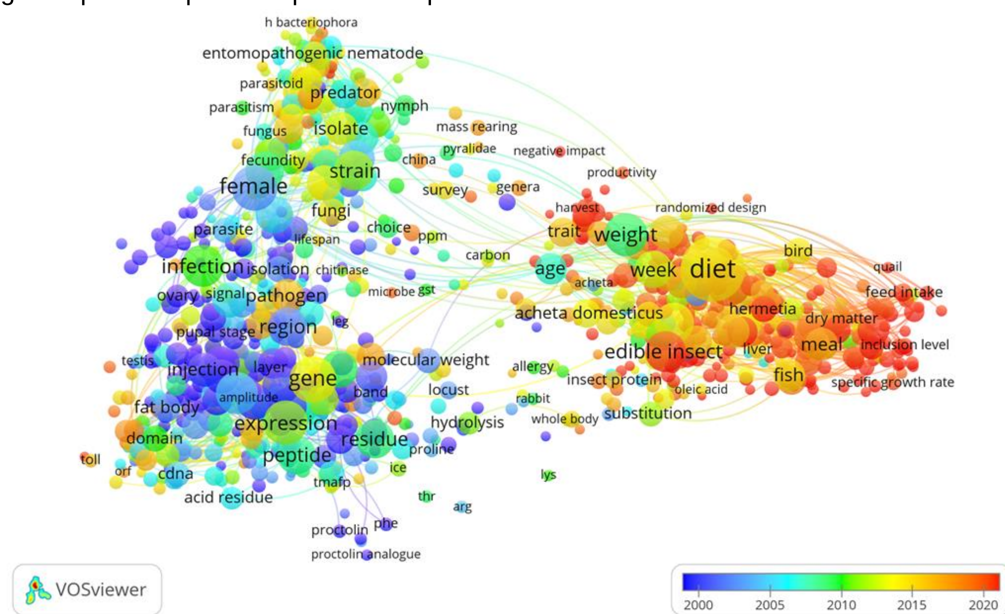

Figure 3. Overlay data from scopus database regarding *Tenebrio molitor*

Data from Top five pairs of terms depending on the value of LSBI:

Analysis of the top five pairs of terms based on their Link strength between items (LSBI) values reveals interesting relationships and associations within the dataset (see Figure 4).

