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Protein and energy digestibility of insect meals for angelfish (*Pterophyllum scalare*)

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ABSTRACT

Insect meal has been the subject of numerous studies in recent years, and its potential in animal feeding has also been investigated. This study aimed to investigate the digestibility coefficients of protein, dry matter, and energy of some insect meals: black soldier fly (*Hermetia illucens*), cricket (*Gryllus* spp.), cricket nymph, and mealworm (*Tenebrio molitor*) for angelfish (*Pterophyllum scalare*). A total of 225 adults of *P. scalare*, weighing 30 ± 2.32 g each, were housed in 15 200-L circular fiber tanks at a density of 15 fish per experimental unit in triplicate. Digestibility was estimated by the indirect method, using chromium oxide as an inert indicator. For feces collection, the modified Guelph system was used. Among the tested insect meals, black soldier fly meal presented a superior digestibility coefficient for dry matter (0.77), protein (0.901), and energy (0.82). The mealworm presented an inferior digestibility coefficient (dry matter = 0.67, protein = 0.88, and energy = 0.77). Cricket (adult and nymph meals) showed similar values of coefficients of dry matter and energy digestibility. These results indicated that the insect meals investigated in this study present suitable alternatives as feed for angelfish.

Keywords: Alternative food; Cricket; Digestibility coefficients; Fishmeal; Fish nutrition; Ornamental fish.

Digestibilidade da proteína e energia de farinhas de insetos para acará-bandeira (*Pterophyllum scalare*)

RESUMO

As farinhas de insetos têm sido objeto de inúmeros estudos nos últimos anos, e seu potencial na alimentação animal também tem sido investigado. Este estudo teve como objetivo investigar os coeficientes de digestibilidade de proteína, matéria seca e energia de farinhas de insetos: mosca soldado-negro (*Hermetia illucens*), grilo (*Gryllus* spp.), ninfa-do-grilo e tenébrio (*Tenebrio molitor*) para acará-bandeira (*Pterophyllum scalare*). Um total de 225 adultos de *P. scalare*, pesando 30 ± 2,32 g cada um, foi alojado em 15 tanques circulares de fibra de 200 L com densidade de 15 peixes por unidade experimental em triplicata. A digestibilidade foi estimada pelo método indireto, utilizando óxido de cromo como indicador inerte. Para coleta de fezes, foi utilizado o sistema Guelph modificado. Entre as farinhas de insetos testadas, a farinha de mosca soldado-negro apresentou coeficiente de digestibilidade superior para matéria seca (0,77), proteína (0,901) e energia (0,82). A farinha de tenébrio apresentou coeficiente de digestibilidade inferior (matéria seca = 0,67, proteína = 0,88 e energia = 0,77). O grilo (farinhas de adultos e ninfa) apresentou valores semelhantes de coeficiente de digestibilidade de matéria seca e energia. Esses resultados indicaram que as farinhas de insetos investigadas neste estudo são alternativas adequadas como alimento para acará-bandeira.

Palavras-chave: Alimento alternativo; Coeficiente de digestibilidade; Farinha de peixe; Grilo; Nutrição de peixes; Peixes ornamentais.

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INTRODUCTION

The production of aquatic organisms for human consumption and ornamental purposes has demonstrated consistent year-on-year growth. According to the Food and Agriculture Organization (2022), the production of aquatic organisms reached a record of 214 million tons in 2020. In parallel, ornamental fish production in 2021 generated a turnover of 5.4 billion USD, with a potential annual growth rate of 8.5% between 2022 and 2030 (Triandafyllidou & McAuliffe, 2019).

The angelfish (*Pterophyllum scalare*), native to the Amazon Basin and a member of the cichlid family, is recognized for its ornamental value, ease of management, and high economic significance. With a maximum length of 15 cm, the angelfish, like its family members, exhibits territorial behavior, favoring water with low hardness and slightly acidic conditions in its natural habitat (Rodrigues & Fernandes, 2006).

In controlled environments, angelfish feeding typically commences in the larval and fry stages with live food, commonly brine shrimp. The juvenile and adult stages of angelfish are fed with extruded pellets with a crude protein content ranging from 290–360 g/kg, with fishmeal being the primary protein source (Ribeiro et al., 2007; Rodrigues & Fernandes, 2006; Zuanon et al., 2009).

