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Evaluation of *β-D-Mannanase* in Modulating Dietary Fiber Breakdown, Growth Efficiency, and Carcass Composition in Hatchling Snails (*Archachatina marginata*): Insights for Animal Biotechnology and Health

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Abstract

African Giant Land Snails (AGLS) are a good source of protein with low-fat content but with slow growth rate which may be due to genetic and nutritional factors. Convectional high fiber feed resources fed to AGLS have a marginal nutrient profile. Growth enhancers have been used to optimise productivity in other animals but reports on their use in AGLS nutrition are scanty. Therefore, the effects of diets supplemented with enzymes on performance of AGLS were investigated in this study. Hatching AGLS (n = 300), weighing 25.60 ± 0.39 g were randomly allotted to four diets containing 0.00 (T_1, control), 0.10 (T_2), 0.15 (T_3) and 0.20/kg (T_4) *β-D-mannanase* in five replicates for 98 days. Using standard procedures, Feed Intake (FI), mean weekly Body Weight Gain (BWG), Feed Conversion Ratio (FCR) and carcass characteristics were determined. Levels of*β-D-mannanase* did not significantly affect FI, but total BWG increased with increasing levels of *β-D-mannanase*. The FCR was not affected by increasing dosage of *β-D-mannanase*. Carcass traits were significantly influenced by the treatments. The feed cost increased with increase in the level of enzyme inclusion ($p < 0.05$), but the cost/weight gain decreased with increasing dosage of the enzyme, with the lowest cost/weight gain observed in 0.15 g *β-D-mannanase* supplementation.

Keywords

β-D-mannanase Performance, Carcass Yield, *Archachatina marginata*,

Hatchling Snails

1. Introduction

Snails are invertebrates with bodies covered by hard calcareous shells and are common animals worldwide. They belong to the phylum Mollusca (soft-bodied animals) and are monogastric, with a digestive system similar to that of poultry but less developed. Their digestive system is adapted to suit a wide variety of diets and feeding behaviours. Snail meat is highly cherished as a delicacy in many parts of the world and serves as an important protein source for rural dwellers in the West African forest regions, particularly in Nigeria and Ghana. If snail farming is practiced under ideal conditions, it holds great potential for meeting the urgent demand for animal protein in developing countries while also offering economic benefits. However, a major challenge in snail rearing is its relatively low growth performance compared to other livestock, necessitating the exploration of strategies to enhance growth and production to meet consumer demand.

Following the ban on the use of antibiotics as growth promoters in animal nutrition in many countries [1], several alternatives have been explored, including exogenous enzymes, prebiotics, probiotics, and organic acids. In-feed enzymes may be administered in monogastric nutrition either to enhance digestibility or as process aids for the removal or conversion of undesirable components in feed. Enzymes are specific protein molecules that act as biological catalysts, controlling the rate of chemical and biochemical reactions without forming part of the end products. They exhibit substrate specificity and facilitate the utilization and conversion of various feed ingredients to improve growth performance and feed efficiency in animals. Monogastric animals, including swine, poultry, and snails, lack the enzymes necessary to degrade β-mannans or β-galactomannans. Mannans, found in soybean meal, are linear polysaccharides with repeating units of β-1,4-mannose and β-1,6-glucose or galactose side chains [2]. They are highly viscous, water-soluble, and heat-stable compounds [3], and are regarded as potent anti-nutritional factors for monogastric animals [4]. In broiler chickens, β-mannans increase digesta viscosity and decrease nutrient digestibility [5]. They can also stimulate the innate immune response, diverting energy away from growth [6], and depress glucose absorption and insulin secretion [7].

β-D-mannanase, a fermentation product of *Bacillus lentus*, breaks down β-mannans in broiler feeds. The enzyme cleaves randomly within the β-1,4-D-mannan backbone of galactomannans, galactoglucomannans, and mannans [8]. While degrading galactomannan polymers, *β-D-mannanase* does not form residues after the reaction. Studies have shown that including 2-4% β-mannans in broiler feed can retard growth, worsen feed conversion ratio (FCR), and reduce feed efficiency [9]. Conversely, *β-D-mannanase* supplementation improves feed-to-gain ratio, reduces water-to-feed ratio, and results in drier faecal output [6]. Supplementa-

tion in broiler diets also decreases intestinal viscosity and enhances growth and feed efficiency [10]. In corn-soybean-based diets, *β-D-mannanase* supplementation has been reported to improve both growth and feed efficiency by approximately 3% [11].

