

Passage Rate of Digesta from Soybean Meal, Wheat Bran and Rice Bran Diets with or without a Multi-enzyme Supplementation in Broiler Chickens

A. F. Agboola^{1*}, A. O. Oke¹ and E. A. Iyayi¹

¹Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author EAI designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AFA reviewed the experimental design and all drafts of the manuscript. Authors AFA and AOO managed the analyses of the study. Authors EAI and AOO performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

The effect of a multi-enzyme (Biomix) supplementation on the rate of feed passage, mean retention time, gut morphology, digesta viscosity and performance of broiler chickens was determined in a 21-day experiment between August and September, 2014.

Two hundred and sixteen one-day-old Arbor Acres broiler chickens were randomly allotted to 6 dietary treatments in a randomized complete block design. Of the 6 diets containing soybean meal, wheat bran and rice bran, 3 were supplemented with the enzyme and 3 without enzyme. Each diet had 6 replicates with 6 birds each. Cumulative and non-cumulative excretion data were calculated from excreta chromium concentration. Viscosity was determined in digesta from birds and a section of the ileum removed for measurement of ileal morphological parameters.

Feed type significantly ($P = .05$) improved body weight gain, feed intake and feed conversion ratio. Time of 50% (T50) and 1% (T1) chromium excretion were significantly ($P = .05$) decreased with enzyme addition. Non-cumulative excretion data yielded similar results. Enzyme supplementation

*Corresponding author: E-mail: adebisi.agboola@gmail.com;

significantly ($P = .05$) reduced retention time by 0.25h and increased ($P = .05$) feed passage rate, villus height and crypt depth in the chickens. Digesta viscosity was also significantly ($P = .05$) reduced by enzyme addition.

In conclusion, results of the study showed that enzyme supplementation of diets improved digesta passage through the gut, improved ileal morphological characteristics and performance in broilers.

Keywords: Feed passage rate; enzyme supplementation; digesta viscosity; broiler chickens.

1. INTRODUCTION

Traditionally, in most research conducted on poultry feeding, dietary fibre has been considered a diluent of the diet [1] with negative influence on voluntary feed intake and nutrient digestibility [2]. Consequently, commercial diets, especially those for young broilers, are formulated to contain less than 3% crude fibre. Polysaccharides are the major components of plant materials used in rations for monogastrics. They are macromolecular polymers of monosaccharides linked by glycosidic bonds. Non-starch polysaccharides (NSP) include cellulose, hemicellulose, pectin and oligosaccharide. They can also be divided into water-soluble and water-insoluble fractions.

Most of the anti-nutritive activities of NSP, which directly affect broiler performance, have been attributed directly to soluble polysaccharides. A majority of polysaccharides, when dissolved in water, give viscous solutions. Increases in digesta viscosity associated with wheat arabinoxylan were noted in poultry [3]. The soluble NSP fraction and not the total NSP fraction are responsible for its anti-nutritive responses. These NSP can bind to large amounts of water and as a result, the viscosity of fluids in the digestive tract is increased. The increased viscosity causes problems in the small intestines because it reduces nutrient availability (particularly fat) and results in increased amount of sticky droppings [4].

Soybean NSPs are composed mainly of a mixture of pectic-polysaccharides such as rhamnogalacturonans (types I and II), arabinogalactan I and xylogalacturonan [4]. The carbohydrates in soybean consist of approximately 10% free sugars (5% sucrose, 4% stachyose and 1% raffinose) and between 20-30% NSP, in which approximately 8% are cellulose and the remaining are pectic-polysaccharides [5]. The NSP levels and the soluble NSP levels of thirteen wheat samples were compared [6]. Wheat bran contains arabinoxylan pentosans, a non-starch

polysaccharide thought to be anti-nutritive to poultry and causes a depression in nutrient utilization and subsequently leads to poor growth [4]. Rice bran also contains an appreciable quantity of arabinoxylan similar to that found in wheat and rye. However, whereas the arabinoxylans are of similar structure, those from wheat are much more viscous in solution than the rice bran arabinoxylan which is probably a reflection of their more branched nature [7].

