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The Effect of Phytase Enzyme in Feed on Growth Performance, Feed Utilization Efficiency, and Survival Rate of Milkfish (*Chanos chanos*) Fingerlings

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to evaluate the effect of phytase enzyme supplementation on the growth performance, feed utilization efficiency, and survival rate of milkfish fingerlings. The experimental fish used in this study were milkfish fingerlings with an average weight of 2.89±0.46 g per fish. The feed used in this research was a formulated pellet feed containing 30% protein, with added phytase

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enzyme. The treatments consisted of different phytase enzyme doses, namely A (0 mg/kg feed), B (250 mg/kg feed), C (500 mg/kg feed), and D (750 mg/kg feed). The results showed that different doses of phytase enzyme in the feed significantly affected (P<0.05) growth performance and feed utilization efficiency but did not significantly affect (P>0.05) the survival rate of milkfish fingerlings. The optimal dose of phytase enzyme for improving growth performance and feed utilization efficiency in milkfish fingerlings was between 502-665 mg/kg feed, resulting in a maximum total feed consumption (TFC) of 140.43 g, Feed Conversion Ratio (FCR) of 1.18, feed efficiency (FE) of 78.16%, protein efficiency ratio (PER) of 2.6, and relative growth rate (RGR) of 4.43%.

Keywords: Efficiency; digestibility; feed; growth; protein.

1. INTRODUCTION

Milkfish (*Chanos chanos*) is a brackish water fish commodity with significant economic value. It exhibits fast growth, can be cultured at high densities, is disease-resistant, and tolerant of environmental changes [1]. In 2021, Indonesia's milkfish production reached 784,941.13 tons, valued at IDR 15.56 trillion [2]. Intensive milkfish farming relies heavily on feed, which currently accounts for 60-70% of the total production costs [3]. To reduce feed costs, plant- based protein sources can be used as a feed protein alternative. However, plant-based ingredients contain an anti-nutritional factor known as phytic acid [4]. Phytic acid, an anti-nutrient present in feed, can inhibit the digestion process, preventing optimal feed utilization by the fish [5]. One strategy to address this issue is the addition of phytase enzyme to the feed.

Phytase enzyme can hydrolyze phytic acid, thereby improving feed utilization efficiency and growth performance. With the breakdown of phytic acid, metabolic processes such as the digestion of protein and mineral complexes in the body can proceed more efficiently [6]. The addition of phytase to the feed has the potential to increase the bioavailability of nutrients and energy, as well as improve feed efficiency and growth parameters [7]. The supplementation of phytase in feed has shown a significant effect on improving growth and feed efficiency in several fish species, including *Cyprinus carpio* [4], *Pangasius hypothalamus* [5], *Cyprinus carpio* [8], and *Piaractus mesopotamicus* [9]. Currently, there is no information available on the addition of phytase enzyme in the feed for milkfish fingerlings, making this study necessary. The aim of this research is to determine the effect of phytase enzyme supplementation on the growth performance, feed utilization efficiency, and survival rate of milkfish fingerlings.

2. MATERIALS AND METHODS

The research was conducted at the Brackishwater Aquaculture Development Center (BBPBAP) in Jepara, Central Java, Indonesia, using an experimental method with a Completely Randomized Design (CRD), consisting of 4 treatments and 3 replicates. The test fish used were milkfish fry obtained from milkfish farmers in Demak Regency, with an average weight of 2.89±0.46 g/fish and a stocking density of 1 fish/2 L. The fry selected for the experiment were uniform in size, healthy, active, and without deformities [8]. The fish were fasted for 1 day before treatment to eliminate metabolic waste.

The feed used was a formulated pellet with a 30% protein content [10], supplemented with phytase enzyme according to the treatments: A (0 mg/kg feed), B (250 mg/kg feed), C (500 mg/kg feed), and D (750 mg/kg feed). The phytase enzyme used was Natuphos E 10,000 G, produced by PT. BASF Indonesia. The feed formulation and proximate analysis of the experimental feed are shown in Table 1.

The experimental units were happas measuring 1 x 0.5 x 0.5 m^3, with 12 happas used in total. Feeding was done to satiation with a feeding frequency of three times a day at 08:00, 13:00, and 17:00 WIB. The rearing medium used was brackish water from the milkfish ponds at BBPBAP Jepara.