The pursuit of alternatives to fishmeal, driven by welfare, ethical, and economic considerations, has been extensively explored in studies (Belghit et al., 2019; Daniel, 2018; Ding et al., 2015; Magalhães et al., 2017; Oliva-Teles et al., 2015). Plant-based protein sources such as soybean protein concentrate, corn gluten, and soybean meal have been examined. However, these alternatives may exhibit undesirable characteristics that impact nutritional efficiency, including low digestibility, reduced palatability, the presence of antinutritional factors (Barrows et al., 2008), and amino acid imbalances (Bulbul et al., 2013).

In this context, insect meal has emerged as a promising substitute for other protein sources, aligning directly with the principles of the circular economy: reduce, reuse, repair, and recycle. The ease with which insects can be bred, the diversity of species that can function as insect meal, the high protein content, and the efficient feed conversion make insect meal a sustainable choice (Vasconcelos, 2021). It can be quickly developed from organic waste and subsequently processed for inclusion in feeds (van Huis, 2020; Vasconcelos, 2021).

However, despite the potential of insect meals to replace conventional fishmeal, the main obstacle lies in the production cost, primarily due to the non-technified and low-volume nature of insect production. Developing more efficient techniques for large-scale production of these feeds necessitates understanding the digestibility of the nutrients in these ingredients and assessing the feasibility of using insect meals in fish feed. Specifically, data on the digestibility of novel insect meals, such as cricket nymph meal, for ornamental species like the angelfish, which can serve as a relevant omnivorous model, remains scarce. Furthermore, a comprehensive understanding of the economic viability of the digestible protein from these insect meals is crucial for their practical application in aquaculture.

Considering these aspects, this study aimed to assess the apparent digestibility coefficients (ADCs) of four insect meals—black soldier fly (*Hermetia illucens*), cricket (*Gryllus* spp.), cricket nymph, and mealworm (*Tenebrio molitor*)—for angelfish, and to evaluate the cost-effectiveness of their digestible protein for aquaculture applications.

MATERIAL AND METHODS

Animals and experimental design

The digestibility trial was conducted to assess the ADCs of dry matter, protein, and energy for four insect meals: black soldier fly meal (*H. illucens*) (BSFM), adult cricket meal (*Gryllus* spp.) (ACM), cricket nymph meal (*Gryllus* spp.) (NCM), and mealworm meal (*Tenebrio molitor*) (MM). The feeding trial was conducted at the Aquaculture Center of Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP) and employed a completely randomized design comprising five dietary treatments (one control without insect meal and four insect meals) with three replications. Each replication contained 15 angelfish with an average weight of 30 ± 2.32 g in every experimental unit. The trial was arranged within a recirculation system featuring 15 200-L fiberglass feeding tanks and nine 200-L fiberglass cylindrical tanks (feces collectors) that were adapted to the modified Guelph system, and equipped with mechanical and biological filtration, constant aeration, and temperature control (27.8 ± 1.3°C).

Diets and feeding

Experimental diets comprised 140 g/kg of the test ingredient (insect meal) and 860 g/kg of the control diet (Table 1), which was a basal diet formulated to meet the nutritional needs of the angelfish based on fishmeal. Additionally, 10 g/kg of chromium oxide (Cr2O3) was incorporated as an inert marker. The control diet served as the reference, and data collected from this group were employed to calculate the ADCs of the test ingredients. Following a seven-day adaptation period to the recirculation system, fish were fed six times a day in the feeding tanks until apparent satiety. Collections began seven days after the start of feeding. After a period of 30 min following the last daily

Table 1. Formulation of the experimental diets used to determine the coefficients of apparent digestibility of protein and energy of insect meals for the angelfish (*Pterophyllum scalare*).

