

Evaluation of Feed Particle Size and Multienzyme Supplementation on Ileal Digesta Viscosity in Broiler Chickens: A Multiple Regression Approach

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Abstract

Feed particle size influences digesta passage rate in broiler chickens, affecting ileal digesta viscosity. Measuring the viscosity of the ileal digesta is laborious but could be simplified with a model. This study evaluated the impact of feed particle sizes and multienzyme supplementation on ileal digesta viscosity in broiler chickens and developed an equation to estimate the ileal digesta viscosity using feed particle size and multienzyme supplementation as predictors. A total of 450 unsexed one-day-old Cobb500 broiler chicks were assigned to nine treatments with three particle sizes (3, 4, and 5 mm) and three levels of multienzyme supplementation (0, 1, and 2 g/kg), with five replicates per treatment, each containing ten birds. Data obtained were subjected to analysis of variance (ANOVA). Pearson correlation (r) between ileal viscosity, particle size, and multienzyme supplementation was performed to determine suitability for multiple linear regression, considering moderate correlation coefficients ($r < 0.8$) to avoid collinearity. Four models were generated using multiple linear regression. The best fit was determined by R^2 values, r , and p -values ($p \leq 0.05$). The model $Y = 0.2734 P + 0.0623 E$ was identified as the best fit, where Y is ileal digesta viscosity, P is particle size, and E is multienzyme supplementation level. In conclusion, larger feed particle sizes decrease ileal digesta viscosity. The derived equation effectively estimates ileal digesta viscosity in broiler chickens fed maize-based diets, using feed particle size and multienzyme supplementation as predictors.

Keywords: Broiler chicken, ileal digesta viscosity, linear regression, particle size, prediction model

Running title: Prediction equation for ileal digesta viscosity in broiler chickens



Évaluation de la taille des particules d'aliment et de la supplémentation multi-enzymatique sur la viscosité du digesta iléal chez les poulets de chair : Une approche par régression linéaire multiple

Résumé

La taille des particules d'aliment influence le taux de passage du digesta dans le tube digestif des poulets de chair, affectant ainsi la viscosité du digesta iléal. La mesure de cette viscosité est fastidieuse, mais un modèle mathématique pourrait simplifier son estimation. Cette étude a évalué l'impact de la taille des particules d'aliment et de la supplémentation multi-enzymatique sur la viscosité du digesta iléal chez les poulets de chair. Elle a également permis de développer une équation permettant d'estimer cette viscosité en utilisant la taille des particules et la supplémentation multi-enzymatique comme variables prédictives. Un total de 450 poussins d'un jour Cobb500 non sexés ont été répartis en neuf groupes expérimentaux recevant des aliments à trois tailles de particules (3, 4 et 5 mm) et supplémentés en multi-enzymes à trois

niveaux (0, 1 et 2 g/kg). Chaque groupe comprenait cinq réplicats de dix oiseaux. Les données obtenues ont été soumises à une analyse de variance (ANOVA). Une corrélation de Pearson (r) a été réalisée pour évaluer la relation entre la viscosité iléale, la taille des particules et la supplémentation multi-enzymatique. Cette analyse visait à garantir l'adéquation des variables à un modèle de régression linéaire multiple, en s'assurant de coefficients de corrélation modérés ($r < 0,8$) afin d'éviter la colinéarité.

Quatre modèles ont été générés par régression linéaire multiple. Le meilleur ajustement a été déterminé en fonction des valeurs de R^2 , du coefficient de corrélation (r) et de la valeur de p ($p = 0,05$). L'équation $Y = 0,2734 P + 0,0623 E$ a été identifiée comme le meilleur ajustement, où Y représente la viscosité du digesta iléal, P la taille des particules et E le niveau de supplémentation multi-enzymatique.