The study began with the weighing of the test fish to determine their initial weight. The fish were then stocked into the experimental units at a density of 1 fish/2 liters. Milkfish fry were reared for 42 days, with feeding done to satiation and at a frequency of 3 times daily at 08:00, 13:00, and 17:00 WIB. Sampling was conducted weekly to monitor weight gain. Water quality parameters, including temperature, pH, dissolved oxygen (DO), and salinity, were measured daily at 07:00 and 15:00 WIB. Ammonia was measured at the beginning, middle, and end of the study.

Table 1. Feed formulation and proximate analysis of experimental feed

Ingredient	Feed Composition (%/100 g Feed)			
	A	B	C	D
Fish meal	30.00	30.00	30.00	30.00
Soybean meal	21.50	21.50	21.50	21.50
Rice bran	16.00	16.00	16.00	16.00
Corn meal	22.00	21.75	21.50	21.25
Tapioca flour	6.50	6.50	6.50	6.50
Fish oil	1.00	1.00	1.00	1.00
Corn oil	1.00	1.00	1.00	1.00
Vit-Min mix	2.00	2.00	2.00	2.00
Phytase	0.00	0.25	0.50	0.75
Total	100.00	100.00	100.00	100.00
Proximate Analysis Results				
Protein (%)*	30.15	30.13	30.11	30.09
NFE (%)*	42.47	42.27	42.07	41.87
Fat (%)*	9.72	9.70	9.69	9.67
Energy (kkal/g)a	290.42	289.72	289.02	288.32
E/P Ratio	9.63	9.62	9.60	9.58

Note:

a) Calculated based on Digestible Energy according to NRC (2011), where 1 g of protein = 3.5 kcal, 1 g of fat = 8.1 kcal, and 1 g of carbohydrate = 2.5 kcal.

**Proximate analysis results from the Animal Nutrition Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University (2024)*

The variables observed in this study included total feed consumption (TFC), feed conversion ratio (FCR), feed utilization efficiency (FUE), protein efficiency ratio (PER), relative growth rate (RGR), and survival rate (SR), referring to NRC [11], all calculated based on the following formulas:

TFC = Initial feed amount (g) + Remaining feed (g)+…+ Feed amount on the nth day (g)

FCR) = Feed intake (g)/Body weight gain (g).
FUE (%) = Final weight – Initial Weight/ Feed consumed x 100

PER = 100 × (Final weight – Initial weight)/ Feed consumed x Protein content of feed

RGR (%) =100 × (Final weight - Initial weight)/(Experiment duration x Initial weight)

SR (%) = 100 x (Final fish count/Initial fish count)

Data analysis was performed using analysis of variance (ANOVA), and if significant effects were observed ($P<0.05$), Duncan's multiple range test was used to determine differences between treatments. The optimum dose of phytase was determined using orthogonal polynomial analysis [12]. Water quality data were analyzed descriptively.

3. RESULTS

Data on total feed consumption (TFC), feed conversion ratio (FCR), feed utilization efficiency (FUE), protein efficiency ratio (PER), relative growth rate (RGR), and survival rate (SR) of milkfish during the study are presented in Table 2.

The results in Table 2 show that milkfish fingerlings fed diets supplemented with phytase enzyme exhibited higher TFC, FCR, FUE, PER, and RGR values compared to those without phytase. Additionally, an increasing dose of phytase enzyme in the feed led to an increase in TFC, FCR, FUE, PER, and RGR values for milkfis juveniles. The addition of 500 mg/kg phytase (treatment C) was the most effective dose for milkfish fry, as it resulted in the highest TFC, FCR, FUE, PER, and RGR compared to other treatments.

The water quality measurements during the study indicated that the conditions were suitable for milkfish fry rearing, based on references regarding the optimal water quality for milkfish. The water quality parameters are presented in Table 3.

