International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **9(3),** 33-37, August **(2020)** *Int. Res. J. Biological Sci.*

Short Communication

Evaluation of the effect of feeding commercial synbiotic and diet-acidifiers on histological and gut morphometric of Arbor-Acre broiler chickens

Arogbodo J.O.*, Adebayo I.A. and Adesida J.A.
Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria
arogbodojos@yahoo.com

Available online at: www.isca.in, www.isca.me
Received 11th March 2020, revised 22th June 2020, accepted 15th July 2020

Abstract

The desire to shift attention from synthetic drugs/growth promoter utilization in livestock feed calls for the exploration of alternative feed additives having less harmful effects on the health of food animals and human consumers. To study the effects of some of these other potential feed additives on broiler production, one hundred and forty four day old broiler chickens were sourced and divided into four groups in a Completely Randomised Design (CRD) experiment and reared for 8 weeks. Four diets were prepared to constitute four experimental groups. Group I was the control with the absence of synbiotic or acidifier. Synbiotic was included in Group II diet while Group III had buffered feed acidifier. Group IV diet was the only one in which Synbiotic and acidifiers were pulled together. Duodenal sections for gut morphometry were taken at the starter and finisher phases of the rearing period while that of histology were done at termination of the experiment. Microscopic examination of the sections revealed no observable lesions in all the duodenal villi from the various groups. However, the villi width as observed in Group IV (at the starter phase) had the longest measurement of 53.33±6.67µm while Group II had the deepest cryptal measurement of 66.67±12.02 µm. At the finisher phase, there was significant difference in the villi width. Group II had a relatively higher villi length (261.67±30.60µm) and deeper cryptal depth (66.67±8.82 µm) when compared with other experimental groups. The length and width of a villus are known to have a direct bearing on the digestion and absorption of nutrients by an animal. From these results, diet-acidifier and synbiotic had no deleterious effects on gut morphology of the experimental broiler chickens and as such could be used to replace antibiotics and anabolic steroids as growth promoters in the rearing of broiler chickens.

Keywords: Villi characteristics, histological examination, synbiotic, diet-acidifiers, broiler chickens.

Introduction

Unarguably, the challenge of antibiotic resistance is a worldwide and ever growing public health predicament. It occurs when strains of bacteria become resistant to antibiotics due to number of reasons, topmost of which is the improper use and abuse of antibiotics[1]. This invariably has become great health hazard concern and therefore envisaged that before long the common antibiotic drugs may not be potent on common infections any longer[2]. Antibiotic resistance is a serious and growing phenomenon in contemporary medicine[3] and has emerged as one of the eminent Public Health Concerns of the 21st century which consequently made the European Union (EU) placed a total ban on the use of antibiotic growth promoters in animal feeds[4].

Owing to the above facts, it is highly imperative and timely to look inward and upward to science in order to find alternatives to antibiotics that will not have adverse effect on the animals and yet on human beings. This act of looking inward and upward to science has brought into limelight biotechnology products such as prebiotics, probiotics, synbiotics and diet-acidifiers.

Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system in many ways claimed to be beneficial to health e.g. Mannan oligosaccharides (mos), Fructooligosaccharide (FOS) and Inulin[5-7]. "Antibiotics" are "against life", and "probiotics" are "for life" hence probiotics are best described as "amibiotics," i.e., "friendly organism" e.g. *Lactobacillus* and *Streptococcus*[8]. Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism, hence synbiotics. Synbiotic was defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract[9].

The synergistic effect of a prebiotic and probiotic has appreciable advantages over either of the two singly[10]. Diet-acidifiers are feed additives with great ability to be acidic and as such may present themselves as organic or inorganic acids. They possess the ability to modulate the pH of feed as well as that of the alimentary canal. With this ability, they are able to inhibit the growth of pathogenic bacteria[11]. Four organic acids commonly used in feed are, formic, acetic, propionic and lactic acid.