Multi-enzymes have been approved for use in poultry feed because they are natural products of fermentation and therefore pose no threat to the animal or the consumer [8]. A considerable amount of nutrients such as starch remains encapsulated in the cell walls in the small intestine of chickens [9] and was removed upon xylanase supplementation. Although the insoluble NSP have mainly been regarded as nutrient diluent in the diet, they can also affect digesta transit time and gut motility. Enzymes with affinity for insoluble NSP can elicit a positive response in growth performance of broilers [10]. The use of chromic oxide as a marker for determining feed passage rate has been reported [11]. Fluorescent dye (Day-Glo) and ferric oxide had been used by [12,13,14] to measure feed passage rate respectively. The rate of feed passage refers to the amount of digesta that passes a point along the alimentary tract in a given time and is an exponential function. Retention time can be defined as the time it takes the nutrients of a meal to be absorbed by the birds in the gastrointestinal tract [15]. This study was conducted to determine digesta passage rate of soybean meal, wheat bran and rice bran in broiler chickens.

2. MATERIALS AND METHODS

2.1 Experimental Site, Diets and Management of Birds

The study was carried out at the Teaching and Research Farm, University of Ibadan. One-day-old Abor acre broiler chicks ($n=216$) were obtained from a reputable commercial hatchery.

The birds were raised in battery cages. The birds were tagged, weighed and allotted to 6 dietary treatments by body weight. Each diet had 6 replicates with 6 birds per replicate. The experimental design was a 2x3 factorial arrangement in a randomized complete block design. Birds were vaccinated following the vaccination schedule for broilers and other routine management practices were carried out.

Diet 1 was a corn-soybean diet without enzyme, diet 2 corn-wheat bran without enzyme, diet 3 corn-rice bran without enzyme, diet 4 corn-soybean supplemented with enzyme, diet 5 corn-wheat bran supplemented with enzyme and diet 6 corn-rice bran supplemented with enzyme. Diets were formulated to meet the nutrient requirements of the birds. The experimental protocol was in accordance with the regulations of the Animal Care and Use Committee of the University of Ibadan.

2.2 Data Collection

2.2.1 Performance indices

The chicks were weighed at day 1 and day 22, the difference between the weights was used to estimate weight gain. Feed intake was calculated as difference between quantity of feed given in and left over. Feed intake and weight gain were used to estimate feed conversion ratio.

2.2.2 Excreta collection

The birds were fed with experimental diets for 21 days and excreta collected on day 22. The birds were exposed to 5 hours of darkness which ended 2 hours (5 am) before chromium II oxide (Cr_2O_3) was administered orally with the use of gelatine capsules at the rate of 0.5 g/ bird. They were monitored for 2 hours after which excreta collection followed immediately. Excreta trays were placed under each cage for excreta collection while the total excreta were scooped out from each tray 2 hours post ingestion of chromium oxide. The collection continued on hourly basis at 2, 3, 4, 5, 6, 8, 12, and 16 hours post ingestion corresponding to 9am, 10 am, 11 am, 12 noon, 1 pm, 3 pm, 7 pm and 11 pm respectively). Fresh samples were weighed, freeze-dried, ground through 1mm sieve, and stored at $-20^{\circ}C$ for dry matter and chromium concentration analysis. The chromium content of samples was determined using an atomic absorption spectrophotometer following the methods of [16].

2.2.2.1 Cumulative excretion curves

Data on excreta chromium concentration from each cage was calculated as cumulative fractions of the total amount of chromium determined during the 16 h collection. The cumulative excretion curves of each diet was plotted and fitted to the Hill equation as recommended by [11]. The mathematical function of the curve was:

$$y = t^m / (t^m + k)$$

Where y = amount of chromium excreted, t = time of collection (hour), m = slope of the line, k = constant.