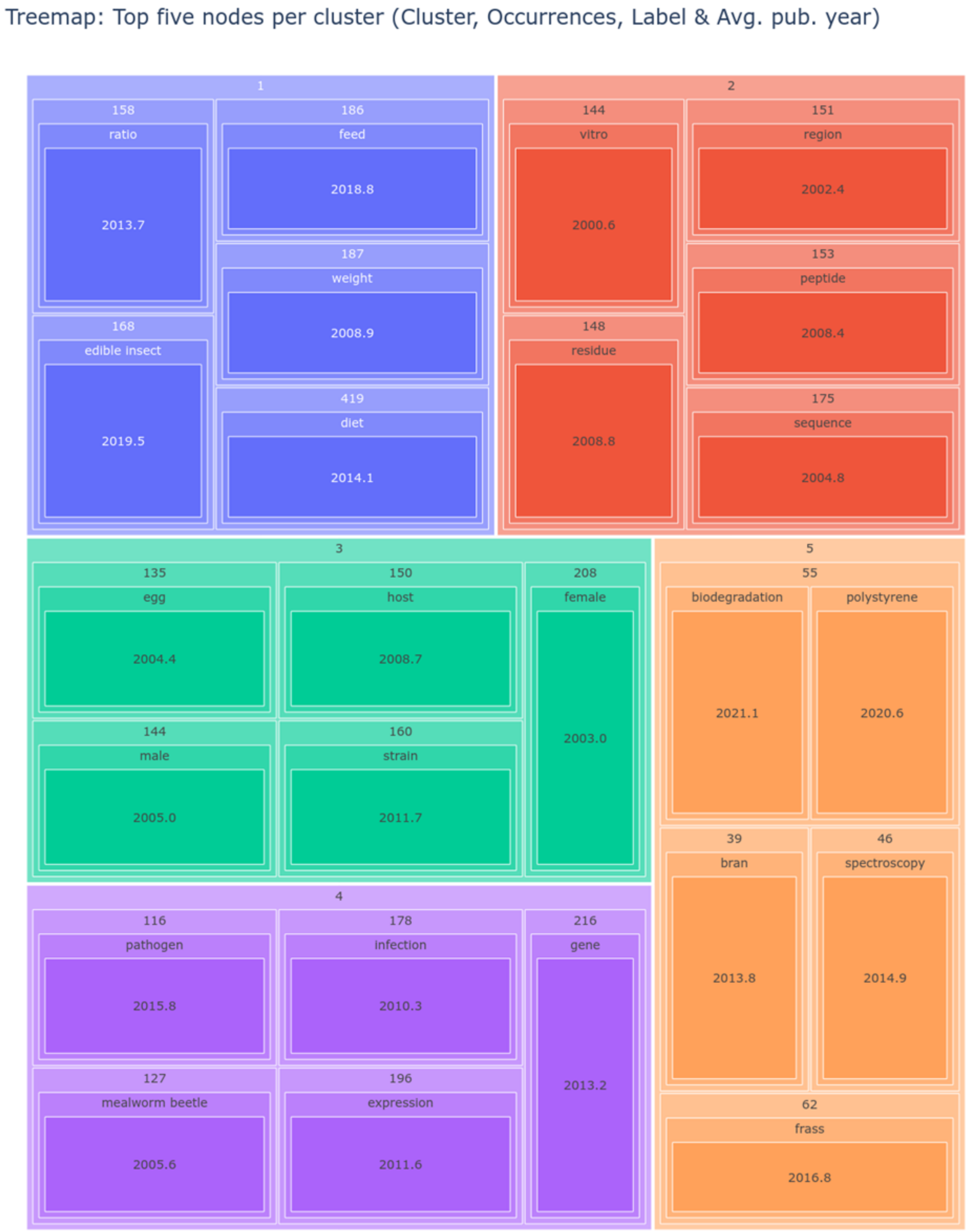

Figure 4. Top five nodes per cluster from Scopus database regarding *Tenebrio molitor*

The first pair consists of the terms 'female vs. male.' These terms, with origins in 2003.03 (t.1) and 2005.01 (t.2), respectively, share a high LSBI value of 105. They are closely connected, indicated by a small Euclidean distance of 0.11. This suggests a significant relationship between female and male subjects in the research, possibly focusing on sexual dimorphism or gender-specific characteristics.

The second pair comprises the terms 'expression vs. gene.' These terms, originating in 2011.56 (t.1) and 2013.24 (t.2), demonstrate a strong association with an LSBI value of 99. Although slightly more distant, with an Euclidean distance of 0.17, they highlight the connection between gene expression and genetic factors in the research, potentially emphasizing the study of gene regulation or molecular mechanisms.

The third pair involves the terms 'diet vs. growth performance.' Emerging from 2014.08 (t.1) and 2019.73 (t.2), these terms exhibit a substantial LSBI value of 95. Despite a relatively larger Euclidean distance of 0.56, the connection between diet and growth performance suggests an investigation into different diets' effects on organisms' growth and development.

The fourth pair includes the terms 'diet vs. weight.' Originating from 2014.08 (t.1) and 2008.85 (t.2), these terms demonstrate an LSBI value of 84. Despite a moderate Euclidean distance of 0.31, the relationship between diet and weight implies a focus on the impact of dietary factors on body weight regulation.

