

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. Digesta viscosity, which is partly correlated with the modulation of gut can be reduced when wheat-based diets is supplemented with NSP degrading enzyme.

In a 42-day trial, an experiment was conducted to evaluate the effect of Roxazyme G2G supplementation on digesta viscosity, gut microbial load, gut pH, and length 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. The diet consisted 4 replicates of 10 birds each. Body weight gain, feed intake, feed conversion ratio and feed efficiency ratio were the growth performance indices measured. 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.

The growth performance of birds fed wheat based-diet with enzyme was identical to those without enzyme and the control diet. 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. 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 improved gut pH, and reduced gut 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 extend the passage rate of digesta in the intestine [3,4], which may lower oxygen tension and favour the proliferation 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. Addition of Roxazyme G2G to poultry diets has been reported to confer numerous beneficial effects, such as improved digestibility, increased utilization of nutrients, reduced digesta viscosity and improved passage rate of digesta [6]. It was therefore the aim 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 0.025% of 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. During the experimental period daily feed intake was determined by deducting the left over from the total quantity of feed supplied to the birds. Weights of birds were monitored weekly were calculated as follows;

$$\text{Feed Efficiency Ratio} = \frac{\text{Weight gain}}{\text{Feed intake}}$$

$$\text{Feed Conversion Ratio} = \frac{\text{Feed intake}}{\text{Weight gain}}$$

Table 1: Gross composition of experimental broiler starter diets (0-21d) with 20% wheat inclusion and Roxazyme G2G supplementation (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 Analysis (%)			
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
Available 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-55mg; calcium pantothenate-11.5mg; vitamin B₆-5mg; vitamin B₁₂-0.025mg; choline chloride-500mg; folic acid-1mg; biotin-0.08mg; manganese-120mg; iron-100mg; zinc-80mg; copper-8.5mg; iodine-1.5mg; cobalt-0.3mg; selenium-0.12mg; Anti-oxidant-120mg.

Source: Agboola et al. [7]

Table 2: Gross composition of experimental broiler finisher diets (22-42 d) with 20% wheat inclusion and Roxazyme G2G supplementation (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 Analysis (%)

Moisture content	8.99	8.64	8.28
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
Available Phosphorus	0.46	0.54	0.54

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

Source: Agboola et al. [7]

2.1 Digesta collection, gut length and pH of gut content

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

2.2 Microbial load analysis

Intestinal content from the duodenum, jejunum, ileum and caeca segments were separately mixed in a 10ml pre-reduced salt medium and serially diluted according to the procedure described by [8] to examine the counts of E-Coli, staphylococcus, lactobacilli (Rogosa, CM 0627, incubated anaerobically 48 h) and coliforms (Mackonkey, CM 0115, incubated aerobically 24 h). The digesta samples were serially diluted from 10^{-3} to 10^{-7} . From each dilution, 0.1 ml of the sample was plated onto the appropriate medium for enumeration of bacteria.

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 Proximate and statistical analyses

The proximate composition of the diets was determined according to the method of AOAC [9]. Data obtained were analyzed using descriptive statistics and ANOVA ($P = .05$) SAS [10]. Mean differences were separated using Duncan multiple range test.

3. RESULTS AND DISCUSSION

The results of performance indices of broilers fed experimental diets are shown in Tables 3. There were significant differences ($P=0.05$) observed in the final weight, weight gain, feed intake and feed conversion ratio of birds on the experimental diets. However, diet had no effect on the feed efficiency ratio of birds at the starter phase. The final weight and weight gain of birds fed 20% wheat-based diet with enzyme supplementation were not significantly different from those on 20% wheat diet without enzyme and the control diet. Feed conversion ratio of birds fed 20% wheat-based diet with addition of enzyme compared favorably with those without enzyme supplementation. Similar feed intake was observed in birds on wheat diet with inclusion of Roxazyme G2G and those on the control diet which were significantly different from birds on wheat based-diet without enzyme supplementation. Similar trends were however recorded for most of the performance indices of birds measured at the finisher's phase. [11] reported that supplementation of poultry diets with enzymes destroys the anti-nutritive factors in cereals, by enhancing the overall digestibility of the feed and nutrient availability. The final weight, weight gain, feed intake and feed conversion ratio of birds fed 20% wheat diet with enzyme supplementation at the starter phase in this study is in variance with the findings of [12] who reported increase in performance parameters measured when birds were fed 20% wheat based-diets supplemented with exogenous enzyme at the starter phase. According to [12], an improved final weight, weight gain and feed intake of birds fed with 10% wheat diet with enzyme supplementation at the finisher phase was reported. In the present study, supplementation of diet with enzyme did not considerably improve the growth performance of birds on the dietary treatments when compared with those on wheat diet without dietary supplemental enzyme. This was contrary to the findings of [13] who reported that enzyme supplementation