These acids have a specific ability to penetrate the bacterial cell wall and kill bacteria by interfering with their metabolism[12]. The mucus membrane of the small intestine consists of numerous tiny finger-like projections known as villi, thrown into folds called plicae thus forming the brush border. Animals with the most rapid digestive and absorptive processes have a more highly developed system of villi to provide a greater surface area for absorption[9]. In line with the above, this experiment was embarked upon to evaluate the integrity of villi from the various groups of the experimental chickens with a view of determining the suitability of synbiotics and diet acidifiers as alternative feed additives to antibiotics and anabolic steroids with myriads of adverse effects on human health.

Materials and methods

The present research was carried out with one hundred and forty four (144) day old Arbor Acre broiler chickens in Akure, Ondo State Nigeria (Longitude 5º8´53.868´´E and Latitude 7º17´35.652´´N). The chicks were reared under standard farm conditions. All chicks were weighed individually upon arrival at the experimental site and continued weekly till 8th week of age using digital electronic top pan balance with 1g accuracy. They were randomly divided into four groups with four formulated diets namely Diet 1 (control), II (synbiotic), III (diet-acidifier), and IV (synbiotic and diet-acidifier) as shown in Tables-1 and 2.

Histopathology and Gut Morphometry: Six chickens per group were sacrificed at the expiration of starter and finisher phases**.** Samples for histopathology were obtained from the duodenum of the sacrificed chickens and immediately stored in the prepared 10% formalin pending the time of tissue processing. The histopathology exercise was carried out and gut morphometry determined according to standard procedures[13, 14]. The length and width of the duodenal villi as well as cryptal depths were measured using well calibrated Zeiss Microscope X452 at X100 magnification.

Table-1: Experimental diets at Starter and Finisher phases (%).

Ingredients	Diet 1		Diet II		Diet III		Diet IV	
	ST	FI	ST	FI	ST	FI	ST	FI
Maize	60	56	60	56	60	56	60	56
Soyabean meal	10	16	10	16	10	16	10	16
Groundnut cake	19	8	19	8	19	8	19	8
Fish meal	5	4	5	4	5	4	5	4
Wheat offal	3	9	3	9	3	9	3	9
Bone meal	2.30	6.30	2.25	6.25	2.20	6.20	2.15	6.15
*Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.250	0.250
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Synbiotic	-	-	**0.05	**0.05	-	-	**0.05	**0.05
Diet-acidifier	-	-	-	-	**0.10	**0.10	**0.10	**0.10
Summation	100	100	100	100	100	100	100	100

Analysis of the experimental diets as per calculation							
Diets	CP (%)	ME (Kcal/KG)	DM (%)	EE (%)	CF (%)	Ca (%)	P (%)
Starter	22.46	3002.90	86.60	4.30	3.11	1.21	0.65
Finisher	20.09	2803.82	82.77	3.86	3.36	2.45	1.20

* Inclusion rate for broiler premix, ** Manufacturer's recommendation. CP= Crude protein, ME= Metabolizable Energy, DM= Dry Matter, EE= Ether Extract, CF= Crude Fibre, P= Phosphorus and Ca= Calcium.

Statistical Analysis: Collated data from the experiment were analyzed using Statistical Analysis System (SAS) software version 9.2 of 2009. The duodenal villi characteristics means were separated by the Duncan's Multiple Range of the same package[15].

Results and discussion

Obtained results from the histological and gut morphometric of broiler chickens fed commercial synbiotic and diet-acidifiers are presented in plates 1 – 4 and Tables-3 and 4.

Histopathological examination of the duodenum: The duodenum receives partially digested food and as such the absorption of nutrients water and electrolytes commences here, being the number one of the three divisions of the small intestine[16]. The duodenal villi of the experimental broiler chickens in the present study showed normal organ architectural presentations as no visible lesion was observed in all the groups (plates 1 – 4). The lamina epithelialis, lamina propria, lamina muscularis, submucosa muscularis and the brunner's glands were visible and normal; indicating the safety of the additives experimented. This has been one of the goals in animal production i.e., the production of meat that is abundant in health benefits to the consumers[17] and yet affordable.