Lastly, the pair 'diet vs. meal' emerges from 2014.08 (t.1) and 2016.85 (t.2). With an LSBI value of 83, this pair showcases the association between diet and meal patterns. Although slightly more distant, with an Euclidean distance of 0.43, it suggests exploring the relationship between dietary habits and specific meals.

These terms provide valuable insights into the interconnectedness of concepts within the dataset, highlighting relationships between gender, gene expression, diet, growth performance, weight, and meals. These findings contribute to understanding the underlying themes and research trends in the field.

Data from the Top five pairs of terms depending on the value of Year:

Analysis of the top five pairs of terms based on their publication years, shown in Figure 5, reveals exciting connections within the dataset.

The first pair consists of 'polyvinyl chloride vs. sole diet.' These terms, originating from 2022.0 (t.1) and 2020.1 (t.2), share a Link strength between items (LSBI) value of 1. They are closely associated, indicated by a small Euclidean distance of 0.11. This suggests a relationship between polyvinyl chloride, a common type of plastic, and the concept of a sole diet. The connection may imply research focusing on the impact of polyvinyl chloride exposure on dietary factors or using polyvinyl chloride as a component of experimental diets.

The second pair comprises the terms 'polyvinyl chloride vs. thermal stability.' These terms, originating from 2022.0 (t.1) and 2019.92 (t.2), share an LSBI value of 1. Although slightly more distant, with an Euclidean distance of 0.49, they highlight the association between polyvinyl chloride and thermal stability. This suggests research exploring polyvinyl chloride's thermal properties and behavior, which is crucial for understanding its performance in various applications.