The aim of the present study was to investigate the effectiveness of *β-D-mannanase* supplementation in diets fed to hatchling African Giant Land Snails (*Archachatina marginata*) in improving feed consumption, growth performance, and carcass characteristics.

2. Materials and Methods

The experiment was conducted at the Mini-Zoo Unit of the Department of Wildlife and Ecotourism Management, University of Ibadan, Nigeria. Snails were reared under an intensive system using cages measuring 50 × 45 × 45 cm (length × width × height). The cages were covered with wire mesh and wood on all sides. Each cage contained five hutches, with floors lined with woven polypropylene layers over wire mesh and filled with loamy soil (8 - 10 cm thick) as bedding. All snails were maintained under similar hygienic and management conditions throughout the study.

A total of 300 hatchling A. marginata snails, with an initial average weight of 25.60 ± 0.39 g, were randomly allocated to four dietary treatments in a completely randomized design. Treatments were as follows: T1 (control): 0.00 g/kg *β-D-mannanase*; T2: 0.10 g/kg *β-D-mannanase*; T3: 0.15 g/kg *β-D-mannanase*; and T4: 0.20 g/kg *β-D-mannanase*. Each treatment had five replicate groups. Prior to the experiment, snails were acclimatized for one week and fed pawpaw leaves. They were given ad libitum access to diets and water, and the environment was kept moist throughout the study. The composition of the basal diets is shown in **Table 1**. Parameters measured included daily feed intake, weekly body weight gain, FCR, carcass characteristics, mortality, and cost analysis. Proximate analyses of the experimental diets and snail excreta were conducted according to AOAC [12] at the Biochemical Laboratory, Department of Animal Science, University of Ibadan. Data were subjected to analysis of variance (ANOVA) using SAS software [13], and treatment means were separated using Duncan's multiple range test at $p < 0.05$.

Table 1. Gross composition of experimental diet fed to snails.

Ingredients (%)	Hatchling
Maize	9.00
Brewers\Dry Grain	29.00
Wheat offal	10.00
Soyabean meal	33.00
Palm kernel cake	10.00

Continued

Fish meal	3.00
Oyster shell	4.45
Dicalcium phosphate	1.00
Salt	0.10
Premix	0.25
L-lysine	0.10
DL-Methionine	0.10
TOTAL	100.00
Calculated Analysis	
Metabolizable energy (kcal/kg)	2367.37
Crude protein (%)	26.11
Calcium (%)	4.45
Phosphorus (%)	0.64

Premix Composition: Vitamin A 10,000 IU, Vitamin D_3: 200,000 IU, Vitamin K_3, 20,000 mg, Vitamin B, 30,000 mg, Vitamin B_2, 50,000 mg, Niacin 4500 mg Calcium Pantothenale 10,000 mg, Vitamin B_6, 40,000 mg, Vitamin B_{12}, 20 mg, Chlorine Chloride 300,000 mg, Biotin, 100 mg, Manganese, 50 mg, Iron, 300,000 mg, Zinc, 120,000 mg, Copper 80,000 mg, Iodine, 15,000 mg, Cobalt, 300 mg, Selenium 130 mg, Antioxidant 120,000 mg, Metabolisable Energy (Kcal/kg) was obtained from prediction equation [(Pauzenga, 1985) ME (kca/kg = 37 x % crude protein + 818 x Ether extract + 35.5 x % Nitrogen – extract)].

3. Results

Table 2 showed the basal proximate composition of the experimental diets fed to snail hatchlings. The basal diet consists of 26.28 ± 0.11 crude protein, 10.48 ± 1.16 crude fibre, 4.29 ± 0.02 Ether extract, 9.84 ± 0.02 Ash and 49.11 ± 1.00 Nitrogen free extract for hatchlings and 24.24 ± 0.04 crude protein, 10.44 ± 0.04 crude fibre 4.77 ± 1.46 Ether extract, 10.52 ± 0.01 Ash and 50.03 ± 0.02 Nitrogen free extract for the grower AGLS.