of wheat-based diets improved broiler performance through increased feed intake and improved nutrient digestibility. The reason for an apparent lack of response is the possibility that the diets fed were of extremely good quality and allows the birds to perform close to their genetic potential. In such cases, enzyme supplementation will likely produce a negligible beneficial response [14]. In many reports, there are frequent instances where enzyme supplementation has failed to result in improved performance. However, it is incorrect to come to the general conclusion, as is often done, that a particular enzyme supplement adds little value to the particular ingredient or component [14]. Dietary constituents may also reduce the activity of supplementary enzymes either because the enzymes attach strongly to a component such as tannin or because a functional cofactor such as a particular trace element is replaced by another that hinders an activity [15].

Table 3: Performance indices (g/b) of broilers fed diets containing 20% wheat inclusion with or without Roxazyme G2G supplementation

		Control	20% whole wheat-enzyme	20% whole wheat+enzyme	SEM
Parameters					
Starter (0 - 21d)	Initial weight	110.05	117.90	112.30	3.62
	Final weight	702.50 ^b	785.00 ^a	737.22 ^{ab}	28.85
	Weight gain	592.45 ^b	667.10 ^a	624.92 ^{ab}	32.37
	Feed Intake	1085.00 ^b	1115.25 ^a	1047.75 ^b	24.94
	Feed conversion ratio	1.83 ^a	1.67 ^b	1.68 ^b	0.07
	Feed efficiency ratio	0.55	0.60	0.60	0.03
Finisher (22 - 42d)	Initial weight	702.50 ^b	785.00 ^a	737.22 ^{ab}	28.85
	Final weight	1927.00 ^b	2143.50 ^a	2085.50 ^a	40.17
	Weight gain	1224.50 ^b	1358.50 ^a	1348.28 ^a	49.89
	Feed Intake	2998.20	2872.10	2880.10	101.75
	Feed conversion ratio	2.45 ^a	2.11 ^b	2.14 ^b	0.10
	Feed efficiency ratio	0.41	0.47	0.47	0.03

^{ab} Means on the same row with different superscripts are significantly (P<0.05) different. g/b- gram per bird, SEM: Standard Error of Mean.
Source: Agboola *et al.* [7]

The results of the gut length, digesta viscosity, and pH of the gut content of birds on experimental diets are shown in Table 4. The results showed that there were no significant differences in the various sections of the Gastro Intestinal Tract (GIT) measured. According to [16], high gut viscosity decreases the rate of diffusion of substrates and digestive enzymes and hinders their effective interaction at the mucosal surface. There were no significant differences observed in the digesta viscosities of different sections of the GIT except at the duodenum. The digesta viscosity at duodenum section of birds fed 20% wheat without enzyme (1.28mPa-s) was significantly similar to those fed control diet (1.15mPa-s) but higher than those fed 20% wheat with enzyme (1.00mPa-s). The reduction in digesta viscosities of duodenal section 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

165 effects of the feed [17]. This result agrees with the reports of [18] and [6] on the benefits of
 166 exogenous enzyme.

