

Original Research Article

Digesta Viscosity and Microbial Load in Broilers fed Wheat-Based Diets Supplemented with Roxazyme G2G

ABSTRACT

Non-starch polysaccharides (NSP) are one of the potential factors reducing nutrient bioavailability in ingredients such as soyabean meal and wheat. Supplementing wheat-based diets with NSP degrading enzyme helps to reduce digest viscosity, which is partly related to the modulation of gut.

The effect of Roxazyme G2G supplementation on digesta viscosity, gut microbial load, gut pH, and length was investigated in a 42-day experiment between October and November 2012 using 120 day-old broiler chickens fed wheat-based diets with or without enzyme supplementation. Brooding of birds was for 7 days after which they were randomly allotted into 3 dietary treatments in a completely randomized design. Each diet had 4 replicates with 10 birds per replicate. On day 42, birds were slaughtered and digesta samples from the duodenum, jejunum, ileum, and caeca were collected for viscosity determination and microbial load analysis. Length of the gastrointestinal tract (GIT) and pH were also measured.

Enzyme supplementation had significant ($P = .05$) influence on the gut microbial loads at different sections of the GIT. Birds fed wheat-based diets supplemented with enzyme had significantly lower microbial loads as compared with birds in other diets. Reduced digesta viscosity was observed in birds fed 20% wheat-based diets supplemented with enzyme. The pH values obtained were slightly acidic (4.40 – 5.10) in birds on enzyme-supplemented diets across the different GIT sections.

Summarily, Roxazyme G2G resulted in reduced digesta viscosity, improved gut pH, and reduced gut and organ microbial loads in birds fed wheat-based diets.

Keywords: Digesta viscosity, gut integrity, wheat, broilers

1. INTRODUCTION

Wheat is an important ingredient in poultry diets, accounting for up to 70% of the metabolizable energy and 40% of the protein requirements of broilers [1]. Its utilization in poultry diets is being hindered by the presence of Non-starch polysaccharides (NSP). NSP are those polysaccharides (complex carbohydrate), other than starches, that are found in foods and wheat contains both soluble and insoluble NSP. Soluble NSP increases the viscosity of the small intestinal digesta whereas insoluble NSP impedes the access of endogenous enzymes to their substrates by physical entrapping [2].

Arabinoxylans, the predominant NSPs in wheat can form highly viscous solutions thus increasing the intestinal digesta viscosity. Increased digesta viscosity also prolong the residence time of digesta in the intestine [3,4], which may decrease oxygen tension and favour the development of anaerobic microflora. These problems can be eliminated by

adding appropriate exogenous enzymes to wheat-based diets for broilers. In addition, [5] reported reduced digesta viscosity in birds via the use of exogenous enzymes.

Roxazyme G2G is a carbohydrase enzyme, which contains β -xylanase and β -glucanase activities. Its addition to poultry diets usually result in numerous beneficial effects, such as increased utilization of nutrients, reduced digesta viscosity and improved passage rate of digesta [6]. It was therefore the objective of this study to determine the effect of Roxazyme G2G on the digesta viscosity and gut microbial load in broilers.

2. MATERIAL AND METHODS

This study was carried out at the poultry unit of the Teaching and Research Farm University of Ibadan, Ibadan, Oyo State. A total of 120-one-day-old broiler chicks (Arbor Acre strain) were used for this study and after 7 days brooding, the birds were randomly allotted to 3 dietary treatments on beddings of wood shavings in a well illuminated poultry house. Each dietary treatment had 4 replicates of 10 birds each in a completely randomized design. The three dietary treatments consisted of treatment 1 which was the control diet without wheat and Roxazyme G2G; treatment 2 which contained wheat at 20% inclusion level without Roxazyme G2G supplementation and treatment 3 which contained wheat at 20% inclusion level with Roxazyme G2G supplementation (Tables 1 and 2). Experimental diets were offered *ad libitum* and birds had free access to clean water in a study that lasted for 42 days.