To determine m and k , curves were transformed into as linear equation as follows:

$$Y = a X + b$$

Where $Y = \ln\{y/(1-y)\}$; $a = m$ (the slope of the line); $x = \ln (t)$; and $b = -\ln k$.

2.2.2.2 Non-cumulated excretion data

Non-cumulative chromium concentration from each cage was calculated as a percentage of total amount of chromium excreted during the 16 h collection. Time of 1% excretion (TI) was the time at which 1% of the chromium intake was excreted, and the T50 was the time necessary to excrete half of the marker administered. These were calculated as in [17] for each cage from the cumulative excretion curve. Retention time (RT) was calculated from the equation proposed by [18]:

$$RT = \sum xi \times ti / \sum xi$$

Where xi is the amount of marker excreted at the i^{th} collection at time t , after administration.

2.2.3 Digesta viscosity

On day 22, three birds were slaughtered from each replicate to collect the digesta samples from the ileum (section between Meckel's diverticulum and 2 cm anterior to ileo-caecocolonic junction). The content was flushed with distilled water. The digesta was centrifuged at 3500 rpm for 15 minutes. The supernatants obtained from each sample were stored separately at $-20^{\circ}C$ until use. The supernatants were thawed on ice before the viscosity was measured using a viscometer. Measurements

were performed at 25°C and at shear rates at spindle 30 rpm with the values expressed in centipoise (cP).

2.2.4 Gut morphology

Intestinal samples of 1 cm in length were taken from the middle of segment of the jejunum. The gut samples were then fixed in 10% buffered neutral formaldehyde solution (pH 7.4), processed, and cut to 6- μ m sections. The sections were stained with hematoxylin and eosin and examined with a light microscope. A digital camera was used to measure the villus height and crypt depth. The villus height was measured by averaging the height of 10 intact villi, and crypt depth was measured by averaging 30 measurements.

2.3 Proximate Analysis

The proximate composition of the test ingredients, diets (Table 2) were analyzed according to the methods of AOAC [16].

2.4 Statistical Analysis

The data collected was subjected to analysis of variance (ANOVA) at (P = .05) using Statistical Analysis System [19]. The significant means were separated using Duncan's Multiple Range Test of the same software.

3. RESULTS

3.1 Performance

The results of performance are shown in Table 2. Diet significantly (P=.05) affected the live weight, body weight gain, feed intake and feed conversion ratio in broilers. These response criteria were better (P = .05) on the SBM compared to WB and RB diets irrespective of enzyme supplementation. There was no difference between the effect of WB and RB on the performance of the birds. Enzyme had no effect on any of the performance criteria, neither was the interaction between diet and enzyme.

Table 1. Gross composition of experimental broiler starter diets (g/kg)

Diets	Without enzyme			With enzyme		
	SBM	WB	RB	SBM	WB	RB
Corn	552.0	396.0	403.0	542.0	386.0	393.0
SBM	303.6	-	-	303.6	-	-
Wheat bran	-	303.6	-	-	303.6	-
Rice bran	-	-	303.6	-	-	303.6
Wheat gluten	50.0	180.0	190.0	50.0	180.0	190.0
Soya bean oil	50.0	70.0	50.0	50.0	70.0	50.0
Dicalcium phosphate	17.0	17.0	17.0	17.0	17.0	17.0
Salt	4.00	4.00	4.00	4.00	4.00	4.00
Limestone	14.0	20.0	23.0	14.0	20.0	23.0
Vitamin min premix	3.00	3.00	3.00	3.00	3.00	3.00
DL-Met	3.60	3.60	3.60	3.60	3.60	3.60
L-Lysine	2.80	2.80	2.80	2.80	2.80	2.80
Enzyme Premix	-	-	-	10.0	10.0	10.0
TOTAL	1000	1000	1000	1000	1000	1000
Calculated nutrients and energy						
ME, kcal/kg	3283	3100	3507	3248	3065	3473
CP, g/kg	237	236	236	236	235	235
Ca, g/kg	6.70	8.77	9.71	6.70	8.77	9.71
Total P, g/kg	4.05	6.04	7.19	4.02	6.01	7.16
NPP, g/kg	1.73	2.37	2.50	1.72	2.36	2.49
Ca:P	1.65	1.45	1.35	1.66	1.45	1.35
Ca:NPP	3.87	3.7	3.88	3.88	3.71	3.89