	Diets				
Ingredients (g/kg)	RD	BSFM	ACM	NCM	MM
Fishmeal	135.0	111.8	111.8	111.8	111.8
Black soldier fly meal	-	140.0	-	-	-
Cricket meal	-	-	140.0	-	-
Cricket nymph meal	-	-	-	140.0	-
Mealworm meal	-	-	-	-	140.0
Corn gluten	36.0	20.0	20.0	20.0	20.0
Soybean meal	400.0	320.0	270.0	300.0	339.0
Rice bran	30.0	38.0	38.0	38.0	38.0
Corn starch	-	19.0	44.0	39.0	-
Corn, grain	290.8	270.0	270.0	270.0	270.0
Soybean oil	30.0	1.0	2.6	1.0	1.0
L-lysine	8.0	8.0	8.0	8.0	8.0
DL-methionine	2.7	2.7	2.7	2.7	2.7
L-arginine	17.5	17.5	17.5	17.5	17.5
L-histidine	7.5	7.5	7.5	7.5	7.5
Dicalcium phosphate	12.0	14.0	14.0	14.0	14.0
Limestone	10.0	10.0	10.0	10.0	10.0
BHT antioxidant	0.5	0.5	0.5	0.5	0.5
Premix1	5.0	5.0	5.0	5.0	5.0
Salt	5.0	5.0	5.0	5.0	5.0
Chromium oxide	10.0	10.0	10.0	10.0	10.0
Total	1 kg	1 kg	1 kg	1 kg	1 kg

RD: reference diet; BSFM: black soldier fly meal; ACM: adult cricket meal; NCM: cricket nymph meal; MM: mealworm meal; [1]composition of the mineral and vitamin supplement: folic acid (1,250 mg); calcium pantothenate (1,200 mg); copper (2,500 mg); iron (15 mg); iodine (375 mg); manganese (12.5 g); selenium (87.5 mg); zinc (12.5 mg); cobalt (125 mg); vitamin A (2,500 IU); vitamin B12 (4,000 mg); thiamine B1 (4,000 mg); riboflavin B2 (4,000 mg); pyridoxine B6 (4,000 mg); vitamin C (50,000 mg); vitamin D3 (600,000 IU); vitamin E (37,500); vitamin K3 (3,750 mg); niacin (122,500 mg); biotin (15 mg).

feeding, fish were transferred to feces collectors. At the base of each collector, a Falcon tube was attached alongside the water outlet and placed in a Styrofoam box with ice to mitigate sample degradation effects. Fecal matter was collected overnight (14 hours of collection). The following morning, animals were moved back to the feeding tanks, and the collected feces were deposited in Petri dishes and subsequently frozen. Collection of feces was carried out until the minimum required quantity for analysis was obtained.

Diets composition and digestibility determination

Analysis of the insect meals (Table 2) and the other ingredients and diets was undertaken to determine their chemical composition, as-fed basis, following standardized methods by the Association of Official Analysis Chemistry (2019) at the Bromatological Analysis Center of the Poultry Science Laboratory at the Faculty of Agricultural and Veterinary Sciences, UNESP, Jaboticabal *Campus*.

Table 2. Chemical composition analysis, as-fed basis, of black soldier fly meal (*Hermetia illucens*), cricket meal (*Gryllus* spp.), cricket nymph meal (*Gryllus* spp.), and mealworm meal (*Tenebrio molitor*) (as-fed basis).

Nutrients (g/kg)	BSFM	ACM	NCM	MM
Dry matter	960.2	942.9	965.0	935.7
Crude protein*	376.1	506.8	422.3	531.4
Crude energy (MJ)	6.041	5.279	6.232	5.829
Crude fat	363.5	146.0	287.0	296.2
Crude ash	96.8	71.7	34.0	35.5

BSFM: black soldier fly meal; ACM: adult cricket meal; NCM: cricket nymph meal; MM: mealworm meal; *crude protein content corrected using a nitrogen-to-protein conversion factor of Kp = 4.76 (Janssen et al., 2017).

The corrected crude protein content of the insect meals and crude protein content of the other ingredients were determined using the Kjeldahl method. A nitrogen-to-protein correction factor of 4.76 (Kp = 4.76) was applied for insect meals due to chitin being a nitrogenous compound with a lower digestibility (Table 3). Using a conversion factor of 6.25 would overestimate the insect meal's protein content (Janssen et al., 2017). For other

Table 3. Analyzed composition of the experimental diets, as-fed basis, to determine the apparent digestibility coefficients of protein and energy of black soldier fly meal (*Hermetia illucens*), cricket meal (*Gryllus* spp.), cricket nymph meal (*Gryllus* spp.), and mealworm meal (*Tenebrio molitor*) for the angelfish (*Pterophyllum scalare*).