The third pair involves the terms 'polyvinyl chloride vs. waste.' Emerging from 2022.0 (t.1) and 2019.77 (t.2), these terms share an LSBI value of 3. An Euclidean distance of 0.21 indicates a connection between polyvinyl chloride and waste management. This bond implies investigations into the generation, disposal, or recycling of polyvinyl chloride waste and its environmental implications.

The fourth pair includes the terms 'polyvinyl chloride vs. powder.' Originating from 2022.0 (t.1) and 2019.76 (t.2), these terms share an LSBI value of 1. Despite a relatively larger Euclidean distance of 0.52, the relationship between polyvinyl chloride and powder suggests a focus on powdered forms or applications of polyvinyl chloride. This association may involve research on processing techniques, material properties, or specific industries using polyvinyl chloride powders.

Lastly, the pair 'polyvinyl chloride vs. yellow mealworm larvae' emerges from 2022.0 (t.1) and 2019.47 (t.2). With an LSBI value of 1 and a Euclidean distance of 0.51, this connection indicates a relationship between polyvinyl chloride and the larvae of yellow mealworms. This suggests research exploring the interactions between polyvinyl chloride and yellow mealworm larvae, potentially investigating aspects such as toxicity, behavior, or the utilization of yellow mealworms in waste management involving polyvinyl chloride.

The mealworm beetle, known as *Tenebrio molitor*, holds significant importance in the scientific community. Its unique characteristics and potential applications have led to extensive research focusing on its nutritional value, growth performance, gene expression, and the effects of different diets on its development. This insect species has shown promise as a sustainable solution for addressing various environmental challenges.

One area of particular interest is the consumption of organic waste materials by *Tenebrio molitor*. This includes traditional dietary components and diverse substances such as plastics. Research has explored the ability of *Tenebrio molitor* larvae to consume and digest polyvinyl chloride (PVC), a common type of plastic. Exploring the relationship between *Tenebrio molitor* and PVC highlights the potential role of these larvae in environmental remediation and waste management efforts. By consuming and breaking down plastics, *Tenebrio molitor* larvae contribute to reducing plastic waste and its associated environmental impact.

Figure 5. Top five most recent nodes per cluster from Scopus database regarding *Tenebrio molitor*

Additionally, *Tenebrio molitor* offers significant potential as an alternative protein source. The larvae should be deeply investigated as a sustainable solution to address protein shortages and improve food security, particularly in economically disadvantaged regions. By utilizing organic waste materials such as coffee grounds as a nutrient source for *Tenebrio molitor* larvae, it is possible to produce protein-rich biomass that can be used as a valuable food source for humans and animals. This approach reduces food waste and provides a sustainable solution for protein production, potentially alleviating hunger and improving the nutritional status of vulnerable populations.

Thus, this study aims to investigate the utilization of *Tenebrio molitor* larvae as a sustainable solution for addressing protein shortages and improving food security, particularly in economically disadvantaged regions. The proposal focuses on feeding the larvae with coffee grounds, a readily available organic waste material, to produce protein-rich biomass. By examining the larvae's growth performance and evaluating the nutritional value of the resulting biomass, we aim to assess the feasibility and potential impact of utilizing *Tenebrio molitor* as a means of reducing food waste, promoting circular economy principles, and providing a valuable protein source for human and animal consumption. The findings of this research will contribute to enhancing food security, sustainable waste management, and resource utilization in the context of *Tenebrio molitor* and coffee ground utilization.

Average values of mean weight for the different treatments with coffee grounds:

The average values of mean weight for the different treatments with coffee grounds are presented in Figure 6. After seven days, all treatments showed weight gain except for T5, which exhibited a loss of 38%. However, the best performance was observed in T1, with a weight gain of 35%. At the end of the experimental period, the control group (TC) showed a weight gain of approximately 25%, followed by T1 with a gain of 10% (0.97 g). No significant weight changes were observed for individuals in treatments T2, T3, and T4.

Additionally, no significant differences were found among these treatments. T5 exhibited a weight loss of approximately 78%. None of the treatments experienced mortality during the experimental period.