C-SBM: corn soybean meal, C-WB: Corn Wheat Bran, C-RB: Corn Rice Bran, ME: Metabolizable Energy, CP: Crude Protein, P: Phosphorus, Ca: Calcium, NPP: Non Phytate Phosphorus. *Composition of premix per kg of diet: vitamin A, 12,500 I.U; vitamin D₃ 2,500 I.U; vitamin E, 40 mg; vitamin K₃ 2 mg; vitamin B₁ 3 mg; vitamin B₂ 5.5 mg; niacin 55 mg; calcium pantothenate, 11.5 mg; vitamin B₆ 5 mg; vitamin B₁₂ 0.025 mg; choline chloride, 500 mg; folic acid, 1 mg; biotin, 0.08 mg; manganese, 120 mg; iron, 100 mg; zinc, 80 mg; copper, 8.5 mg; iodine, 1.5 mg; cobalt, 0.3 mg; selenium, 0.12 mg; Anti-oxidant, 120 mg

3.2 Digesta Passage Rate

3.2.1 Cumulative excretion curves

The cumulated chromium excretion curves (Figs. 1 and 2) were sigmoidal. The excretion patterns of chickens fed SBM, WB and RB diets without enzyme (Fig. 1) or with enzyme (Fig. 2) were similar. Estimation of times for the excretion of 1% (T1) and 50% (T50) of the chromium for all treatments is shown in Table 3. The excretion times were not significantly different between chickens fed diets with SBM, WB or RB. However, for chickens fed the diets supplemented with enzyme, the times for excretion of 1 and 50% of the marker were significantly ($P = .05$) reduced compared with those fed diets with no enzyme. The T1 of chickens fed diets supplemented with enzyme was reduced by 0.57 h (SBM), 0.34 h (WB), 0.22 h (RB), respectively, compared with those of chickens fed diets without enzyme supplementation. The T50 of chickens fed enzyme-supplemented diets was also significantly ($P = .05$) reduced. Mean values of retention time for birds fed SBM, WB and RB diets with enzyme were 5.80 h, 5.56 h, 5.88 h, respectively while for birds fed the same diets but without enzyme, the mean retention times were 5.43 h, 4.85 h, and 3.85 h, respectively.

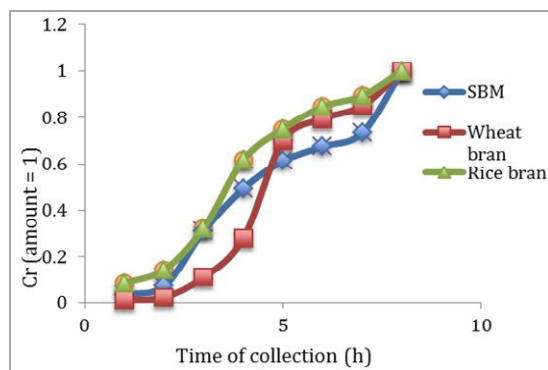


Fig. 1. Cumulative chromium excretion curves for diets without enzyme supplementation

3.2.2 Non-cumulative data of marker excretion

Results of non-cumulative chromium excretion are shown in Figs. 3 and 4. A delay of 2 h in excretion of chromium was observed in chickens fed the diets without enzyme supplementation than chickens fed the diets with enzyme. In chickens fed SBM, WB and RB diets without enzyme, maximum excretion occurred at 4 h, 6

h, and 5 h, respectively. When enzyme was added to the diets, maximum excretion occurred at 11-12 h. The peak excretion was also higher when enzyme was included. After 8 h, the excretions of Cr from chickens of all dietary treatments were not significantly different.