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

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172 **Table 4: Gut length, Digesta Viscosity, and pH of gut content of broilers fed diets**
 173 **containing 20% wheat inclusion with or without Roxazyme G2G supplementation**

	Segment	control	20% whole wheat-enzyme	20% whole wheat+enzyme	SEM	P value
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 ^a	1.28 ^a	1.00 ^b	0.15	0.04
	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

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

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177 Table 5 shows the results of the microbial load of gut content of broilers fed wheat-based
 178 diets. The microorganisms in which the samples collected were analyzed for were Bacillus,
 179 *E. coli*, Staphylococcus, and Coliform. There were significant differences observed in most of
 180 the microbes at the different sections of the gastrointestinal tract (GIT). At the duodenal
 181 section, the concentration of Bacillus in birds fed 20% wheat without enzyme
 182 supplementation was significantly higher than those fed other diets with the least observed in
 183 those fed 20% wheat with enzyme supplementation. However, diets had no effect on the
 184 Bacillus concentration at the Jejunum. The population of Bacillus was similar at the ileal
 185 segment for birds fed control and 20% wheat without enzyme but significantly higher than
 186 those fed 20% wheat with enzyme inclusion. At the caeca, the bacillus population in birds
 187 fed control was significantly higher than those fed other diets with the least observed in
 188 those fed 20% wheat with enzyme supplementation. The population of staphylococcus was

not affected by the diets at the ileal and caeca segments. Similar trends were recorded for *E. coli* at the caeca section and coliform at the duodenal and jejunal sections. However, the concentrations of staphylococcus and *E. coli* were significantly higher at the duodenum of bird fed control diet than those fed other diets while least values were recorded in birds on 20% wheat with enzyme inclusion. At the jejunum, the concentration of staphylococcus was similar at the ileal segment for birds fed control and 20% wheat without enzyme but significantly higher than those fed 20% wheat with enzyme inclusion. The population of *E. coli* at the jejunum followed similar trend. 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 [19] who reported that there was no relationship between rye-based diet and microbial loads in some organs of birds fed such diets. However, it is known that the profile of gut microflora can be affected by a variety of factors such as diet composition [19], animal strain [20] and digesta flow rate [21].

Table 5: Microbial load (cfu x 10⁴/ml) of gut contents of broilers fed diets containing 20% wheat inclusion with or without Roxazyme G2G supplementation

	Segment	Treatment 1 (control)	Treatment 2 (20% whole wheat-enzyme)	Treatment 3 (20% whole wheat+enzyme)	SEM	P value
Bacillus	Duodenum	4.75 ^b	8.38 ^a	3.00 ^c	0.24	0.04
	Jejunum	2.00	2.86	3.75	0.34	0.87
	Ileum	24.50 ^a	23.80 ^a	11.88 ^b	2.78	0.04
	Caeca	6.75 ^a	4.00 ^b	0.25 ^c	0.43	0.02
Staphylococcus	Duodenum	9.50 ^a	2.00 ^b	1.25 ^c	0.33	0.03
	Jejunum	16.25 ^a	10.00 ^a	4.75 ^b	7.62	0.04
	Ileum	15.00	10.00	7.00	6.60	0.41
	Caeca	20.75	18.80	19.80	4.58	0.41
<i>E. coli</i>	Duodenum	10.00 ^a	3.75 ^b	0.75 ^c	1.20	0.02
	Jejunum	0.50 ^b	0.50 ^b	0.35 ^a	0.09	0.03
	Ileum	2.75 ^a	1.75 ^b	0.95 ^b	0.50	0.01
	Caeca	11.75	13.50	10.00	2.18	0.55
Coliform	Duodenum	17.55	23.90	22.30	3.24	0.61
	Jejunum	17.00	16.00	18.80	3.54	0.06
	Ileum	31.50 ^a	23.80 ^b	14.50 ^c	3.11	0.82
	Caeca	69.75 ^a	68.00 ^a	63.00 ^b	6.66	0.04

*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 did not positively influence the growth performance and the digesta viscosity of birds fed 20% wheat-based diets. However, the pathogenic load was reduced with enzyme inclusion rate. It is therefore recommended that, enzyme supplementation may be required in reduction of pathogenic load for 20% whole-wheat inclusion in broiler diets at finisher phase.