En conclusion, des particules d'aliment de plus grande taille réduisent la viscosité du digesta iléal. L'équation dérivée permet d'estimer efficacement la viscosité du digesta iléal chez les poulets de chair nourris avec des régimes à base de maïs, en utilisant la taille des particules d'aliment et la supplémentation multi-enzymatique comme facteurs prédictifs.

Mots-clés : Poulet de chair, viscosité du digesta iléal, régression linéaire multiple, taille des particules, modèle de prédiction

Introduction

Feed intake in broiler chickens is affected by feed particle sizes, with coarse particles being more favored to increase the gastrointestinal passage rate (Downs *et al.*, 2022; Kareem *et al.* 2022a). This can significantly impact the viscosity of the ileal digesta (Yasar, 2003; Amerah *et al.*, 2008; Kareem *et al.*, 2022b) and consequently, production performances and digestibility of the nutrients. Other factors such as feed characteristics, environmental temperature, housing type and birds' health status have also been reported to affect maximum feed intake in broiler chickens (Dozier *et al.*, 2010).

According to Morgan and Bedford (1995), poultry diets containing enzymes, especially those that originate from cereals and rich in non-starch polysaccharides (NSPs) such as rye, wheat, and barley, reduce digesta viscosity. However, more recent studies reported that xylanase, amylase, and glucanase supplementation in poultry diets did not impact its digesta viscosity (Perić *et al.*, 2002; Iji *et al.*, 2003; Kareem *et al.*, 2022b). The interaction of multienzyme and particle sizes were also reported as not significantly affecting ileal digesta viscosity (Kim *et al.*, 2005; Amerah *et al.*, 2008; Kareem *et al.*, 2022b).

Reports are available on the impacts of feed particle size, enzyme supplementation, and their interaction on the ileal digesta viscosity of broiler

chickens. Measuring the viscosity of the ileal digesta can be laborious, and this can be offset by fitting prediction model(s) that estimates the ileal viscosity of the birds from the relative impact weighting of the principal variables (particle size and enzyme supplementation levels). This study was therefore aimed at generating a prediction equation for broiler chickens fed maize-based diets using the feed particle sizes and level of enzyme supplementation as predictors.

Materials and methods

Study area

The experiment was carried out at the Poultry Unit of the Directorate of University Farms (DUFARMS) of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State Nigeria. The site is located in the rainforest vegetation zone of South-Western Nigeria at latitude 7°10'N and longitude 3°2'E (Google Earth, 2019).

Ethical permit

The practices adopted in the conduct of the study were according to the guidelines approved by the Project Review Committee of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State.

Experimental design

The feeding trial was conducted with 450 unsexed 1-day-old Cobb500 broiler chicks arranged into nine dietary treatments with three

particle sizes (3, 4, and 5mm) and three graded levels of multienzyme supplementation (0, 1, and 2 g/kg) of 5 replicate each. Each replicate contained 10 birds reared in floor pens equipped with drinkers and feeders and offered feed and water *ad libitum*. Standard commercial starter diet was fed to the birds for the first two weeks while the experimental diets were offered from week 2 to 6 post-hatch. The multienzyme (MultiZyme Pro®) used in the study contains xylanase, β -glucanase, α -amylase, and 6-phytase, compositions of which are reported in Kareem *et al.* (2022a) and Kareem *et al.* (2024).

The method for granulometric analyses to determine the geometric mean diameter (GMD) and geometric standard deviation (GSD) of the feed particles, as well as the diet information, has been extensively described in Kareem *et al.* (2022a).

Data collection

Viscosity of the ileal digesta

At 42-day-old, four birds per replicate whose weights were closest to the average weight were slaughtered via cervical dislocation and dissected to expose the celomic cavity to sample the intestinal content. The digesta content was collected on the third distal section of the ileum. The same amount of digesta content was taken from each sample using a sensitive scale and diluted with 200mL distilled water. The viscosity of the supernatant from digesta was measured at 29.1°C and a shear rate of 60rpm using a viscometer with coaxial cylinders and a mobile rotor (model NDJ-8S) (Omidiwura and Agboola, 2016).