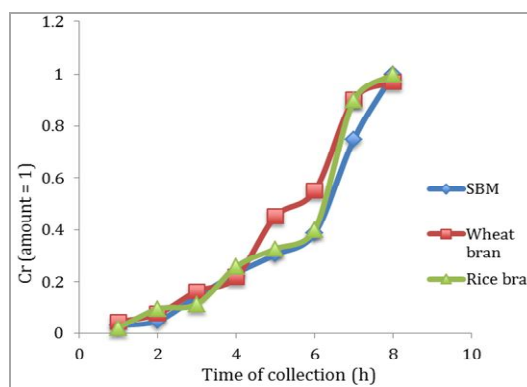


Fig. 2. Cumulative chromium excretion curves for diets with enzyme supplementation

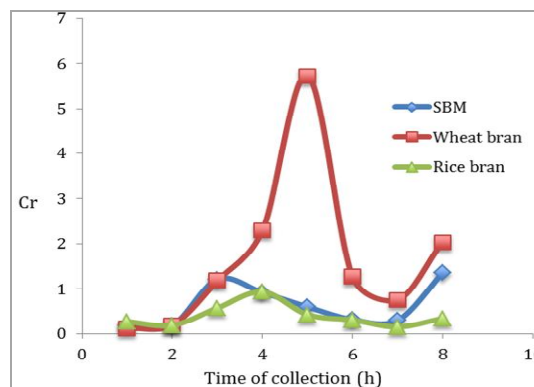


Fig. 3. Non-cumulative chromium excretion curves for diets without enzyme supplementation

3.3 Gut Morphology

The effects of diets on the villus height and crypt depth of section of small intestine are shown in Table 4. Feed type and enzyme supplementation significantly ($P < .0001$) affected villus height. Birds fed the SBM diet had the highest villi followed by WB and RB diets. The crypt depth of birds on dietary treatments differed significantly ($P = .05$). Birds fed with enzyme-supplemented diets had similar crypt depth. The highest crypt value was obtained in birds fed SBM with enzyme while birds fed RB without enzyme had the shortest crypt depth.

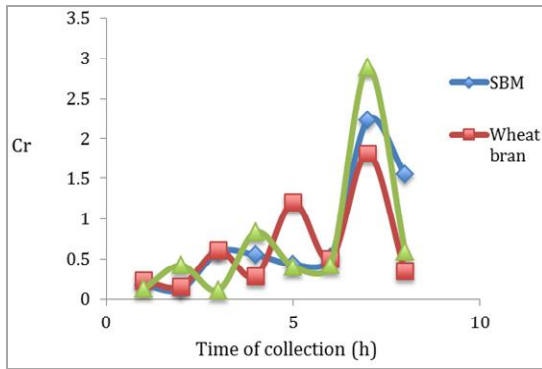


Fig. 4. Non-cumulative chromium excretion curves for diets with enzyme supplementation

3.4 Digesta Viscosity

The effects of dietary treatments on digesta viscosity of birds are shown in Table 4. Significantly ($P = .05$) reduced viscosity (1841.6, 1902.9, 2116.5 mPa-s) was observed in broiler chickens fed SBM, WB and RB diets supplemented with enzyme respectively compared with birds on diets without enzyme supplementation.

4. DISCUSSION

4.1 Growth Performance

Choct and Sinlae [20] reported degradation of β -mannan and 70% NSPs into soluble metabolizable products for monogastrics when xylanase was added to high fibre diets. Cleavage of the NSPs in fibre fraction is expected to improve digestion and nutrient absorption but this is contrary to what was observed in this study, growth performance of chicks fed diets with or without multi-enzyme supplementation was similar. Also, lower weights of birds fed increasing levels of fibrous diet without enzyme supplementation has been reported [21,22]. The interaction effects of diets and enzyme on live weight, body weight gain, feed intake and feed

conversion ratio were not significant. This could be as a result of some factors such as the strain of the birds, management, enzyme type.