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REFERENCES

1. Annison G, Choct M. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies for minimizing their effects. *World Poult Sci.* 1991: 60: 232-242.
2. Bedford MR. Mechanism of action and potential environmental benefits from the use of enzymes. *Anim. Feed. Sci. Technol.* 1995: 53:145-155.
3. Gohl B, Gohl I. In: *Feed Non-Starch Polysaccharides: Chemical Structures and Nutritional Significance.* (Choct, M.ed). *feed Milling International.* pp. 13-26. feeding. *Int. J. Poult. Sci.* 1977: 7: 311-314.
4. Van Der Klis JD, Van Voorst A, Van Cruyningen C. Effect of a soluble polysaccharide (carboxymethyl cellulose) on the physicochemical conditions in the gastrointestinal tract of broilers. *British Poult. Sci.* 1993:34: 971-983.
5. Ouhida IJ, Perez JF, Gasa J, Puchal F. Enzymes (β -glucanase and arabinoxylanase and/or Sepiolite supplementation and nutritive value of maize-barley-wheat based diets for broiler chickens. *British Poultry Science.* 2000: 41:617-624.
6. Broz J, Ward NE. Effect of enzyme supplementation of feed enzymes in combating metabolic challenges and disorders. *J. Applied Poult. Res.*, 2007: 16: 150-159.
7. Agboola AF, Fagbemi MA, Oluwadamilare EI, Majolagbe OH. Effect of Roxazyme G2G supplementation on performance and carcass characteristics of broilers fed wheat-based diets. *Inter J Agri Biosci.* 2013: 2(6): 321-326. www.ijagbio.com.
8. Engberg RM, Hedemann MS, Steinfeldt S, Jensen BB. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poult. Sci.* 2004: 83, 925-938.
9. AOAC. Official Methods of Analysis Chemists. 16th Ed. Arlington, V.A, USA. 2000.
10. SAS Institute. SAS Users Guide Statics. Version 9.2. Ed. SAS Institute Inc., Cary, NC. USA. 2012
11. Choct M, Hughes J, Bedford MR. Effects of a xylanase on individual bird variation, starch digestion through the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. *Br Poult Sci*, 1999: 40: 419-422.
12. Agboola AF, Awoyemi MO, Inyang UO, Ogunbode SM. Growth response and nutrient digestibility of broiler chickens fed wheat-based diets supplemented with an exogenous enzyme. *Inter J Agri Biosci.* 2014: 3(2): 95-101. www.ijagbio.com.
13. Lázaro R, García M, Aranibar MJ, Mateos GG. Effect of enzyme addition to wheat-barley- and rye- based diets on nutrient digestibility and performance of laying hens. *Br Poult Sci*, 2003: 44: 256-265.
14. Acamovic T. Commercial application of enzyme technology for poultry production. *World's Poult Sci J*, 2001: 57: 225-243.
15. Bedford MR, Classen HL. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration of broiler chicks. *J Nutr*, 1992: 122: 560-569.
16. Ikegami S, Tsuchihashi F, Harada H, Tsuchihashi N, Nishide E, Innami S. Effect of various indigestible polysaccharides on pancreatic – biliary secretion and digestive organs in rats. *J. Nutr.*, 1990: 120: 353-360.
17. Bedford MR, Classen HL. An in-vitro assay for prediction of broiler intestinal viscosity and growth when fed rye based diets in the presence of exogenous enzymes. *Poultry Science.* 1993: 72: 137-143.
18. Agboola AF, Inyang, UO, Awoyemi, MO. Digesta viscosity and ileal morphology of broilers fed wheat-based diets supplemented with Roxazyme G2G. In: Abdullah, AR, Tayo, GO, Okubango, AO, Akinsoyinu, OA (Eds). *Proceedings of the 39th Annual conference of Nigerian Society of Animal Production (NSAP)*, Babcock University, Ilishan-Remo, Ogun state. 16th-19th March 2014. Pp 352-355.

- 271 19. Apajalahti JH, Kettune AA, Graham H. Characteristics of the gastrointestinal
272 microbial communities, with special reference to the chicken. *World's Poult. Sci. J.*
273 2004; 60:223-232.
- 274 20. Zoetendal EG., Collier CT, Koike S, Mackie RI, Gaskins HR. Molecular ecological
275 analysis of the gastrointestinal microbiota: a review. *J. Nutr.* 2004. 134:465-472.
- 276 21. Danicke S, Vahjen W, Simon O, Jeroch H. Effects of dietary fat type and xylanase
277 supplementation to rye-based broiler diets on selected bacterial groups adhering to
278 the intestinal epithelium, on transit time of feed, and on nutrient digestibility. *Poult.*
279 *Sci.* 1999. 78:1292-1299.