Table 2. Mean values of total feed consumption (TFC), feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER), relative growth rate (RGR), and survival rate (SR) of milkfish during the study

Variable	Treatment			
	A	B	C	D
TFC (g)	104,23±4,26	121,58±3,87	149,39±2,96	127,96±5,49
FCR	1,94±0,05	1,59±0,08	1,13±0,05	1,24±0,01
FUE (%)	45,58±2,30	56,77±2,29	83,72±3,18	75,01±1,36
PER	1,52±0,08	1,89±0,08	2,79±0,11	2,50±0,05
RGR (%/hari)	1,94±0,05	2,99±0,09	5,03±0,23	3,89±0,17
SR (%)	76,67±2,89	81,67±2,89	85,00±5,00	83,33±2,89

Note: Different superscripts indicate significant differences (P<0.05) between treatments.

Table 3. Water quality parameters measured during the study

Water Quality Parameter	Range		Standard (SNI 01.6148.1999.)
	Morning	Afternoon	
Dissolved oxygen(mg/L)	3,82	3,82	3
Temperature (oC)	28	30	28-32
pH	7,71	7,74	7,0-8,5
Salinity (ppt)	29	29	5-35
Ammonia (mg/L)	< 0,018		<0,02

4. DISCUSSION

The results of this study indicate that the addition of phytase enzyme in the feed had a significant effect (P<0.05) on the total feed intake (TFC) of milkfish juveniles. The increase in feed consumption suggests that the palatability of the feed was improved [13]. The highest TFC value was observed in treatment C (500 mg/kg of feed phytase enzyme) at 149.39±2.96 g, while the lowest TFC was found in treatment A (0 mg/kg of feed phytase enzyme) at 104.23±4.26 g. This is likely due to the increased feed palatability in treatment C, leading to higher feed consumption. Salem et al. [14] reported that the addition of phytase enzyme in feed can improve palatability by breaking down the phytate-protein-mineral complex, thereby increasing feed consumption. The Orthogonal Polynomial test resulted in a quadratic equation ($Y = -155.11x^2 + 155.93x + 101.25$) and $R^2 = 0.793$ (Fig. 1). The R^2 value of 0.79 indicates that 79.29% of the TFC is influenced by the addition of phytase enzyme, while the remaining 20.71% is affected by other factors. The optimal dose of phytase enzyme for TFC is 502 mg/kg feed, yielding a maximum TFC of 140.43 g.

Feed conversion ratio (FCR) is related to the efficiency of feed utilization, where optimal feed utilization results in a better FCR [11]. The addition of phytase enzyme in the feed significantly affected (P<0.05) the FCR of milkfish juveniles. This is likely due to phytase's ability to hydrolyze phytic acid, thereby improving feed utilization and reducing FCR. The best FCR value was obtained in treatment C (500 mg/kg phytase enzyme) at 1.13±0.05, likely because 500 mg/kg of phytase is the optimal dose to break down phytic acid, increasing nutrient absorption and resulting in a lower FCR. This study showed that feed with added phytase enzyme yields better FCR compared to feed without it. Yang et al. [15] stated that a low FCR is due to phytase's ability to break down phytic acid, which otherwise inhibits nutrient absorption. Rachmawati and Istiyanto [5] also reported that the addition of phytase in feed can lower FCR and increase feed efficiency. Similar results have been reported in various fish species, including *Carassius auratus* [4], *Sparus aurata* [14], *Clarias gariepinus* var Sangkuriang [10], and *Penaeus monodon* [16]. The Orthogonal Polynomial test resulted in a quadratic equation ($Y = 1.84x^2 - 2.404x + 1.974$) and $R^2 = 0.9252$ (Fig. 2). The R^2 value indicates that 92.52% of the FCR is influenced by the addition of phytase enzyme, while 7.48% is affected by other factors. The optimal phytase enzyme dose for FCR is 650 mg/kg of feed, yielding a maximum FCR of 1.18.