Statistical analysis and model

Normality (Shapiro-Wilk) and outlier tests were carried out on the ileal viscosity data at $p \leq 0.05$, after which Pearson correlation (r) was performed between ileal viscosity, particle size, and enzyme supplementation to determine their suitability for multiple linear regression. To ensure non-collinearity, moderate correlation coefficient ($r < 0.8$) was considered appropriate (Kim, 2019). Four models were generated using multiple linear regression of Minitab 19 and compared with the study of Kareem *et al.* (2022b). The R^2 values, r , and p -value ($p \leq 0.05$) based on the comparison were used to determine the best-fit model.

The models generated go thus;

$$Y = b_1X_1 + b_2X_2 \quad \text{---} \quad \text{Model 1}$$

$$Y = a + b_1X_1 + b_2X_2 \quad \text{---} \quad \text{Model 2}$$

$$Y = b_1X_1 + b_2X_2 + b_3X_1X_2 \quad \text{Model 3}$$

$$Y = a + b_1X_1 + b_2X_2 + b_3X_1X_2 \quad \text{---Model 4}$$

Where Y is the viscosity (dependent variable), X_1 is the particle size variable, and X_2 is the enzyme supplementation level. Parameter a is the intercept, and b_1 , b_2 , and b_3 are the slopes for their corresponding variables.

Bar graphs of the ileal digesta viscosity of the of broiler chickens were plotted using Microsoft 365 (Excel).

Results and discussion

Normality test

The ileal viscosity data was observed to be normally ($p > 0.05$) distributed, which implies that it could be subjected to a parametric test as shown in Fig. 1.

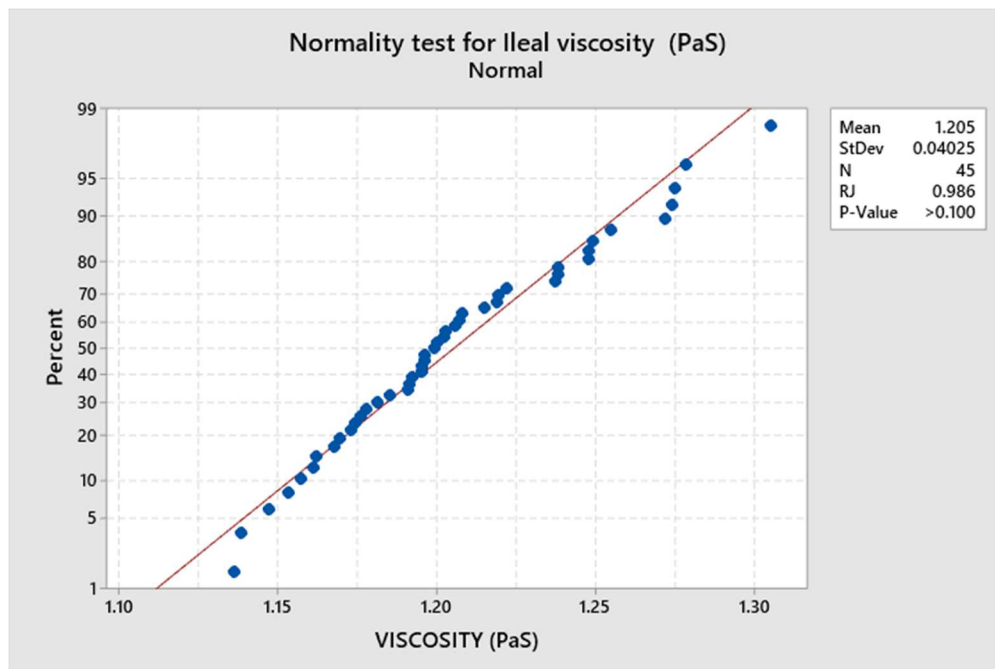


Fig. 1. Normality test for the ileal digesta viscosity

Main and interaction effects of feed particle size and multienzyme supplementation on ileal digesta viscosity