Gut Morphometry: The duodenal villi length and width as well as the cryptal depth were determined at the starter and finisher phases as presented in Tables 3 and 4 respectively. The results showed no significant differences ($P > 0.05$). However, Group IV had an average villi width of 53.33 ± 6.67 µm at the Starter Phase while Group II recorded a cryptal depth of 66.67 ± 12.02 µm during the same phase. The finisher phase presented a significant difference ($P < 0.05$) of the villi width. The significant difference ($p < 0.05$) noticed in the villi width of the broiler chickens at the end of this experiment is at variance with the previous report[18] that no significant difference occurred in villi size and cryptal depth between control and other groups though with phytogenic feed additives. Group II had relatively longer villi length (261.67 ± 30.60 µm) and cryptal depth (66.67 ± 8.82 µm) when compared with other experimental groups. All these showed that the functionality and morphology of the duodenal sections were not compromised by the feeding of these additives. This result agrees with other authors[19] who reported the advantages of probiotics, improved haeamatological parameter and healthy gut of broiler chickens[20]. The simple deduction from above is that probiotics are promising alternative to antibiotic growth promoters (AGP)[21]. The longest villi length recorded in group II (probiotics group) also agrees with the findings of previous authors[22-24] that probiotics and diet- acidifiers (with appreciable cryptal depth of 46.67 ± 16.07 µm at the finisher phase) are additives once had beneficial effect on the gut health of poultry birds through increament in villus height and villus to crypt ratio. Physiologically, the exposed surface area of a villus is directly proportional to efficient digestion and absorption of food (nutrients) for optimum growth performance[25]. On the other hand, cryptal depth has to do with the secretion and storage of digestive enzymes which ultimately contribute to efficient feed utilization and improved feed conversion ratio as the preponderance of these crypts (glands) is indicative of competent mucosal functions during the process of digestion. Most of the duodenal glands (Brunners glands and Goblet cells) are known to be actively involved in the absorption of triglycerides which are preferentially taken up by lacteals rather than blood capillaries[26].

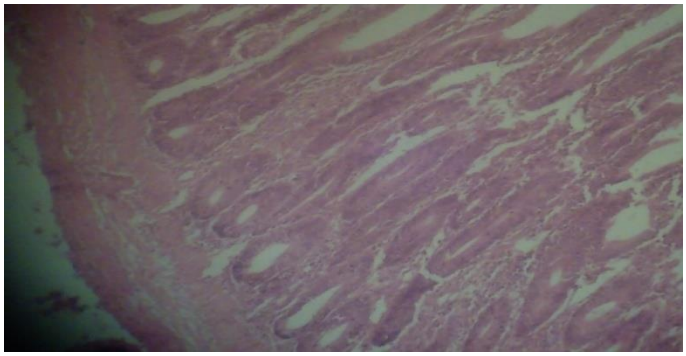

Figure-1a: Group I (control).

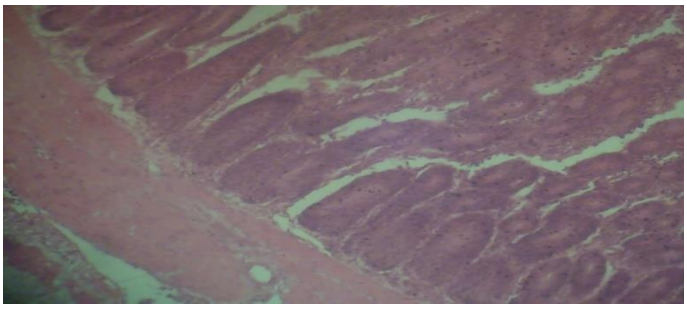

Figure--1b: Group II.