Table 1: Gross composition of experimental wheat-based broiler starter diets supplemented with Roxazyme G2G (g/100gDM)

Ingredients	Control	20% wheat - Roxazyme)	20% wheat + Roxazyme)
Maize	58.50	38.50	38.50
Wheat	0.00	20.00	20.00
Soyabean meal	19.00	19.00	19.00
Groundnut cake	16.40	16.40	16.40
Fish meal (72% CP)	2.50	2.50	2.50
Limestone	1.10	1.10	1.10
DCP (23%Ca, 18%P)	1.50	1.50	1.50
Salt	0.25	0.25	0.25
*Broiler Premix	0.25	0.25	0.25
Lysine	0.25	0.25	0.25
Methionine	0.25	0.25	0.25
Roxazyme G2G	-	-	0.025
Total	100.00	100.00	100.00
Calculated Nutrients (%)			
Crude protein	23.01	23.43	23.43
Energy (kcal/gME)	3.03	2.94	2.94
Ether extract	4.12	3.30	3.30
Crude fibre	3.25	2.85	2.85
Lysine	1.28	1.31	1.31
Methionine	0.59	0.60	0.60
Calcium	0.99	0.97	0.97
Phosphorus	0.49	0.57	0.57

DCP- Dicalcium phosphate *Composition of premix per kg of diet: vitamin A-12,500 I.U; vitamin D3-2,500 I.U; vitamin E-40mg; vitamin K₃-2mg; vitamin B₁-3mg; vitamin B₂-5.5mg; niacin-

53 55mg; calcium pantothenate-11.5mg; vitamin B₆-5mg; vitamin B₁₂-0.025mg; choline chloride-500mg;
 54 folic acid-1mg; biotin-0.08mg; manganese-120mg; iron-100mg; zinc-80mg; copper-8.5mg; iodine-
 55 1.5mg; cobalt-0.3mg; selenium-0.12mg; Anti-oxidant-120mg.

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 57 **Table 2: Gross composition of experimental wheat-based broiler finisher diets**
 58 **supplemented with Roxazyme G2G (g/100gDM)**

Ingredients	Control	20% wheat - Roxazyme)	20% wheat + Roxazyme)
Maize	66.50	46.50	46.50
Whole wheat	0.00	20.00	20.00
Soyabean meal	19.50	19.50	19.50
Groundnut cake	8.30	8.30	8.30
Fish meal (72% CP)	2.00	2.00	2.00
Limestone	1.20	1.20	1.20
DCP (23% Ca, 18% P)	1.50	1.50	1.50
Salt	0.25	0.25	0.25
*Broiler Premix	0.25	0.25	0.25
Lysine	0.25	0.25	0.25
Methionine	0.25	0.25	0.25
Roxazyme G2G	-	-	0.025
Total	100.00	100.00	100.00
Calculated Nutrients (%)			
Crude protein	20.02	20.44	20.44
Energy (kcal/g ME)	3.09	3.00	3.00
Ether extract	3.93	3.13	3.13
Crude fibre	3.03	2.63	2.63
Lysine	1.17	1.19	1.19
Methionine	0.56	0.57	0.57
Calcium	0.95	0.96	0.96
Phosphorus	0.46	0.54	0.54

59 DCP- Dicalcium phosphate *Composition of premix per kg of diet: vitamin A-12,500 I.U;
 60 vitamin D3-2,500 I.U; vitamin E-40mg; vitamin K₃-2mg; vitamin B₁-3mg; vitamin B₂-5.5mg; niacin-
 61 55mg; calcium pantothenate-11.5mg; vitamin B₆-5mg; vitamin B₁₂-0.025mg; choline chloride-500mg;
 62 folic acid-1mg; biotin-0.08mg; manganese-120mg; iron-100mg; zinc-80mg; copper-8.5mg; iodine-
 63 1.5mg; cobalt-0.3mg; selenium-0.12mg; Anti-oxidant-120mg.

64 65 2.1 Digesta collection

66
 67 On day 42, four birds from each pen were selected, weighed, and slaughtered. The digestive
 68 tract was carefully excised and duodenum, jejunum, ileum, and caeca contents were gently
 69 flushed out with distilled water into containers on replicates basis.