Mean plots of the ileal viscosity of the broiler chickens presented in figures 2, 3, and 4 shows the main and interaction effects of multienzyme and particle sizes, respectively. The results showed that multienzyme supplementation had no significant ($p > 0.05$) impact on the ileal

viscosity, while the particle sizes significantly ($p < 0.05$) affected the ileal viscosity of the broiler chickens, with the ileal content of birds fed 3 mm particle sizes being the most viscous, which is 4% higher than those fed with 4 mm and 5 mm feed particles. The interaction of multienzyme and particle sizes did not significantly affect the ileal viscosity of the broiler chickens.

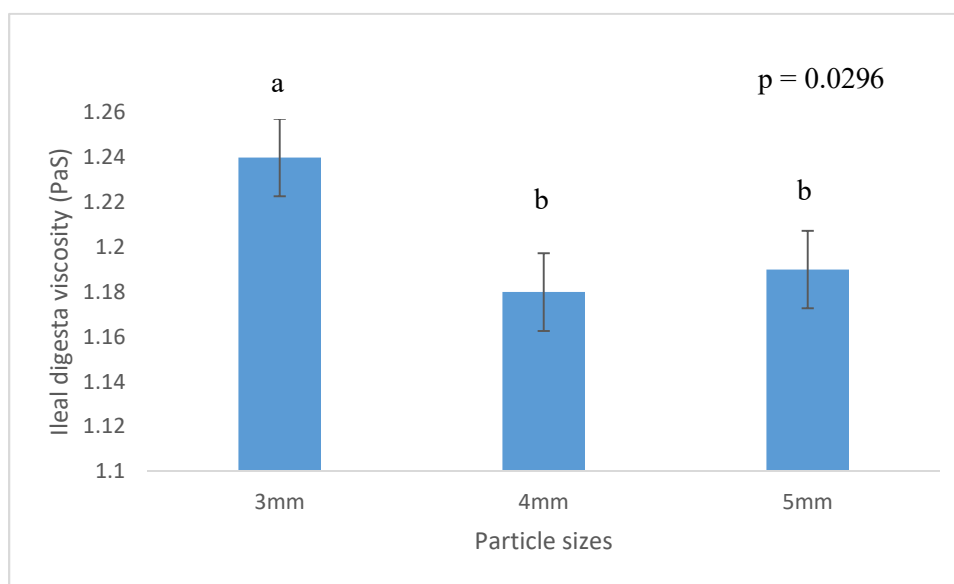


Fig. 2. Ileal digesta viscosity of broiler chickens fed different feed particle sizes