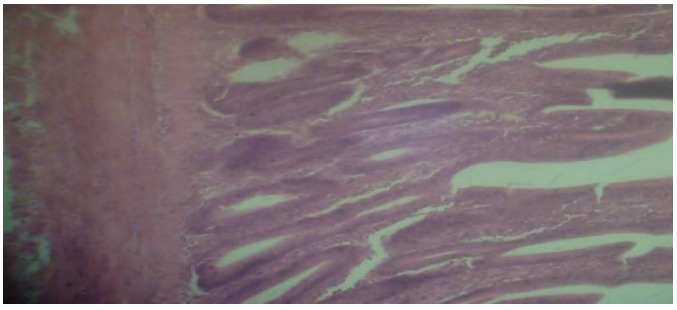

Figure-1c: Group III.

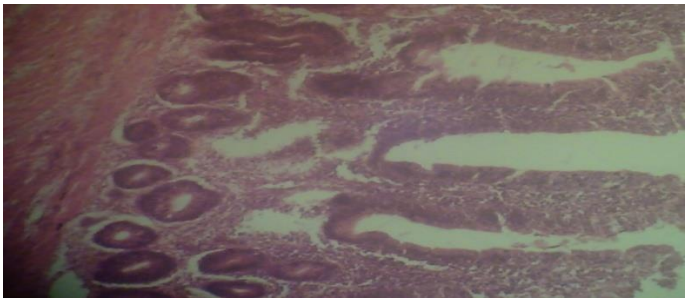

Figure--1d: Group IV.

Photomicrograph of the duodenal section of the experimental broiler chickens (Group I to IV) stained with H&E (X100), showing normal architectural morphology of the organ

Table-3: Duodenal morphometry (µm) of experimental broiler chickens (starter phase).

Treatments	Villi length	Villi width	Cryptal depth
G1	153.33 ± 25.17	38.33 ± 10.41	60.00 ±17.32
G2	125.00 ± 35.00	40.00 ± 10.00	66.67 ±20.82
G3	84.00 ± 101.43	41.67 ± 29.30	46.67 ± 30.14
G4	126.67 ± 64.29	53.33 ± 11.55	31.67 ± 10.41
Mean	122.25 ± 60.25	43.33 ± 16.00	51.25 ± 22.68
Remark	NS	NS	NS

G=Group and NS = Not significantly different (P > 0.05)

Table-4: Duodenal morphometry (µm) of experimental broiler chickens (finisher phase).

Treatments	Villi length	Villi width	Cryptal depth
G1	256.67±51.32	63.33± 25.17^{a}	100.00 ± 65.57
G2	261.67±52.99	33.33 ± 5.77^{b}	66.67 ± 15.28
G3	206.67±55.08	41.67 ± 12.58^{ab}	46.67 ± 16.07
G4	236.67±66.58	36.67 ± 5.77^{ab}	43.33 ± 12.58
Mean	240.42±53.45	43.75 ± 17.47	64.17 ± 38.13
Remark	NS	*	NS

Where: G= Group, * = Significantly different (P < 0.05), NS = Not significantly different (P > 0.05), Means ± SD (Standard deviation) with different superscripts within the same column are significantly different (P < 0.05)

Conclusion

The morphometric and histological findings showed normal architectural display of the critical segment of the small intestine (duodenum) when compared with the control. This can therefore form the premise by which inference could be made, that the two feed additives had no detrimental effects on the mucosal integrity of the experimental broiler chickens.

Recommendation: The experimented commercial additives (Diet-acidifiers and synbiotics) can be said to possess enviable qualities that qualify them as alternatives to antibiotics and anabolic steroids growth promoters.

References

1. ACP (2013). American College of Physicians. International Medicine/Doctors for Adults. 190 North Independence mall west, Philadelphia, 1-5.

2. Adetokunbo, O. L. and Hebert, M. G. (2003). Short Text Book of Public Health Medicine for the Tropics. 4th edition. Printed and Bound in Malta by Gutenberg Press Ltd. 40-45.

3. Herbs 200.com (2002). Use and Misuse of Antibiotics. 2002-2013, herbs 200.com. 1-4. http:www.whatarebacteria.com./streptococcus-faecalis (2014). 1-4.