Ingredients (g/kg)	Diets				
	RD	BSFM	ACM	NCM	MM
Dry matter	917.9	926.4	932.8	922.3	918.8
Crude energy (MJ)	4.06	4.43	4.35	4.41	4.40
Crude protein*	-	404.9	415.6	407.6	412.9
Crude fat	30.5	88.8	60.0	81.2	82.6
Ash	105.6	99.9	96.6	96.1	97.5

RD: reference diet; BSFM black soldier fly meal; ACM: adult cricket meal; NCM: cricket nymph meal; MM: mealworm meal; *consideration of the corrected crude protein content of the insect meals, calculated using a nitrogen-to-protein conversion factor of Kp = 4.76 (Janssen et al., 2017).

ingredients, the correction factor was 6.25 (Kp = 6.25). Gross energy was determined through a calorimetry bomb, dry matter through gravimetric analysis (drying in a forced-air oven at 105°C for 16 hours), ash content through gravimetric analysis (samples were incinerated in a muffle furnace at 550°C for 4 hours), and fat through gravimetric analysis (samples were washed in an ANKOMXT15 automatic fat extractor). The samples were subjected to digestion in a solution of nitroperchloric acid to determine the chromium oxide and were subsequently subjected to atomic absorption analysis at the Technology Laboratory of UNESP, in Jaboticabal *Campus*.

The ADCs of the reference and test diets were calculated, as-fed basis, employing the formula proposed by Nose (1966) (Eq. 1):

$$ADC = [(\text{indicator in the diet} / \text{indicator in the feces}) \times (\text{nutrient in the feces} / \text{nutrient in the diet})] \quad (1)$$

The calculation of the apparent digestibility of nutrients in the feed (DNF) followed the formula described by Forster (1999) (Eq. 2):

$$DNF\ ingredient = [\ (a + b) \times ADCdiet\ test - (a) \times ADCdiet\ reference] / b \quad (2)$$

where: a: the contribution of the nutrient from the reference diet to the nutrient content of the test diet; b: the contribution of the nutrient from the test ingredient to the nutrient content of the base diet (concentration of the nutrient in the test ingredient).

The concentrations of digestible dry matter (DDM), digestible protein (DP), and digestible energy (DE) for each ingredient were calculated by multiplying the gross content of the respective nutrient by its apparent digestibility coefficient.

Cost-effectiveness analysis of digestible protein

The cost per kilogram of DP (USD/kg) was determined based on the market prices of each insect meal, according to research conducted in June 2025. The cost of DP was determined by dividing the cost of the insect meal per kilogram (USD/kg) by its DP content (kg DP/kg meal), as derived from the apparent digestibility coefficients (ADCs) obtained in this study.

Statistical analysis

The results are presented as the average and standard deviation of the means. Normality and homoscedasticity were assessed using the Shapiro–Wilk's and Bartlett's tests, respectively. Subsequently, the data underwent analysis of variance, followed by the Tukey's test mean comparison test at a significance level of 5%. All statistical analyses were performed using RStudio.

RESULTS

The ADCs of dry matter, protein, and energy for the evaluated insect meals are presented in Table 4. MM exhibited the lowest ADC values for all evaluated nutrients for the angelfish ($p < 0.05$). ACM also showed the lowest protein ADC for the species. Conversely, BSFM and NCM demonstrated the highest digestibility for dry matter, protein, and energy for the angelfish ($p < 0.05$).

Table 4. Apparent digestibility coefficients of dry matter, protein, and energy, as-fed basis, of black soldier fly meal (*Hermetia illucens*), cricket meal (*Gryllus* spp.), cricket nymph meal (*Gryllus* spp.), and mealworm meal (*Tenebrio molitor*) for adult angelfish (*Pterophyllum scalare*)*.

Nutrient	BSFM	ACM	NCM	MM	p-value
Dry matter	0.77 ± 0.00 a	0.77 ± 0.00 a	0.75 ± 0.00 a	0.67 ± 0.02 b	0.0007
Protein	0.91 ± 0.00 a	0.87 ± 0.00 b	0.90 ± 0.00 a	0.88 ± 0.01 b	0.0007
Energy	0.82 ± 0.00 a	0.82 ± 0.00 a	0.83 ± 0.00 a	0.77 ± 0.01 b	0.0009

BSFM: black soldier fly meal; ACM: adult cricket meal; NCM: cricket nymph meal; MM: mealworm meal; *different letters on the same line indicate a significant difference between the insect meals.

Table 5 provides the digestible nutrient values for each evaluated insect meal. Additionally, the cost per kilogram of DP is presented in Table 6.

Table 5. Results of digestible dry matter, digestible protein, and digestible energy calculated for the evaluated insect meals, as-fed basis.