Table 2. Basal Proximate composition of the experimental diets fed to hatchling snails.

Parameters	Composition (%)
Dry matter	92.83 ± 1.03
Crude protein	26.28 ± 0. 11
Crude Fibre	10.48 ± 1.16
Ether extract	4.29 ± 0.02
Ash	9.84 ± 0.02
Nitrogen free extract	49.11 ± 1.00

The results of performance characteristics of African giant land snail (AGLS) hatchlings fed diets supplemented with varying inclusion levels of fibre degrading enzymes, (*β-D-mannanase*) are shown in **Table 3**. No significant ($p > 0.05$) effect of *β-D-mannanase* dosage was observed on Feed Intake (FI) of AGLS hatchlings. A contrary trend was observed in Final Weight (FW), and Total Weight Gain (TWG) with significant increase with respect to increasing dosage of *β-D-mannanase*. No significant effect was observed for feed conversion ratio (FCR) of AGLS hatchlings fed diets supplemented with *β-D-mannanase*. Increasing dosage of *β-D-mannanase* significantly ($p < 0.05$) increased FW and TWG in a linear fashion. Moreover, the shell dimensions; shell length, and shell width were not ($p > 0.05$) affected by *β-D-mannanase* dosage. The results of the cost analysis revealed that there was a significant ($p < 0.05$) difference in the cost/g feed of the snails fed experimental diets with increasing enzymes inclusion. However, the cost/ body weight gain decreased as the levels of *β-D-mannanase* inclusion in the diets increased, demonstrating an inverse relationship. The lowest cost/body weight gain was obtained in the diet containing 0.15 g *β-D-mannanase* /kg feed (H_3). Based on the cost/body weight gain record therefore, it would be expedient to advise snail farmers to go for diets with inclusion of 0.15 g *β-D-mannanase* /kg feed to accelerate the growth of snails at this phase of growth.

Table 3. Growth performance of African giant land snail hatchlings (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of *β-D-mannanase*.

PARAMETERS	T1 BD + 0.00 g/kg feed	T2 BD + 0.01 g/kg feed	T3 BD + 0.15 g/kg feed	T4 BD + 0.20 g/kg feed	SEM
Total feed intake (g/day/snail)	343.22	344.70	345.30	347.30	7.20
Initial weight (g)	26.01	25.90	25.03	25.46	3.90
Final weight (g)	100.14[b]	103.71[b]	108.84[a]	109.58[a]	5.60
Total weight gain (g/snail)	74.13[c]	77.81[b]	83.81[a]	84.12[a]	4.50
Feed conversion ratio	4.63[a]	4.43[b]	4.12[c]	4.13[c]	0.02
Total shell length (mm)	19.13	19.21	19.28	19.31	2.30
Total shell width (mm)	16.29	16.31	16.33	16.38	2.10
Cost/g feed (N)	0.05[b]	0.05[ab]	0.06[a]	0.07[a]	1.50
Cost/g body weight gain (N/g)	82.13[a]	80.27[a]	78.12[d]	79.48[c]	3.90

[a,b,c]are the averages with diverse superscripts along the similar row which are significantly different ($p < 0.05$) BD—Basal diet; SEM—Standard error of means.

Figure 1 shows the relationship between varying inclusion levels of *β-D-mannanase* and feed conversion ratio of the hatchling snails. It was observed that a negative linear relationship exists between *β-D-mannanase* and FCR of AGLS hatchlings. The R^2 value (0.91) represents the proportion of variance in the dependent variable (FCR) that is predictable from the independent variable (enzyme dosage). A unit increase in *β-D-mannanase* dosage will result in a 1.8 decrease in FCR of AGLS hatchlings. The intercept is set at 4.64 when the *β-D-mannanase* value is at zero.