4.2 Digesta Passage Rate

The representations of the experimental data fit a sigmoidal curve. The Hill equation used is only one of a large number of sigmoidal equations [23] that can be used to calculate the rate of feed passage. Differences in TI and T50 between diets were significant. With the improved intestinal characteristics, the rate of feed passage in chickens fed the enzyme-supplemented diets was decreased compared with those of chickens fed the diets without enzyme. Chickens fed enzyme-supplemented diets irrespective of type of fibre source took 0.25 h to excrete 50% of the administered marker, whereas chickens fed the same diets but without the multi-enzyme took an additional 0.25 h to excrete the same amount of marker. Mean retention time (MRT) calculated from Equation (3) showed an inverse proportionality between rate of feed passage and retention time. The mean retention time showed the same tendencies as rate of feed passage. The slower retention time in chickens fed with enzyme supplemented diet indicated a higher rate of passage, which could have improved nutrient, digestibility, absorption and utilization. These results agree with those of [24], where the effect of β -glucanase addition was to increase the rate of feed passage in broiler chicks at 2 weeks of age; however, in Leghorn chicks at 16 week of age, there was no difference in rate of feed passage.

In a study carried out by [11], the addition of β -glucanase significantly decreased the time for excretion of 50% of the administered marker by 3 h in 3-week-old chickens. The rate of feed passage is suggested to be related to the intestinal viscosity [11]. The T50 and MRT values obtained in the current study are similar to those reported by [25,26] for complete diets fed to

Table 2. Performance of broiler chickens fed soybean meal, wheat bran, rice bran without or with multi-enzyme supplementation

	Without enzyme			With enzyme			SEM	P value		
	SBM	WB	RB	SBM	WB	RB		Diet	Enzyme	Diet*enzyme
Live weight, g	709.0 ^a	215.0 ^b	186.5 ^b	699.7 ^a	227.1 ^b	175.1 ^b	39.8	<.0001	0.9267	0.8812
Body weight gain, g	665.9 ^a	172.1 ^b	143.0 ^b	656.6 ^a	184.1 ^b	127.3 ^b	39.7	<.0001	0.9180	0.8815
Feed intake, g	1062.8 ^a	469.4 ^b	500.0 ^b	1046.1 ^a	519.4 ^b	447.2 ^b	31.2	<.0001	0.8133	0.3080
FCR	1.62 ^c	2.73 ^b	3.66 ^a	1.64 ^c	2.87 ^b	3.32 ^a	0.212	<.0001	0.7382	0.5597

^{abc}Means on the same row with different superscripts are significantly ($P < 0.05$) different. SBM = soybean meal, WB = Wheat bran, RB = Rice bran

Table 3. Excretion and retention times of experimental diets without or with multi-enzyme supplementation in 3 weeks broiler chickens

	Without enzyme			With enzyme			SEM
	SBM	WB	RB	SBM	WB	RB	
T1, h	0.66 ^a	0.46 ^a	0.44 ^a	0.08 ^b	0.12 ^b	0.22 ^b	0.03
T50, h	0.42 ^a	0.25 ^a	0.36 ^a	0.25 ^b	0.24 ^b	0.19 ^b	0.09
Mean retention time, h	5.81	5.56	5.88	5.43	4.86	3.86	0.06

^{abc}Means on the same row with different superscripts are significantly ($P < 0.05$) different.

SBM = soybean meal, WB = Wheat bran, RB = Rice bran.

T1%: time of appearance of 1% of marker in excreta, T50%: time of excretion of 50% of marker in excreta

Table 4. Ileal morphological and digesta viscosity in broiler chickens fed soybean meal, wheat bran, rice bran without or with multi-enzyme supplemented diets

	Without enzyme			With enzyme			SEM	P value		
	SBM	WB	RB	SBM	WB	RB		Diet	Enzyme	Diet*enzyme
Villus height, μm	385.4 ^{ab}	329.9 ^c	256.7 ^d	410.4 ^a	347.7 ^b	322.1 ^c	10.90	<.0001	<.0001	0.2905
Crypt depth, μm	41.9 ^b	34.4 ^{cd}	32.1 ^d	55.3 ^{ab}	38.4 ^{cb}	38.9 ^{cb}	1.729	<.0001	<.0001	0.0544
Viscosity, mPa	3630.8 ^a	3706.7 ^a	3662.9 ^a	1841.6 ^c	1902.9 ^{cb}	2116.5 ^b	84.39	0.2720	<.0001	0.3154