Ingredient (g/kg)	DDM	DP	DE (MJ)
Hermetia illucens	741.0	341.0	4.97
Gryllus spp.	741.8	442.8	4.86
Gryllus spp. nymph	725.6	379.5	5.15
Tenebrio molitor	631.9	465.5	4.66

DDM: digestible dry matter; DP: digestible protein; DE: digestible energy.

Table 6. Cost per kilogram of insect meal and cost per kilogram of digestible protein for the evaluated insect meals.

Insect meal	Cost/kg (USD)	Cost/kg digestible protein (USD)
Hermetia illucens	8.96	26.27
Gryllus spp.	57.45	129.74
Gryllus spp. nymph	57.45	151.38
Tenebrio molitor	3.59	7.71

DISCUSSION

BSFM has been widely recognized for its high nutritional value, as indicated in various studies (Dietz & Liebert, 2018; Kroeckel et al., 2012; Liu et al., 2017; NRC, 2011). In the present study, the digestibility coefficients of protein, energy, and dry matter of BSFM were comparable to values previously reported in the literature for tambaqui (*Colossoma macropomum*) (Santos et al., 2023) and gilthead seabream (*Sparus aurata*) (Piccolo et al., 2017). In contrast, for carnivorous fish such as European sea bass (*Dicentrarchus labrax*), digestibility coefficients were lower those obtained in the present study (Basto et al., 2020). Notably, concerning Atlantic salmon (*Salmo salar*), the protein digestibility of BSFM was lower compared to that for angelfish. One of the main reasons for these discrepancies, particularly when compared to carnivorous fish, can be attributed to the presence of chitin in the exoskeleton of the black soldier fly. The angelfish has an omnivorous feeding habit, similar to *C. macropomum*, naturally feeding on zooplankton, microcrustaceans, and insects (Corrêa et al., 2007; Ikeda et al., 2017; Oliveira et al., 2006). This suggests that angelfish are more capable of digesting the chitin present in the exoskeleton of insects. This ability may be enhanced over time, as evidenced in previous studies with *Danio rerio* (Lanes et al., 2021), indicating a remarkable capacity for adaptation to dietary composition and highlighting the angelfish's potential as a suitable model for nutritional studies involving chitin-rich ingredients for other omnivorous aquaculture species.

Significantly, no existing studies in the literature have addressed the digestibility of nutrients in NCM in fish, making the present findings a novel contribution to aquaculture nutrition research. Our study observed that the coefficients of dry matter and energy digestibility for NCM were similar to those of ACM. In contrast, it was found that the protein digestibility of NCM exceeded that of ACM, despite the latter having a higher protein and amino acid concentration and an equivalent amount of chitin (Finke, 2002, 2007).

Angelfish exhibited superior potential nutrient utilization for both ACM and NCM when compared to Nile tilapia fed with ACM (Fontes et al., 2019; Hanan et al., 2022). Specifically, angelfish showed higher values for energy digestibility (0.47) and dry matter (0.43) in ACM, while protein ADC (0.85) remained consistent (Fontes et al., 2019). In contrast, when compared to juvenile tilapia, angelfish displayed elevated dry matter digestibility values (0.91), surpassing those observed in both ACM and NCM (Hanan et al., 2022). Moreover, the angelfish demonstrated superior utilization of ACM and NCM in comparison to the African catfish (*Clarias gariepinus*), despite its carnivorous nature.

Currently, MM stands out as the most widely adopted insect meal in animal nutrition due to its positive results in nutrient digestibility and fish performance, as pointed out by Belforti et al. (2015) and van Huis (2013). However, as evidenced in Table 2, this insect meal showed the lowest ADC values for the angelfish. In the case of Nile tilapia, which also has the same feeding habit as the angelfish, lower ADC values were recorded for energy (0.82) and dry matter (0.96) (Fontes et al., 2019). In the context of carnivorous fish, such as European sea bass (*D. labrax*), Basto et al. (2020) reported a dry matter digestibility coefficient of 0.77, a similar value that was found in this study. On the other hand, Tran (2021) reported a higher ADC (0.97) for dry matter and a lower ADC for protein (0.71) for MM compared to the results of this study when evaluating European perch (*Perca fluviatilis*).