70 71 2.2 Microbial load analysis

72
 73 Intestinal content from the duodenum, jejunum, ileum and caeca segments were separately
 74 mixed in a 10ml pre-reduced salt medium and serially diluted according to the procedure
 75 described by [7] to examine the counts of lactobacilli (Rogosa, CM 0627, incubated
 76 anaerobically 48 h), coliforms (Mackonkey, CM 0115, incubated aerobically 24 h) and
 77 Clostridium per fringes (CM 0543 OPSP, incubated anaerobically for 7 days). Gut tissue
 78 samples were serially diluted from 10⁻⁷ to 10⁻³. From each dilution, 0.1 ml of the sample
 79 was plated onto the appropriate medium for enumeration of bacteria.

80 81 2.3 Digesta viscosity

Duodenum, jejunum, ileum, and caeca digesta were diluted (1:1) with distilled water and homogenized for 20 min at room temperature before centrifugation at 3500 RPM for 15 min. The viscosity of the supernatant from digesta were measured at 29.1°C and at a shear rate of 60rpm using a viscometer with coaxial cylinders and a mobile rotor (model NDJ-8S).

2.4 Gut length and pH of gut content

The pH of fresh content of the duodenum, jejunum, ileum and caecum were determined using a Model pH meter (E-HI98128) immediately after collection from various sections while the lengths of the duodenum, jejunum, ileum and caecum were measured using a measuring tape.

2.5 Proximate and statistical analyses

The proximate composition of the diets was determined according to the method of AOAC [8]. Data obtained were analyzed using descriptive statistics and ANOVA ($P = .05$) SAS [9]. Mean differences were separated using Duncan multiple range test.

3. RESULTS AND DISCUSSION

The results of the gut length, digesta viscosity, and pH of the gut content of birds on experimental diets are shown in Table 3. The results showed that there were no significant differences in the various sections of the Gastro Intestinal Tract (GIT) measured. However, the lengths of jejunum and ileum of birds fed wheat-based diets supplemented with Roxazyme G2G were longer than those of birds on the other diets.

There were no significant differences observed in the digesta viscosities of different sections of the GIT. However, duodenal, jejunum and ileal viscosity in birds fed 20% wheat-based diets without Roxazyme G2G supplementation were higher than birds on control diet. According to [10], high gut viscosity decreases the rate of diffusion of substrates and digestive enzymes and hinders their effective interaction at the mucosal surface. Higher viscosity value of 1.28, 1.29, and 1.29mPa-s recorded respectively in the duodenum, jejunum and ileum of birds fed 20% whole wheat without Roxazyme G2G supplementation was probably due to high insoluble fibre as well as soluble Non-starch polysaccharides (NSPs), which usually results into viscous nutrient digesta. The reduction in digesta viscosities of different sections of the GIT in birds fed 20% whole wheat with Roxazyme G2G supplementation was achieved primarily by reducing the molecular weight through hydrolysis of xylan backbone by endo-xylanase into smaller compounds and thus reduction in viscous effects of the feed [11]. This result corroborates with the reports of [Inyang UO. Unpublished M.Sc thesis result, Department of Animal Science, University of Ibadan. Nigeria] and [6] on the benefits of exogenous enzyme. Low gut viscosity increases the rate of diffusion of substrates and digestive enzymes thereby allowing their effective interaction at the mucosal surface. The resultant effect is improved growth and feed conversion ratio.

Dietary treatments had an effect ($P = .05$) on the pH values on the duodenum contents of birds. The lower pH value (4.68) in the duodenum section was observed in birds on the control diet which was similar to the pH value (4.93) recorded with birds on wheat-based diets supplemented with Roxazyme G2G.

Table 3: Gut length, Digesta Viscosity, and pH of gut content of broilers fed wheat-based diets.