^{abc}Means on the same row with different superscripts are significantly ($P < 0.05$) different. SBM = soybean meal, WB = Wheat bran

broilers. However, methodology has varied among research determining rate of passage in poultry. In addition to diet composition and form, rate of passage can be affected by the strain and age of the bird [13,25], fasting period, ambient temperature and marker type and administration [13,17,27]. Additionally, interpretations of results can be made when considering different measurements of rate of passage, such as first appearance of feed or marker, the time required to reach a specified amount of marker excretion such as T1 or T50 [17], or the time required for a feed or marker substance to pass through a specified section of the gastrointestinal tract [18].

An inconsistent relationship among the rate of passage measurements was observed as T1 and T50 indicated faster initial rate of passage for diets without the multi-enzyme. The determination of T1 resulted in analyzing low concentrations of Cr in the excreta, possibly leading to increased variability. Furthermore, [17] found no differences in T1 of birds fed chromium-mordanted wheat bran particles varying in particle size, while differences were observed in MRT for both particle size and ingredient source. Thus, T50 and MRT may be less variable and more accurate indicators of rate of feed passage than T1.

4.3 Gut Morphology

The results of the present study showed that the absorptive function in the jejunum of chickens fed the multi-enzyme supplemented diets were higher compared with those on the same diets but with no enzyme added, which is an indication

that the enzyme used in this study enhanced digestion and absorption of available nutrients. No significant differences in villus heights and crypt depths of chickens fed diets with different viscosities during the first period of the experiment (7 d) were found [28] but at the end of the second period (21 d), the ileal villi were significantly longer in chickens fed a less viscous diet. It was reported [29] that increasing villus height suggests an increased surface area for the greater absorption of available nutrients while deeper crypt is implicated in a greater production of enterokinase, which is the precursor for the production of trypsin. Trypsin is needed for digestion of protein, which culminates into increased availability of amino acids vital for birds' performance. Thus the results of this study show that the use of multi-enzyme in soybean meal, wheat bran, rice bran based diets improved the surface area for absorption of available nutrients but much more improved in soybean meal supplemented with enzyme. This could be due to the low level of NSPs present in SBM compared to WB and RB. The results are supported by those of [30] and [31] who showed that carbohydrase can improve performance and nutrient digestibility when added to a viscous diet. The lowest villus height observed in birds on diets without enzyme indicates a reduction of absorption of available nutrients and consequently reduced performance.

4.4 Digesta Viscosity

The lowest digesta viscosity observed, increased the nutrient digestibility and performance of birds on the SBM, WB and RB supplemented diets,

but best for chickens on SBM + Enzyme diet. This is supported by reports of [32,33,34]. The authors reported the beneficial effects of using feed enzymes to poultry diets which include; reduction in digesta viscosity, enhanced digestion and absorption of nutrients especially fat and protein, improved apparent metabolizable energy value of the diet, reduced water content of excreta, reduced production of ammonia from excreta and reduced output of excreta, including reduced N and P. These findings showed that the addition of multi-enzyme that contains β -glucanase, xylanase, cellulose and pentosanase to broiler diets based on soybean meal, wheat bran and rice bran were adjusted to reduce intestinal viscosity and improve nutrient digestibility.

5. CONCLUSION

The study showed that exogenous enzyme did not improve the performance of the birds despite higher passage rate and lower mean retention time. Feeding the fibrous diet slowed down digesta passage in the gut but this was improved by enzyme supplementation. Multi enzyme supplementation also reduced digesta viscosity resulting in higher villus height and improved crypt depth as a result of higher nutrient absorption but surprisingly did not improved performance of the birds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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