Comparison with fishmeal is essential to evaluate insect meals, as it is the standard protein ingredient in aquaculture (Macedo-Viegas & Souza, 2004). However, fishmeal digestibility varies widely, influenced by the fish species, its feeding habit, and the quality of the meal itself (Tacon, 1987). This variability is evident in the literature: Köprücü and Özdemir (2005) reported an ADC of 0.90 for fishmeal protein in juvenile tilapias. This value was the same as the ADCs obtained for all the insect meals tested in the present study and the results observed for guppies (*Poecilia reticulata*) by Perera and Bhujel (2022). In contrast, when analyzing European sea bass (*D. labrax*), Basto (2021) identified higher ADCs for fishmeal, specifically, 0.79 for dry matter, 0.92 for protein, and 0.88 for energy, compared to the values for all insect meals investigated in this study. These discrepancies can be directly attributed to the feeding habits of the species evaluated. For carnivorous fish, the use of fishmeal may represent an advantage, as their digestive systems are fully adapted to process animal-derived proteins, and the absence of chitin in fishmeal makes the protein more readily available for digestion. On the other hand, for omnivorous fish like the angelfish, as mentioned earlier, which naturally feed on microcrustaceans and insects, the ability to digest chitin present in the exoskeleton of insects can improve over time, further supporting the potential of insect meals as highly suitable protein sources for this group of fish. Taufek et al. (2016) observed the apparent digestibility of nutrients from ACM for angelfish surpassed the values observed for fishmeal.

Beyond nutritional performance, the economic viability of feed ingredients is paramount for aquaculture adoption. The cost per kilogram of digestible protein varied significantly among the insect meals. While cricket meals (ACM and NCM) demonstrated high digestibility, their substantially higher costs per kilogram of digestible protein (USD 129.74 and USD 151.38, respectively) currently pose a significant barrier to their widespread application, unless production costs are substantially reduced. In contrast, mealworm meal, despite exhibiting the lowest digestibility coefficients in this study, stood out with the lowest cost per kilogram of digestible protein (USD 7.71). This highlights a critical trade-off between digestibility and economic feasibility. Black soldier fly meal also offered a competitive cost per kilogram of digestible protein (USD 26.27) while maintaining high digestibility, positioning it as a highly promising alternative.

Throughout this research, it was generally observed that the insect meals analyzed showed comparable or even higher ADCs for dry matter, protein, and energy compared to the coefficients obtained for fishmeals examined in previous studies when considering fish with feeding habits similar to the angelfish. The remarkable utilization of BSFM and NCM stands out in this context. Given the promising digestibility profiles, particularly for BSFM and NCM, and considering the varied economic profiles, further studies are strongly recommended to evaluate optimal levels of fishmeal replacement by these promising alternatives in the context of angelfish nutrition, with a focus on comprehensive growth performance, long-term health, and feed cost-effectiveness analyses under commercial conditions. Such research is crucial to bridge the gap between experimental digestibility data and practical feed formulation strategies for sustainable aquaculture.

CONCLUSION

Black soldier fly meal and cricket nymph meal presented the highest apparent digestibility coefficients for corrected protein and energy in angelfish. Mealworm meal, despite lower digestibility, offered the best cost-benefit regarding the cost per kilogram of digestible protein.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

All data were generated or analyzed in this study.

AUTHORS' CONTRIBUTIONS

Conceptualization: Fernandes, J.B.K.; **Data Curation:** Sayão, R.S.B., Oliveira, T.S.; **Formal Analysis:** Sayão, R.S.B., Oliveira, T.S., Silva, H.J.; **Investigation:** Sayão, R.S.B., Silva, H.J.; **Writing – Original Draft:** Sayão, R.S.B., Oliveira, T.S., Romaneli, R.S.; **Supervision:** Oliveira, T.S., Fernandes, J.B.K.; **Writing — Review & Edition:** Oliveira, T.S., Romaneli, R.S., Fernandes, J.B.K.; **Resources:** Fernandes, J.B.K.; **Project Administration:** Fernandes, J.B.K.; **Final Approval:** Fernandes, J.B.K.

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DECLARATION ON USE OF ARTIFICIAL INTELLIGENCE TOOLS

Artificial Intelligence (AI) tools, specifically Gemini, were utilized to assist in language refinement and stylistic suggestions during the drafting of this manuscript. This process was performed prior to language and grammar review by editage. All scientific content, data analysis, and interpretation remain the sole responsibility of the authors.

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