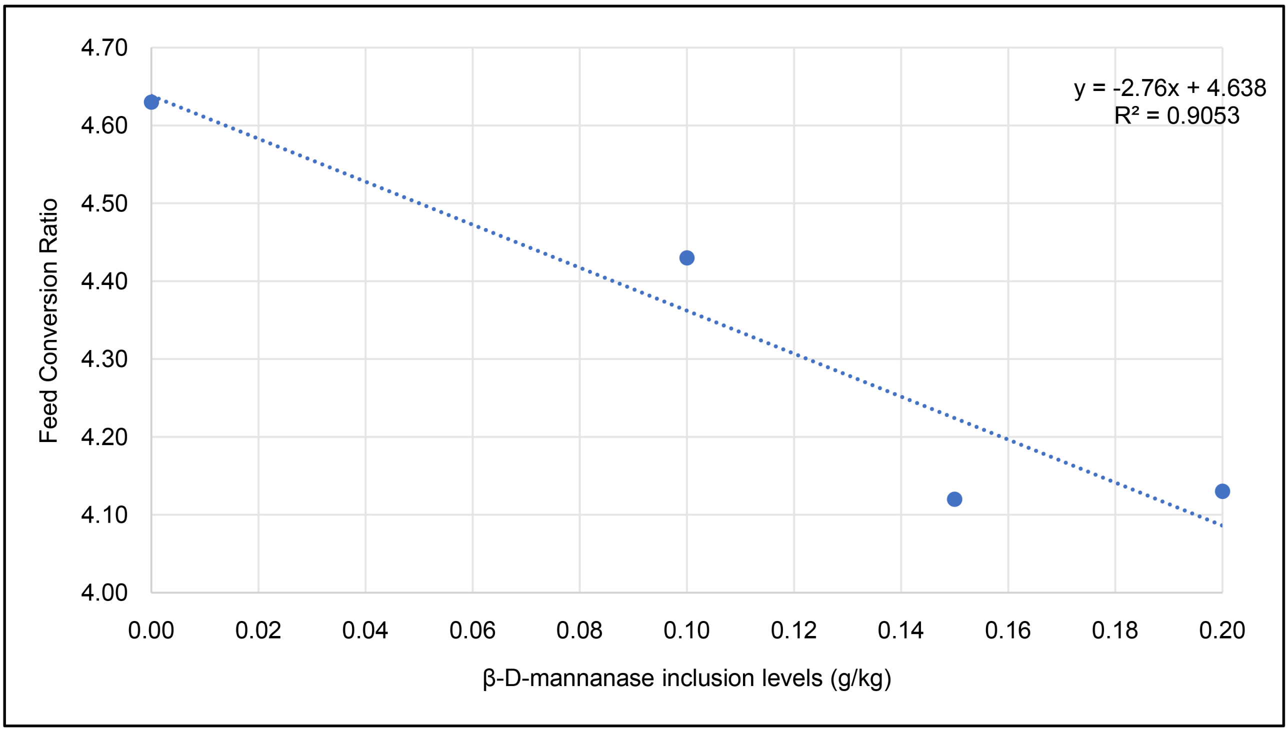

Figure 1. Relationship between varying inclusion levels of *β-D-mannanase* and feed conversion ratio of hatchling snails.

The relationship between varying inclusion levels of *β-D-mannanase* and the Body Weight Gain (BWG) of AGLS hatchlings is shown in **Figure 2**. A positive linear relationship exists between *β-D-mannanase* and the BWG of AGLS hatchlings. Increasing level of *β-D-mannanase* resulted in a corresponding increase in the body weight gain of AGLS hatchlings. The R^2 value (0.92) represents the proportion of variance in the dependent variable (BWG) that is predictable from the independent variable (enzyme dosage). A unit increase in *β-D-mannanase* will result in a 128.29 g weight gain.

The results of carcass yield of AGLS hatchlings fed diets supplemented with varying inclusion levels of *β-D-mannanase* are summarized in **Table 4**. The carcass yield differed significantly ($p < 0.05$) across the treatment groups. Increasing levels *β-D-mannanase* resulted in a corresponding increase in live weight of the AGLS hatchings. A similar trend was observed on the foot (most edible portion) weight. The foot weight increased as the level of *β-D-mannanase* dosage increased. The highest value for foot weight (53.84 g) was recorded for treatment T_4 with 0.20/kg feed *β-D-mannanase*. The dressing percentage increased as the level of *β-D-mannanase* inclusion increased.