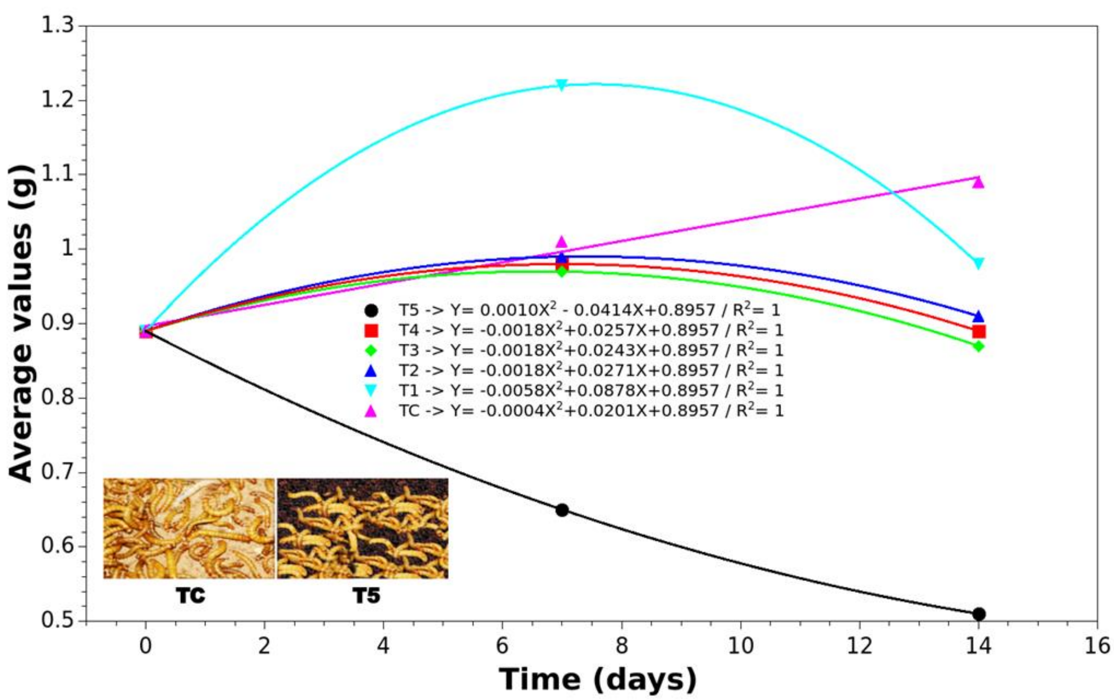

Figure 6: Average weight values as a function of the different treatments with coffee grounds. Means followed by the same lowercase letter do not differ statistically according to Tukey's test at a 5% probability level. The upper right inset shows larvae on TC (only substrate) and T5 (only coffee grounds).

The findings depicted in Figure 6 provide compelling evidence supporting the superior performance of condition T1, which exhibited a remarkable increase in mass during the initial 7-day period. Notably, the observed mass gain in T1 surpassed that observed in the control group over the entire 14-day experimental duration. These results strongly suggest that adding coffee grounds is a powerful stimulant for larval growth, particularly during the critical early developmental stage. Early exposure to coffee grounds likely enhances the larvae's nutritional intake and metabolic processes, promoting accelerated growth. However, it is essential to note that beyond the

7-day mark, the larvae tend to experience heightened stress levels, which may account for the subsequent weight loss observed in some treatments. This phenomenon underscores the significance of timely harvesting and processing of the larvae to maximize their growth potential.

4. CONCLUSION

In conclusion, the culmination of this study sheds light on the multifaceted potential of Tenebrio molitor in addressing pressing challenges at the intersection of waste management, protein scarcity, and sustainable food production. By investigating the utilization of coffee grounds as a viable nutrient source for *Tenebrio molitor* larvae, this research introduces a groundbreaking avenue towards mitigating protein shortages, minimizing food waste, and fortifying food security. The intrinsic value of this study lies in its pioneering approach to repurposing coffee grounds, a widely abundant byproduct, into a resource of substantial nutritional value. The experimental findings resonate as a testament to the capacity of *Tenebrio molitor* larvae to thrive on coffee grounds. Notably, treatment T1, comprising a strategic blend of 5% coffee grounds and 95% substrate, showcased exceptional mass gain during the initial 7-day span, surpassing the control group. These results present a promising solution to the imperative need for sustainable protein sources. However, the subsequent weight loss observed in specific treatments beyond the 7-day threshold introduces an intriguing dimension, underlining the need for precise temporal management of larval harvesting and processing. This insight underscores the intricate interplay between *Tenebrio molitor*, coffee grounds, and their potential in protein production and waste management. Remarkably, this research embodies the quintessential circular economy principle, harnessing the latent potential within organic waste streams to cultivate a protein-rich resource.

This methodology encompasses a holistic approach that aligns harmoniously, intending to establish self-sustaining systems for food production and waste reduction. The novelty of this study lies not only in its scientific contribution but also in its potential real-world applications. The symbiotic relationship between *Tenebrio molitor* and coffee grounds presents an innovative perspective on addressing global challenges. Integrating these findings into practical solutions can pave the way for transformative shifts in how we approach protein provisioning and waste valorization. This research illuminates a path forward that amalgamates ecological soundness with nutritional sufficiency. The convergence of *Tenebrio molitor* and coffee grounds exemplifies a holistic paradigm shift in our approach to protein sourcing and waste utilization. As we venture into an era of heightened sustainability consciousness, the insights derived from this study beckon us to redefine possibilities, beckoning a future where novel applications spring forth from the synergy of nature and innovation.