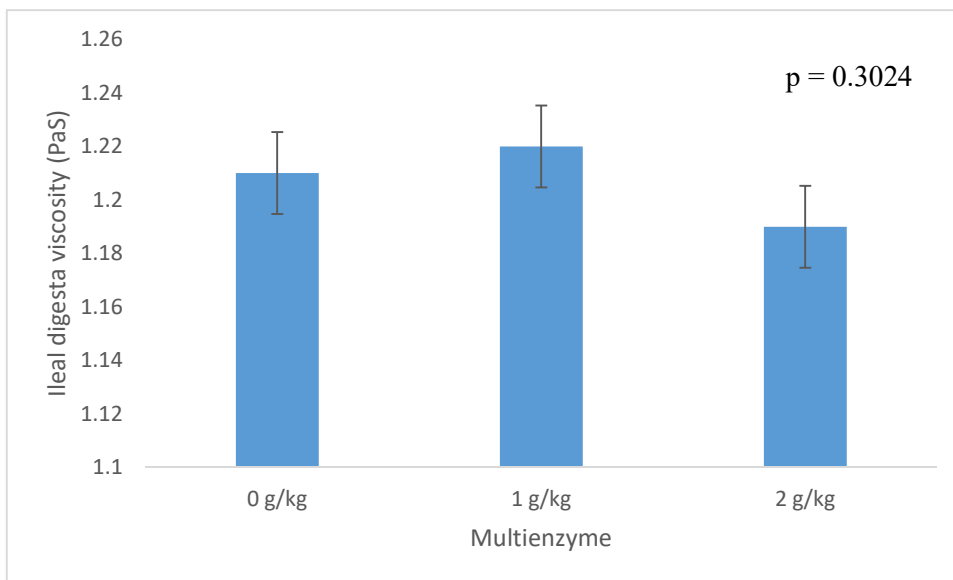


Fig. 3. Ileal digesta viscosity of broiler chickens fed graded levels of multienzyme

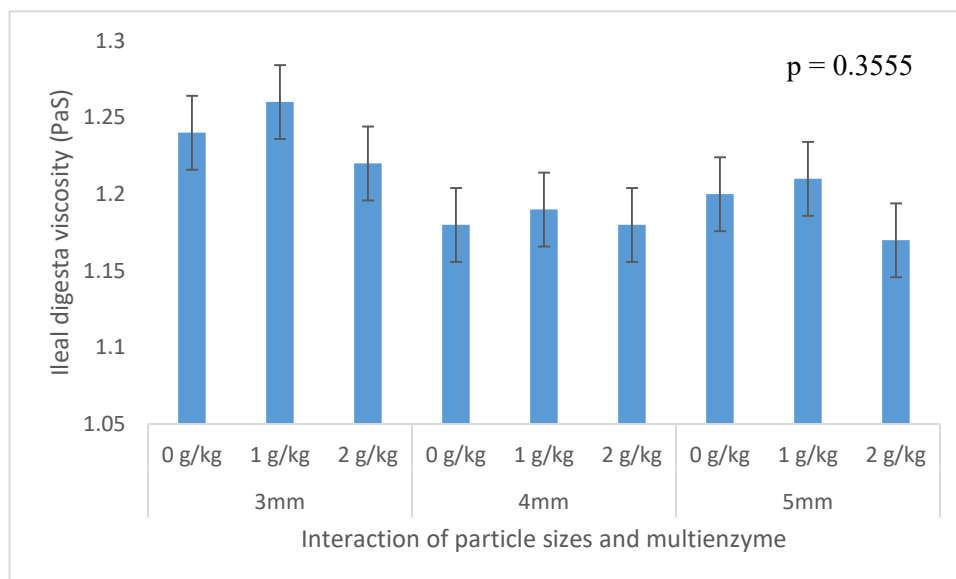


Fig. 4. Ileal digesta viscosity of broiler chickens fed diets of different feed particle sizes with graded levels of multienzyme

Multiple linear regression models and Pearson correlation coefficients of the ileal digesta viscosity

The regression models generated for the ileal digesta viscosity of broiler chickens in the study and their correlation coefficients are described in Table 1. Model 1 shows that the particle size significantly ($p < 0.05$) impacted the model, while the enzyme supplementation does not, with an R^2 value of 95.51%. Model 2 was modeled to have a

constant value and was observed to be also significantly ($p < 0.05$) affected by the particle size; however, it has an R^2 value of 25.62%. Model 3 has a high R^2 value of 98.07%, but all parameters (particle size, enzyme, and their additive effects) significantly ($p < 0.05$) affected the model. Model 4 combines constant value with additive effects of particle sizes and multienzyme supplementation, has no significant ($p > 0.05$) effect on the model, and an R^2 value of 25.74%.