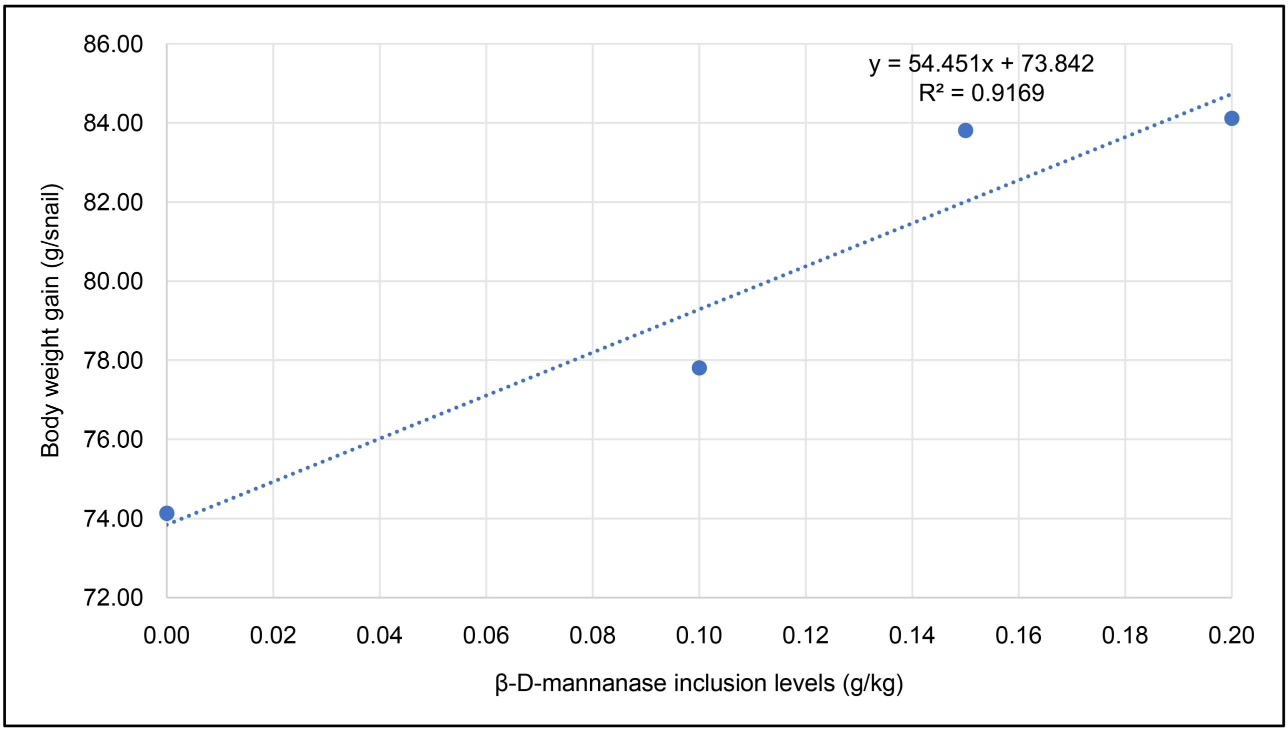

Figure 2. Relationship between varying inclusion levels of *β-D-mannanase* and body weight gain of hatchling snails.

Table 4. Carcass characteristics of African giant land snail hatchlings fed diets supplemented with varying inclusion levels of *β*-D-*mannanase*.

Parameters	T1	T2	T3	T4	SEM	P value
Live weight (g)	99.84^{d}	102.70^{c}	108.30^{b}	108.79^{a}	0.01	<0.0001
Foot weight (%)	50.15^{d}	51.33^{c}	53.45^{b}	53.84^{a}	0.01	<0.0001
Shell weight (%)	20.05^{b}	20.51^{a}	19.95^{c}	19.93^{c}	0.01	<0.0001
Offal weight (%)	26.22^{c}	24.88^{d}	27.30^{b}	27.56^{a}	0.01	<0.0001
Shell: live weight	0.20^{b}	1.20^{a}	0.18^{b}	0.18^{b}	0.01	0.0003
Offal: live weight	0.26^{a}	0.24^{c}	0.25^{b}	0.25^{b}	0.09	0.0003
Visceral weight (%)	29.80^{a}	28.16^{b}	26.59^{c}	26.23^{d}	0.01	<0.0001
Dressing percentage	50.23^{a}	50.16^{b}	49.35^{d}	49.46^{c}	0.01	<0.0001

^{abcd}Averages of treatments along a row with different superscript differed significantly ($p < 0.05$). T1—0.00 g/kg, T2—0.10 g/kg, T3—0.15 g/kg, T4—0.20 g/kg, *β*-D-*mannanase* SEM—Standard error of means, P value—probability.