CONFLICT OF INTEREST

There is no conflict of interest.

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REFERENCES

1. SOREN, Siba, CHAKROBORTY, Subhendu, MAHALIK, Rakesh Ranjan, PARHI, Purnendu, PAL, Kaushik, BEHERA, Debendra, SAHOO, Chita Ranjan, PADHY, Rabindra N., AULAKH, Manpreet Kaur, SAREEN, Shweta and KRISHNA, Suresh Babu Naidu. Evaluation of the antimicrobial potential of cerium-based perovskite (CeCuO3) synthesized by a hydrothermal method**. New Journal of Chemistry**. v. 46, n. 40, p. 19147–19152. 2022. DOI 10.1039/D2NJ03646K.
2. PAL, Kaushik, CHAKROBORTY, Subhendu and NATH, Nibedita. Limitations of nanomaterials insights in green chemistry sustainable route: Review on novel applications**. Green Processing and Synthesis**. v. 11, n. 1, p. 951–964. 2022. DOI 10.1515/gps-2022-0081.
3. RAJAN, Ramya, PAL, Kaushik, JAYADEV, D., JAYAN, Jitha S., U, Aathira, APPUKUTTAN, Saritha, DE SOUZA, Fernando Gomes, JOSEPH, Kuruvilla and KUMAR, Saran S. Polymeric Nanoparticles in Hybrid Catalytic Processing and Drug Delivery System**. Topics in Catalysis**. v. 65, n. 19, p. 1860–1884. 2022. DOI 10.1007/s11244-022-01697-0.
4. WONG, C.W., CHAN, Y.S., JEEVANANDAM, J., PAL, K., BECHELANY, M., ABD ELKODOUS, M. and EL-SAYYAD, G.S. Response Surface Methodology Optimization of Mono-dispersed MgO Nanoparticles Fabricated by Ultrasonic-Assisted Sol–Gel Method for Outstanding Antimicrobial and Antibiofilm Activities**. Journal of Cluster Science**. v. 31, n. 2, p. 367–389. 2020. DOI 10.1007/s10876-019-01651-3. Scopus
5. ABIC. *Associação Brasileira das Indústrias do Café. Relatório mensal dezembro de 2017, Conselho dos Exportadores de Café do Brasil* [online]. 2021. Conselho dos Exportadores de Café do Brasil. Available from: https://static.poder360.com.br/2022/01/Cafe_2021.pdf.
6. RAMBO, M.K.D., AMORIM, E.P. and FERREIRA, M.M.C. Potential of visible-near infrared spectroscopy combined with chemometrics for analysis of some constituents of coffee and banana residues**. Analytica Chimica Acta**. v. 775, p. 41–49. 2013. DOI 10.1016/j.aca.2013.03.015.
7. ZUORRO, Antonio and LAVECCHIA, Roberto. Spent coffee grounds as a valuable source of phenolic compounds and bioenergy**. Journal of Cleaner Production**. v. 34, p. 49–56. 2012. DOI 10.1016/j.jclepro.2011.12.003.
8. PANUSA, Alessia, ZUORRO, Antonio, LAVECCHIA, Roberto, MARROSU, Giancarlo and PETRUCCI, Rita. Recovery of Natural Antioxidants from Spent Coffee Grounds**. Journal of Agricultural and Food Chemistry**. v. 61, n. 17, p. 4162–4168. 2013. DOI 10.1021/jf4005719.
9. RAMALAKSHMI, K., RAO, L. Jagan Mohan, TAKANO-ISHIKAWA, Yuko and GOTO, Masao. Bioactivities of low-grade green coffee and spent coffee in different in vitro model systems**. Food Chemistry**. v. 115, n. 1, p. 79–85. 2009. DOI 10.1016/j.foodchem.2008.11.063.
10. MARQUES, Fernanda D., SOUZA JR., F.G. and OLIVEIRA, Geiza E. Oil sorbers based on renewable sources and coffee grounds**. Journal of Applied Polymer Science**. v. 133, n. 11, p. 43127–43134. 2016. DOI 10.1002/app.43127.
11. SILVA, Johny C., OLIVEIRA, G. E., TOLEDO FILHO, Romildo D. and SOUZA JR., F.G. Oil Spill Clean-Up Tool Based on Castor Oil and Coffee Grounds Magnetic Resins**. Macromolecular Symposia**. v. 380, n. 1, p. 1800095. 2018. DOI 10.1002/masy.201800095.
12. PANDEY, A., SOCCOL, C. R., NIGAM, P., BRAND, D., MOHAN, R. and ROUSSOS, S. Biotechnological potential of coffee pulp and coffee husk for bioprocesses**. Biochemical Engineering Journal**. v. 6, n. 2, p. 153–162. 2000. DOI 10.1016/s1369-703x(00)00084-x.
13. LINS JR, Juracy, FONTANA, Luisa and PEDERIVA, Kamila. DESENVOLVIMENTO DE LARVAS DE Tenebrio molitor L. EM DIFERENTES DIETAS VISANDO A PRODUÇÃO DE INSETOS PARA CONSUMO HUMAN**. CONNECTION LINE - REVISTA ELETRÔNICA DO UNIVAG**. 2018. DOI 10.18312/1980-7341.n18.2018.822.
14. MENEZES, Claubert Wagner Guimarães de, CAMILO, Silma da Silva, FONSECA, Arley José, ASSIS JÚNIOR, Sebastião Lourenço de, BISPO, Diego Faustolo and SOARES, Marcus Alvarenga. A dieta alimentar da presa Tenebrio molitor (Coleoptera: Tenebrionidae) pode afetar o desenvolvimento do predador Podisus nigrispinus (Heteroptera: Pentatomidae)?**. Arquivos do Instituto Biológico**. v. 81, p. 250–256. 2014. DOI 10.1590/1808-1657001212012.
15. HONG, Jinsu, HAN, Taehee and KIM, Yoo Yong. Mealworm (Tenebrio molitor Larvae) as an Alternative Protein Source for Monogastric Animal: A Review**. Animals**. v. 10, n. 11, p. 2068. 2020. DOI 10.3390/ani10112068.
16. ABOELKHEIR, Mostafa G., VISCONTE, Leila Y., OLIVEIRA, Geiza E., TOLEDO FILHO, Romildo D. and SOUZA, Fernando G. The biodegradative effect of Tenebrio molitor Linnaeus larvae on vulcanized SBR and tire crumb**. Science of The Total Environment**. v. 649, p. 1075–1082. 2019. DOI 10.1016/j.scitotenv.2018.08.228.
17. MABELEBELE, M., KOLOBE, S. D., MALEMATJA, E., SEBOLA, N. A. and MANYELO, T. G. A comprehensive review of the importance of selected trace elements present in edible insects**. Biological**