The study also revealed that the addition of phytase enzyme in the feed had a significant

effect (P<0.05) on the feed utilization efficiency (FUE) of milkfish juveniles. The highest FUE was observed in treatment C (500 mg/kg phytase enzyme) at 83.72±3.18%, while the lowest FE was in treatment A (0 mg/kg phytase enzyme) at 45.58±2.30%. This is likely because treatment C (500 mg/kg phytase enzyme) provided the appropriate dose to hydrolyze the phytic acid contained in plant-based ingredients, thereby enhancing feed absorption and utilization. Phytase can improve protein digestibility in fish, leading to better nutrient absorption and feed efficiency compared to treatments without phytase [17]. Maas et al. (2020) stated that phytase in feed can hydrolyze phytic acid, improving protein digestibility, which can then be efficiently utilized by fish for growth. Similar findings were reported in several fish species, including *Cirrhinus mrigala* (Naz et al., 2019), *Acipenser baerii* [18], and *Sparus aurata* (Morales et al., 2017). The Orthogonal Polynomial test resulted in a quadratic equation ($Y = -79.573x^2 + 105.78x + 43.011$) and $R^2 = 0.8382$ (Fig. 3). The R^2 value indicates that 83.82% of FUE is influenced by the addition of phytase enzyme in the feed, while 16.18% is affected by other factors. The optimal phytase enzyme dose for FUE is 664 mg/kg feed, yielding a maximum FUE of 78.16%.

Protein efficiency ratio (PER) is an indicator of the amount of protein absorbed by the fish and used for growth [19]. The study showed that the addition of phytase enzyme in the feed significantly affected (P<0.05) the PER of milkfish juveniles. The highest PER was found in treatment C (500 mg/kg phytase enzyme) at 2.79±0.11, while the lowest PER was observed in treatment A (0 mg/kg phytase enzyme) at 1.52±0.08. This is likely due to the breakdown of anti-nutritional factors such as phytic acid by the phytase enzyme. Joudaki et al. [20] reported that phytase enzyme in the feed can break down phytic acid and separate its complex bonds with protein and minerals, thus enhancing the activity of digestive enzymes responsible for breaking down protein into its amino acids. This allows for optimal protein absorption and utilization for growth, thus improving the PER. The addition of phytase enzyme improved protein digestibility, which in turn enhanced nutrient digestibility and PER [21]. Similar findings were reported in various fish species, including *Oreochromis niloticus* [17], *Psetta maxima* [22], *Cyprinus carpio* [8], and *Sciaenops ocellatus* [23]. The Orthogonal Polynomial test resulted in a quadratic equation ($Y = -2.6533x^2 + 3.53x + 1.4342$) and $R^2 = 0.8379$ (Fig. 4). The R^2 value indicates that 83.79% of PER is influenced by the addition of phytase enzyme in the feed, while 16.21% is affected by other factors. The optimal phytase enzyme dose for PER is 665 mg/kg feed, yielding a maximum PER of 2.6.

The addition of phytase enzyme to the feed had a significant effect (P<0.05) on the relative growth rate (RGR) of milkfish (*Chanos chanos*) fingerlings. The highest RGR was observed in treatment C (500 mg/kg feed of phytase enzyme) at 5.03±0.23%/day, while the lowest value was found in treatment A (0 mg/kg feed of phytase enzyme) at 1.94±0.05%/day. The highest RGR in treatment C (500 mg/kg feed of phytase enzyme) was attributed to the highest values of protein efficiency ratio (PER), feed utilization efficiency (FUE), and feed conversion ratio (FCR) compared to other test feeds. According to Kumar et al. (2018), the addition of phytase enzyme to feed can enhance feed utilization efficiency, thus promoting growth. Maas et al. [24] further explained that phytase enzyme can hydrolyze complex phytate-protein compounds into amino acids that are easily digested for fish growth. Hussain et al. [25] also stated that phytate in feed hydrolyzed by phytase enzyme made the feed more easily absorbed by fish, resulting in better growth. In contrast, treatment A (0 mg/kg feed of phytase enzyme) showed the lowest RGR, presumably because the phytate in the feed was not hydrolyzed, which inhibited the growth of milkfish fingerlings. Phytate can reduce nutrient bioavailability, impair growth performance and metabolism in fish, and increase nitrogen and other nutrient excretions into the aquatic environment [26]. Similar results were reported by Adeshina et al. [27] on *Clarias gariepinus*, Rachmawati et al. [8] on *Cyprinus carpio*, and Nie et al. [28] on *Carassius auratus*. Orthogonal polynomial analysis resulted in a quadratic equation ($Y = -8.7467x^2 + 9.72x + 1.7317$) and $R^2 = 0.8229$ (Fig. 5). The R^2 value indicates that 82.29% of the RGR is influenced by the addition of phytase enzyme to the feed, while the remaining 17.71% is affected by other factors. The optimum dose of phytase enzyme for RGR was found to be 555.6 mg/kg feed, producing a maximum RGR of 4.43%/day.