Figure 3 shows the relationship between varying inclusion levels of *β-D-mannanase* and live weight of AGLS hatchlings fed diets supplemented with *β-D-mannanase.* A positive linear relationship exists between *β-D-mannanase* and the live weight of the hatchling snails. The intercept is set at 99.43 when the *β-D-mannanase dosage* was zero increasing levels of *β-D-mannanase* resulted in a corresponding increase in live weight of AGLS hatchlings. The R^2 value (0.91) represents the proportion of variance in the dependent variable (Live weight) that is predictable from the independent variable (enzyme dosage). A unit increase in *β-D-mannanase* will result in 148.10 g live weight of AGLS hatchlings.

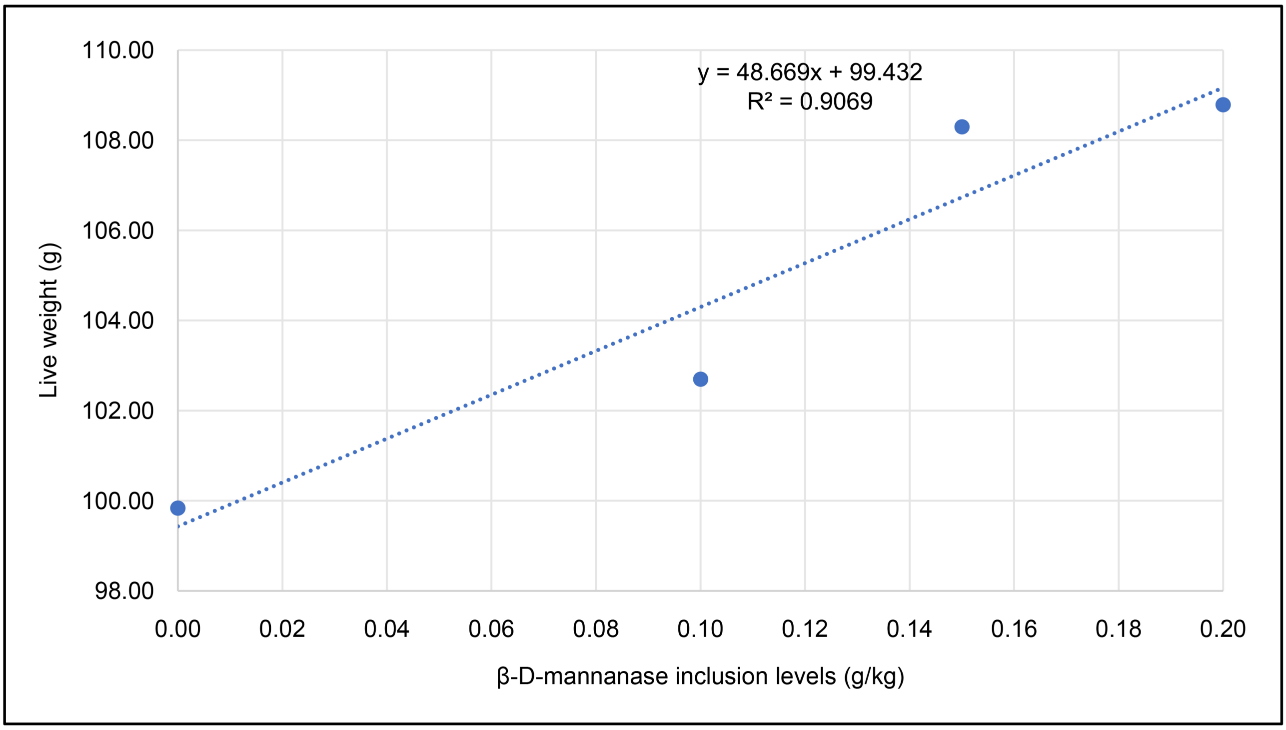

Figure 3. Relationship between varying inclusion levels of *β-D-mannanase* and live weight of hatchling snail.