Segment	Treatment 1 (control)	Treatment 2 (20% whole wheat-enzyme)	Treatment 3 (20% whole wheat+enzyme)	SEM	Pvalue
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Gut length (cm)	Duodenum	29.13	31.86	31.50	0.93	0.13
	Jejunum	74.28	78.40	80.75	2.20	0.17
	Ileum	82.83	80.75	86.15	2.67	0.39
	Caeca	21.13	19.73	20.78	0.72	0.40
Viscosity (mPa-s)	Duodenum	1.15	1.28	1.00	0.15	0.46
	Jejunum	1.12	1.29	1.14	0.18	0.74
	Ileum	1.12	1.29	1.14	0.18	0.56
	Caeca	1.07	1.05	0.97	0.11	0.81
pH	Duodenum	4.68 ^b	5.45 ^a	4.93 ^b	0.13	0.01
	Jejunum	4.33	4.75	4.40	0.13	0.09
	Ileum	5.45	5.53	5.10	0.19	0.29
	Caeca	7.15	7.00	6.93	0.09	0.28

*Means on the same row with different superscripts are significantly ($P<0.05$) different. SEM-Standard Error of Mean, P-Probability

The results of the microbial load analysis of gut content of broilers fed wheat-based diets are shown in Table 4. The microorganisms in which the samples collected were analyzed for were *Bacillus*, *E. coli*, *Staphylococcus*, and Coliform. There were no significant differences observed in most of the microbes at the different sections of the gastrointestinal tract (GIT). However, *E. coli* in the duodenum and caeca of birds fed 20% wheat without Roxazyme G2G supplementation were higher than birds on the other diets. In addition, *E. coli* in the jejunum of birds in the control diet were significantly higher (700 cfu) than birds on the other diets. The Coliform and *Staphylococcus* in birds on the control diet were significantly higher than those fed whole wheat with and without Roxazyme G2G supplementation. The inclusion of wheat in the diet without Roxazyme G2G supplementation had a negative correlation with the microbes in the different sections of the GIT. This could be because of high digesta viscosities measured along the GIT, which enhanced proliferation of these microorganisms. This was contrary to the findings of [11] who reported that there was no relationship between rye-based diet and microbial loads in some organs of birds fed such diets.

Table 4: Microbial load of gut contents of broilers fed wheat-based diets (cfu).

	Segment	Treatment 1 (control)	Treatment 2 (20% whole wheat-enzyme)	Treatment 3 (20% whole wheat+enzyme)	SEM	P value
<i>Bacillus</i>	Duodenum	4.75	8.38	3.00	4.02	0.64
	Jejunum	2.00	2.86	3.75	2.34	0.87
	Ileum	24.50	2.38	1.88	7.82	0.12
	Caeca	6.75	0.25	4.00	4.33	0.42
<i>E. coli</i>	Duodenum	175.50	239.00	223.00	203.44	0.61

	Jejunum	700.00 ^a	16.00 ^b	1.80 ^b	235.45	0.05
	Ileum	31.50	23.80	14.50	131.16	0.82
	Caeca	69.75	133.00	18.00	166.44	0.42
Staphylococcus	Duodenum	9.50	2.00	1.25	3.53	0.24
	Jejunum	16.25	0.00	4.75	7.62	0.34
	Ileum	150.00	0.00	0.00	86.60	0.41
	Caeca	207.50	1.80	19.80	114.58	0.41
Coliform	Duodenum	0.75	0.00	3.75	1.20	0.12
	Jejunum	0.00	0.50	0.00	0.29	0.41
	Ileum	22.75 ^a	1.75 ^b	0.00 ^b	4.50	0.01
	Caeca	1.75	3.50	0.00	2.18	0.55

*Means on the same row with different superscripts are significantly ($P<0.05$) different. cfu- colony forming unit, SEM-Standard Error of Mean

4. CONCLUSION

The results of this study showed that enzyme supplementation reduced the digesta viscosity in birds fed 20% wheat-based diets while the gut integrity was not affected at this inclusion rate. It is therefore recommended that, enzyme supplementation may not be required for 20% whole-wheat inclusion in broiler diets at both starter and finisher phases.

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