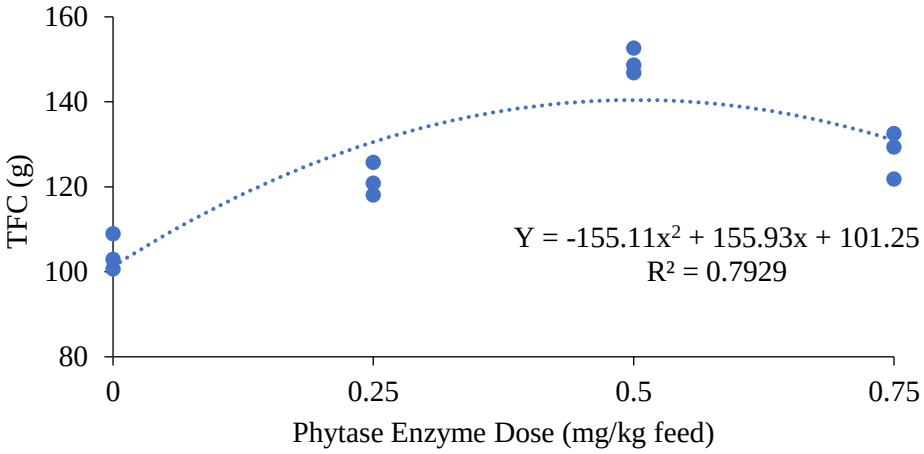

Fig. 1. Orthogonal polynomial graph of TFC in milkfish juveniles

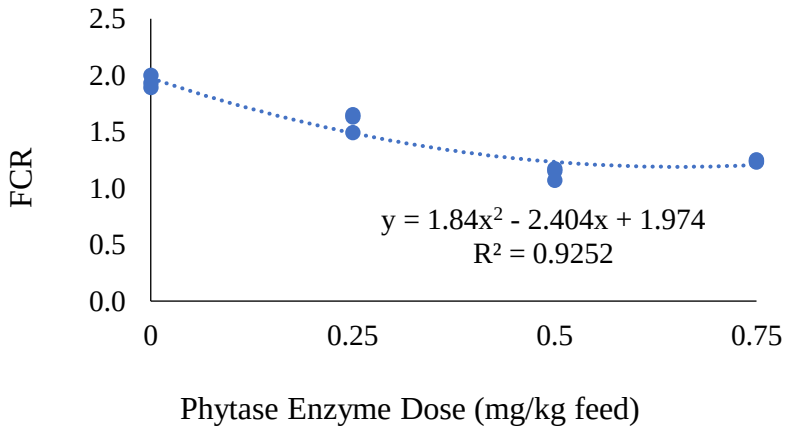

Fig. 2. Orthogonal polynomial graph of FCR in milkfish juveniles

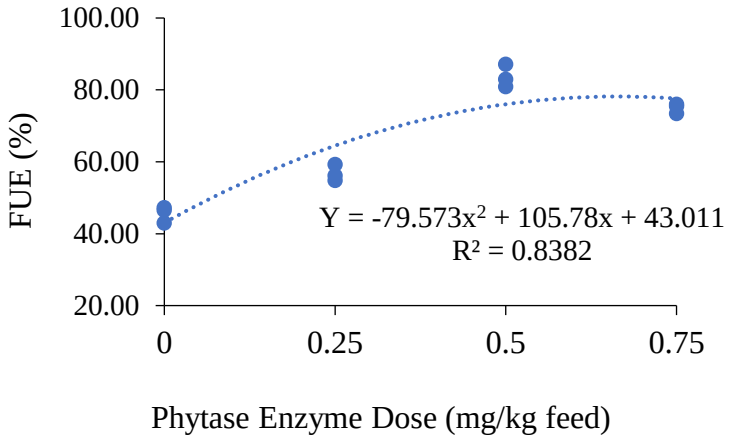

Fig. 3. Orthogonal polynomial graph of FUE in milkfish juveniles

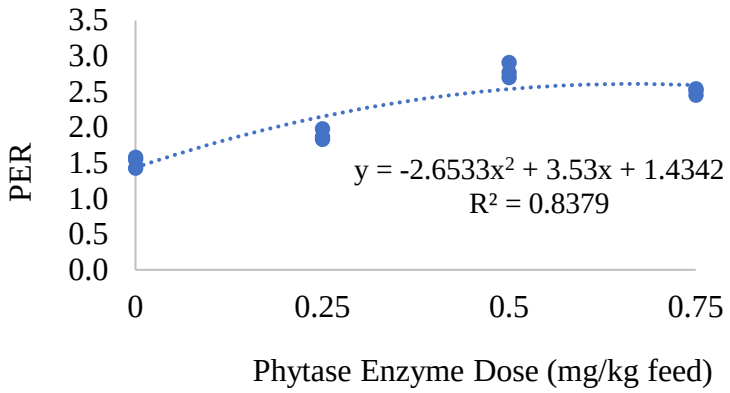

Fig. 4. Orthogonal polynomial graph of PER in milkfish juveniles

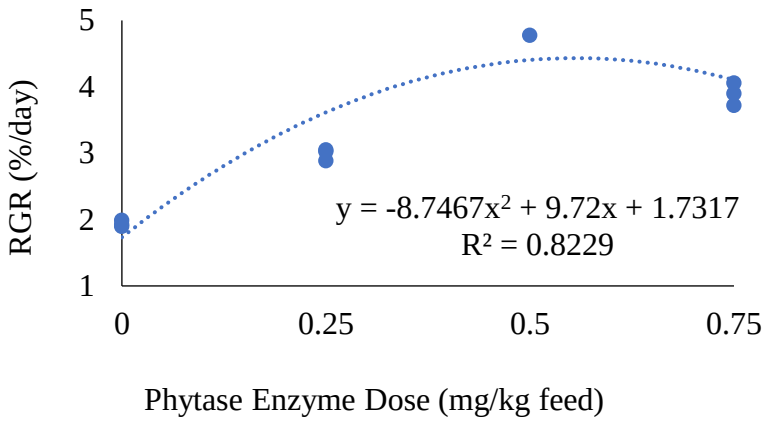

Fig. 5. Orthogonal polynomial graph of RGR in milkfish fingerlings

The addition of phytase enzyme to the feed did not significantly affect (P>0.05) the survival rate of milkfish fingerlings. The results (Table 2) show that the highest survival rate was achieved in fish fed test feed C (500 mg/kg phytase enzyme) at 85.00 ± 5.00%, followed by treatment D (750 mg/kg phytase enzyme) at 83.33 ± 2.89%, treatment B (250 mg/kg phytase enzyme) at 81.67 ± 2.89%, and treatment A (0 mg/kg phytase enzyme) at 76.67 ± 2.89%. These results indicate that the survival rate of milkfish fingerlings ranged from 76% to 85%, which is categorized as good. Pereira et al. [29] stated that a fish survival rate above 50% is considered good, a survival rate below 30% is poor, and a survival rate between 30-50% is considered moderate. The high survival rate was influenced by the environmental conditions during the maintenance period [30]. Observations of water quality parameters (Table 3), including dissolved oxygen, temperature, pH, salinity, and ammonia, showed favorable values for the growth and survival of milkfish fingerlings, as they were within the optimal range according to SNI 01.6148.1999 [31] (water quality standards for milkfish maintenance), thus ensuring suitable water quality for rearing milkfish [32,33].

5. CONCLUSION

This study concludes that the addition of phytase enzyme to feed had a significant effect (P<0.05) on the growth performance and feed utilization efficiency but did not significantly affect (P>0.05) the survival rate of milkfish fingerlings. The optimum dose of phytase enzyme in feed for growth performance and feed utilization efficiency ranged from 502 to 665 mg/kg feed, yielding maximum values for total feed consumption (TFC), feed conversion ratio (FCR), feed utilization efficiency (FUE), protein efficiency ratio (PER), and relative growth rate (RGR) of 140.43 g; 1.18; 78.16%; 2.6; and 4.43%/day, respectively.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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