Table 1: Regression models and correlation coefficients for the ileal digesta viscosity

S/N	Model	R ²	p-value
1	$Y = 0.2734 P + 0.0623 E$	95.51%	Particle size; $p = 0.000$ Enzyme; $p = 0.186$ Regression; $p = 0.000$
2	$Y = 1.3039 - 0.02181 P - 0.01154 E$	25.62%	Particle size; $p = 0.002$ Enzyme; $p = 0.082$ Regression; $p = 0.002$
3	$Y = 0.2912 P + 0.774 E - 0.1886 P * E$	98.07%	Particle size; $p = 0.000$ Enzyme; $p = 0.000$ P*E; $p = 0.000$ Regression; $p = 0.000$
4	$Y = 1.2956 - 0.0197 P - 0.0032 E - 0.00208 P * E$	25.74%	Particle size; $p = 0.064$ Enzyme; $p = 0.922$ P*E; $p = 0.797$ Regression; $p = 0.006$

Pearson Correlation Coefficients (r)

	Ileal viscosity	
	r	p-value
Particle Size	-0.447	0.002
Enzyme	-0.237	0.117

Y: Ileal viscosity (PaS); P: Particle size (mm); E: Enzyme (g/kg); r: Pearson correlation coefficients

Discussion

Enzyme supplementation has been reported by Morgan and Bedford (1995) to reduce digesta viscosity in broiler chickens. This is however in contrast with the present study which showed no significant effect of multienzyme supplementation on ileal digesta viscosity of the birds as evident in fig. 2. While the result of the current study contradicts some established reports, it is in conformity with some other studies (McNab and Bernard, 1997; Perić *et al.*, 2002; Iji *et al.*, 2003; Amerah *et al.*, 2008) that registered no positive effect of multienzyme supplementation on digesta viscosity of broiler chickens. The particle sizes on the other hand, significantly affected the ileal viscosity of the broiler chickens, with the large particle size significantly reducing the ileal viscosity of the

digesta (fig. 1). This discovery is in line with the report of Yasar (2003) who recorded higher digesta viscosity with birds fed fine mash diets compared to those fed medium or coarse wheat diets. The interaction of feed particle sizes and multienzyme supplementation which elicited no significant effect on the ileal digesta viscosity of the broiler chickens conforms with the studies of Amerah *et al.* (2008) who reported no effect of xylanase supplemented to wheat based diets of two different particle sizes on digesta viscosity of broiler chickens and Kim *et al.* (2005) who found interaction effect of particle size and multienzyme on digesta viscosity of pigs. The coefficient of determination (R²) of models 2 and 4 in the current study were below the 50% mark, thus not suitable for statistical prediction (Ojedapo *et al.*, 2022). This leaves only models 1

and 3 for consideration. The reports of Yasar (2003) and the current study showed significant effects of particle size on ileal digesta viscosity of broiler chickens, and the current study further showed that enzyme supplementation and the interaction of particle size and multienzyme supplementation had no significant effect on ileal viscosity. Despite the higher R^2 (98.07 %) of model 3, it had significant values for enzyme and additive effect of enzyme and particle size, contradicting the correlation coefficient result that only particle size significantly impacts the model. This also contradicts the result obtained in the current study, that interaction of feed particle size and multienzyme supplementation had no significant effect on the ileal viscosity of broiler chickens. Only model 1 fits the criteria of high R^2 and correlation significance. Hence, based on the data available for this study, model 1, $Y = 0.2734 P + 0.0623 E$, is opined to be the best to predict the ileal digesta viscosity of broiler chickens,

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- using feed particle size and level of enzyme supplementation as predictors. Although further studies that consider more factors such as digesta transit time/ passage rate and whole intestine length may give more insight and make the model more accurate.
- Conclusion and recommendation**
Based on the result of the study, $Y = 0.2734 P + 0.0623 E$ ($R^2 = 95.51$), where Y, ileal viscosity; P, particle size; and E, enzyme supplementation level, is the best model to predict the ileal viscosity of broiler chickens when enzyme supplementation level and feed particle sizes are the predictors. Also, an increase in particle size of maize-based diets leads to a reduction in the viscosity of the ileal digesta.
- Conflict of interest**
None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.
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