Trace Element Research. v. 201, n. 7, p. 3520–3527. 2023.

18. AMOAH, Isaac, COBBINAH, Jesse Charles, YEBOAH, Jacqueline Afua, ESSIAM, Fiifi Amoako, LIM, Jia Jiet, TANDOH, Marina Aferiba and RUSH, Elaine. Edible insect powder for enrichment of bakery products–A review of nutritional, physical characteristics and acceptability of bakery products to consumers**. Future Foods**. P. 100251. 2023.
19. TEIXEIRA, Carla SS, VILLA, Caterina, COSTA, Joana, FERREIRA, Isabel MPLVO and MAFRA, Isabel. Edible Insects as a Novel Source of Bioactive Peptides: A Systematic Review**. Foods**. v. 12, n. 10, p. 2026. 2023.
20. BOUKID, F., SOGARI, G. and ROSELL, C. M. Edible insects as foods: mapping scientific publications and product launches in the global market (1996-2021)**. Journal of Insects as Food and Feed**. v. 9, n. 3, p. 353–368. 2023.
21. KRONGDANG, Sasiprapa, PHOKASEM, Patcharin, VENKATACHALAM, Karthikeyan and CHAROENPHUN, Narin. Edible Insects in Thailand: An Overview of Status, Properties, Processing, and Utilization in the Food Industry**. Foods**. v. 12, n. 11, p. 2162. 2023.
22. DEVI, Wahengbam Deepanita, BONYSANA, Rajkumari, KAPESA, Kokho, MUKHERJEE, Pulok Kumar and RAJASHEKAR, Yallappa. Edible insects: as traditional medicine for human wellness**. Future Foods**. P. 100219. 2023.
23. TANGA, Chrysantus M. and KABABU, Margaret O. New insights into the emerging edible insect industry in Africa**. Animal Frontiers**. v. 13, n. 4, p. 26–40. 2023.
24. MANNOZZI, Cinzia, FOLIGNI, Roberta, MOZZON, Massimo, AQUILANTI, Lucia, CESARO, Cristiana, ISIDORO, Nunzio and OSIMANI, Andrea. Nonthermal technologies affecting techno-functional properties of edible insect-derived proteins, lipids, and chitin: A literature review**. Innovative Food Science & Emerging Technologies**. P. 103453. 2023.
25. PARK, E.-S., KANG, M.-H. and CHOI, M.-K. Nutritional composition and antioxidant properties of edible insects sold in Korea**. Journal of Insects as Food and Feed**. v. 9, n. 2, p. 245–254. 2023.
26. KOLOBE, Sekobane D., MANYELO, Tlou G., SEBOLA, Nthabiseng A. and MONNYE, Mabelebele. Scope and present status of rearing edible insects for animal feeding in Africa**. All Life**. v. 16, n. 1, p. 2206542. 2023.
27. NOWAK, Verena, PERSIJN, Diedelinde, RITTENSCHOBER, Doris and CHARRONDIERE, U. Ruth. Review of food composition data for edible insects**. Food Chemistry**. v. 193, p. 39–46. 2016. DOI 10.1016/j.foodchem.2014.10.114.
28. CAMPOS-VEGA, Rocio, LOARCA-PIÑA, Guadalupe, VERGARA-CASTAÑEDA, Haydé A. and OOMAH, B. Dave. Spent coffee grounds: A review on current research and future prospects**. Trends in Food Science & Technology**. v. 45, n. 1, p. 24–36. 2015. DOI 10.1016/j.tifs.2015.04.012.
29. DREYER, Moritz, HÖRTENHUBER, Stefan, ZOLLITSCH, Werner, JÄGER, Henry, SCHADEN, Lisa-Marie, GRONAUER, Andreas and KRAL, Iris. Environmental life cycle assessment of yellow mealworm (Tenebrio molitor) production for human consumption in Austria–a comparison of mealworm and broiler as protein source**. The International Journal of Life Cycle Assessment**. v. 26, p. 2232–2247. 2021.
30. ERRICO, Simona, SPAGNOLETTA, Anna, MOLITERNI, Stefania, DIMATTEO, Salvatore, VERARDI, Alessandra, SANGIORGIO, Paola, BALDACCHINO, Ferdinando and BALDUCCHI, Roberto. Tenebrio molitor as a new promising alternative in the production of feed and food**. Environmental Engineering and Management Journal**. v. 21, n. 10, p. 1657–1671. 2022.
31. ERRICO, Simona, SPAGNOLETTA, Anna, VERARDI, Alessandra, MOLITERNI, Stefania, DIMATTEO, Salvatore and SANGIORGIO, Paola. Tenebrio molitor as a source of interesting natural compounds, their recovery processes, biological effects, and safety aspects**. Comprehensive Reviews in Food Science and Food Safety**. v. 21, n. 1, p. 148–197. 2022.
32. KHANAL, Prabhat, PANDEY, Deepak, NÆSS, Geir, CABRITA, Ana RJ, FONSECA, António JM, MAIA, Margarida RG, TIMILSINA, Bishnu, VELDKAMP, Teun, SAPKOTA, Rumakanta and OVERREIN, Hege. Yellow mealworms (Tenebrio molitor) as an alternative animal feed source: A comprehensive characterization of nutritional values and the larval gut microbiome**. Journal of Cleaner Production**. v. 389, p. 136104. 2023.
33. KUAN, Zhi-Jue, CHAN, Barnabas Kuan-Nang and GAN, Samuel Ken-En. Worming the circular economy for biowaste and plastics: Hermetia illucens, Tenebrio molitor, and Zophobas morio**. Sustainability**. v. 14, n. 3, p. 1594. 2022.
34. MEYER, Sandra, GESSNER, Denise K., BRAUNE, Maria S., FRIEDHOFF, Theresa, MOST, Erika, HÖRING, Marcus, LIEBISCH, Gerhard, ZORN, Holger, EDER, Klaus and RINGSEIS, Robert. Comprehensive evaluation of the metabolic effects of insect meal from Tenebrio molitor L. in growing pigs by transcriptomics, metabolomics and lipidomics**. Journal of Animal Science and Biotechnology**. v. 11,

n. 1, p. 1–19. 2020.