4. Discussion

Regarding the growth performance characteristics of AGLS hatchlings fed *β-D-mannanase* supplemented diets, no significant effect of *β-D-mannanase* was observed on feed intake of AGLS hatchlings. A contrary trend was observed in final body weight (FBW), and total body weight gain (TBWG). Increasing dosage of *β-mannanase* significantly increased FBW and TBWG in a linear fashion, while improving FCR from 4.63 (T_1) to 4.13 (T_4). This result agrees with the report of [11], who evaluated the effects of *β-D-mannanase* addition to diets of weanling pigs on growth performance and found improved feed:gain ratio compared with pigs fed diets without *β-D-mannanase*. Observations from the current study suggest that *β*-D-mannanase improved growth performance and enhanced efficiency of converting diets to weight gain in AGLS hatchlings. Similarly, [1] reported that pigs fed corn-SBM diets with *β-D-mannanase* showed enhanced feed conversion ratios. [1] also found that dietary *β-D-mannanase* supplementation improved performance of broilers fed corn-soya bean meal during the growing period, although feed intake was affected.

The shell dimensions—shell length and shell width—were unaffected by *β-D-mannanase* addition. Since shell development in snails depends mainly on calcium levels, this result may be attributed to calcium deficiency in the diets. On cost analysis, there was a significant increase in cost per gram of feed of snails fed experimental diets with increasing enzyme inclusion, implying higher production costs and eventual rise in market price. However, cost per gram of weight gain decreased with higher *β-D-mannanase* dosage, showing an inverse relationship. The lowest cost per weight gain was recorded at 0.15 g *β-D-mannanase*/kg feed. Therefore, it is advisable for snail farmers to use diets containing 0.15 g *β-D-mannanase*/kg feed to accelerate growth of AGLS hatchlings.

Carcass characteristics of AGLS hatchlings fed *β-D-mannanase* supplemented diets were positively influenced. Live width and foot weight (the main edible portion) increased with increasing *β-D-mannanase* dosage. Dressing percentage showed similar trends, with treatments T_3 (50.16) and T_4 (50.23) statistically similar. These observations may be attributed to the beneficial effects of *β-D-mannanase* inclusion [14]. The positive performance exhibited by AGLS hatchlings indicates that the fibre-degrading enzyme *β-D-mannanase* is effective at the hatchling phase and could be used to enhance snail hatchling growth.

The non-significant effect of *β-D-mannanase* dosage on feed intake, while improving body weight gain could be due to the exogenous *β-D-mannanase* degrading *β-D-mannans*, galactomannans, and other fibres present in diets, preventing or suppressing viscous digesta formation and allowing better nutrient utilization. The findings align with [15], who found that *D-mannanase* reduced intestinal digesta viscosity, improving feed efficiency and growth in grower AGLS fed guar meal diets. [16] reported that exogenous enzyme addition reduces intestinal digesta viscosity, increasing nutrient uptake and animal performance. According to [17], improved performance of monogastrics fed NSP-rich diets supplemented with exogenous enzymes like *β-D-mannanase* results not only from the release of simple sugars but also from prevention of viscous digesta formation, reducing viscosity and improving nutrient uptake and animal performance. [18] noted that adding *β-D-mannanase* to poultry diets high in *β*-mannans could increase beneficial intestinal bacteria, improve mannan digestibility, boost immunity, and slow harmful bacterial growth, thus improving nutrient absorption. These findings are comparable to those of [11] and [1]. Shell length and width in grower AGLS were not significantly affected, and this is likely due to dependence on calcium and phosphorus availability.

Carcass traits were positively influenced by *β-D-mannanase*. Over the 98-day experimental period, live weight and foot weight increased with enzyme dosage, as did dressing percentage, demonstrating *β-D-mannanase*'s potential beneficial effects on growth performance, health, and nutrient utilization. Supplementing exogenous enzymes is therefore a useful strategy to mitigate anti-nutritive effects of viscous diets [19] [20].

5. Conclusion

The addition of the fibre-degrading enzyme *β-D-mannanase* to diets of snails, enhances performance and productivity by improving feed efficiency and health. It degrades galactomannans, mannans, and other fibres in corn-soybean meal diets while reducing digesta viscosity. Dietary *β-D-mannanase* supplementation improved performance and health status of hatchling AGLS fed corn-SBM based diets. The optimal dosage was found to be 0.15 g *β-D-mannanase* per kg feed.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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