35. MONTALBÁN, Ana, SÁNCHEZ, Cristian Jesús, HERNÁNDEZ, Fuensanta, SCHIAVONE, Achille, MADRID, Josefa and MARTÍNEZ-MIRÓ, Silvia. Effects of agro-industrial byproduct-based diets on the growth performance, digestibility, nutritional and microbiota composition of mealworm (Tenebrio molitor L.)**. Insects**. v. 13, n. 4, p. 323. 2022.
36. MORUZZO, Roberta, RICCIOLI, Francesco, ESPINOSA DIAZ, Salomon, SECCI, Chiara, POLI, Giulio and MANCINI, Simone. Mealworm (Tenebrio molitor): potential and challenges to promote circular economy**. Animals**. v. 11, n. 9, p. 2568. 2021.
37. VASILOPOULOS, Stylianos, GIANNENAS, Ilias, SAVVIDOU, Soumela, BONOS, Eleftherios, RUMBOS, Christos I., PAPADOPOULOS, Elias, FORTOMARIS, Paschalis and ATHANASSIOU, Christos G. Growth performance, welfare traits and meat characteristics of broilers fed diets partly replaced with whole Tenebrio molitor larvae**. Animal Nutrition**. v. 13, p. 90–100. 2023.
38. KARIM, Darboe Abdou. A review of non-conventional animal protein sources as poultry feeds**.** . 2023.
39. TOVIHO, Odunayo A., IMANE, Moutia, TÜNDE, Pusztahelyi and PÉTER, Bársony. Effect of Duckweed (Spirodela polyrhiza)-Supplemented Semolina on the Production Parameters and Nutrient Composition of Yellow Mealworm (Tenebrio molitor)**. Agriculture**. v. 13, n. 7, p. 1386. 2023.
40. CUNHA, Nair, ANDRADE, Vanda, RUIVO, Paula and PINTO, Paula. Effects of Insect Consumption on Human Health: A Systematic Review of Human Studies**. Nutrients**. v. 15, n. 14, p. 3076. 2023.
41. CHEWAKA, Legesse Shiferaw, PARK, Chan Soon, CHA, Youn-Soo, DESTA, Kebede Taye and PARK, Bo-Ram. Enzymatic Hydrolysis of Tenebrio molitor (Mealworm) Using Nuruk Extract Concentrate and an Evaluation of Its Nutritional, Functional, and Sensory Properties**. Foods**. v. 12, n. 11, p. 2188. 2023.
42. SZOPA, Daniel, IZYDORCZYK, Grzegorz, CHOJNACKA, Katarzyna and WITEK-KROWIAK, Anna. Evaluation of the Feasibility of Using Inorganic and Organic Acids for the Extraction of Amino Acids from High-Protein Material (Mealworm Larvae) by Chemical Hydrolysis**. Available at SSRN 4530553**.
43. KRÖNCKE, Nina and BENNING, Rainer. Influence of Dietary Protein Content on the Nutritional Composition of Mealworm Larvae (Tenebrio molitor L.)**. Insects**. v. 14, n. 3, p. 261. 2023.
44. HAMMER, Laila, MORETTI, Diego, ABBÜHL-ENG, Lychou, KANDIAH, Pabiraa, HILAJ, Nikolin, PORTMANN, Reto and EGGER, Lotti. Mealworm larvae (Tenebrio molitor) and crickets (Acheta domesticus) show high total protein in vitro digestibility and can provide good-to-excellent protein quality as determined by in vitro DIAAS**. Frontiers in Nutrition**. v. 10, p. 1150581. 2023.
45. KANG, Yifei, APPLEGATE, Catherine C., HE, Fei, OBA, Patricia M., VIESON, Miranda D., SÁNCHEZ-SÁNCHEZ, Lorena and SWANSON, Kelly S. Yellow mealworm (Tenebrio molitor) and lesser mealworm (Alphitobius diaperinus) proteins slowed weight gain and improved metabolism of diet-induced obesity mice**. The Journal of Nutrition**. 2023.
46. PANIZZI, A. R., PARRA, J. R. P. and ANTONIO RICARDO PANIZZI, CNPSO; ESALQ. **Bioecologia e nutrição de insetos: base para o manejo integrado de pragas.** [online]. Brasília, DF: Embrapa, 2013., 2013. Accessed 31 May 2023. ISBN 978-85-7035-290-3. Available from: http://www.alice.cnptia.embrapa.br/handle/doc/1076988.
47. GOMES SOUZA, Fernando, PAL, Kaushik, AMPAH, Jeffrey Dankwa, DANTAS, Maria Clara, ARAÚJO, Aruzza, MARANHÃO, Fabíola and DOMINGUES, Priscila. Biofuels and Nanocatalysts: Python Boosting Visualization of Similarities**. Materials**. v. 16, n. 3, p. 1175. 2023. DOI 10.3390/ma16031175.
48. MILLER, Jane Charlotte and MILLER, James N. **Statistics for Analytical Chemistry**. Ellis Horwood PTR Prentice Hall, 1993. ISBN 978-0-13-030990-7.
49. NETO, Pedro Luiz de Oliveira Costa. **Estatística**. Editora Blucher, 2002. ISBN 978-85-212-1522-6.