4. World Health Organization (2011). Tackling antibiotic resistance from a food safety perspective in Europe. Regional office for Europe scherfigsvej 8, DK-2100 Copenhagen, Denmark.

5. Yusrizal, Y. and Chen, T. C. (2003). Effect of Adding Chicory Fructans in Feed on Broiler Growth Performance, Serum Cholesterol and Intestinal Length. *Int. J. Poult. Sci*., 2003, 2(3), 214-219.

6. Rahmani, H.R. and Speer, W. (2005). Natural Additives influence the Performance and Humoral Immunity of Broilers. *Int. J. Poult. Sci*., 2005, 4(9), 713-717.

7. Fuller, R. (1992). Probiotics. The Scientific basis. Ed. Fuller, R. Champman and Hall. London. *Proceedings of 33rd Annual Conference, Nigerian Society for Animal Production.* 90-92.

8. Banerjee, G. C. (2012). A Text Book of Animal Husbandry. Eighth Edition. Oxford and IBH Publishing Company Pvt. Ltd. 113-B Shahpur Jat. Asian Games Village Side New Delhi 110049, India. 927-930.

9. Awad, W. A., Ghareeb, K., Abdel-Rahhem, S. and Bohm, J. (2009). Effects of Dietary Inclusion of Probiotic and Synbiotic on growth Performance, Organ Weights, and Intestinal Histomorphology of Broiler Chickens. *Poult. Sci.*, 88(1), 49-56.

10. Gallaher, D. D. and Khil, J. (1999). The Effect of Synbiotic on Colon Carcinogenesis in Rats. *J. Nutr.*, 129 (Suppl. 7), 1483S-1487S.

11. Martins, F.S., Nardi, R.M., Arantes, R.M., Rosa, C.A., Neves, M.J. and Nicoli, J.R. (2005). *Journal of Genetics and Applied Microbiology*, 51, 83-89.

12. Corne, Van der Ejik (2002). Acidifiers and Antibiotic growth Promoters Compared. *Feed mix*, 10(6), 34-36. www.AgriWorld.nl.

13. Lamb, G. B. (1981). Manual of Veterinary Techniques in Kenya. Published by CIBA-GEGY. 127-147.

14. Yu, B., Tsai, CC., Hsu, J. C., and Chiou, P W S (1998). Effect of Different Sources of Dietary Fibre on Growth Performance, Intestinal Morphology and Caecal carbohydrate of Domestic Geese. *Br Poultry Science,* 39, 560-567.

15. SAS Institute (2009). Statistical Analysis System (SAS), users' Guide. Release 9.2 Ed. SAS Institute Inc., Cary, NC.

16. Kim, B. (2020). Duodenum: Anatomy, histology, composition, functions. www.kenhub.com. Acessed on 10/03/2020.

17. Ivana, P., Maja, M., Mirela, P., Ksenija, M., Valerija, B., Ivan, M and Matija, D. (2019). Intestinal Morphology in Broiler Chickens Supplemented with Propolis and Bee Pollen. *Animals MDPI* 9, 301; doi: 10.3390/ani9060301.

18. Mounia, M., Nadir, A and Omar, B. (2018). Effects of phytogenic products on gut morpho-histology of broiler chickens. *Int . J. Vet Sci Res,* 4(1), 009-0011.

19. Yang, Y., Iji, P. A. and Choct, M. (2009). Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poult. Sci. J.,* 65, 97-114.

20. Biswas, A., Junaid, N., Kumawat, M., Qureshi, S. and mandal, A B. (2018). Influence of Dietary Supplementaion of Probiotics on Intestinal Histomorphometry, Blood Chemistry and Gut Health Status of Broiler Chickens. *South African Journal of Animal Science,* 48(5), 967-976.

21. Widya, P. L., Teguh, B. P., Anam, A., Soeharsono, S., Sri, H., Nenny, H., Rifqy, N., Khoirul, H., Hana, C. P. W., Nabil, F. N. R and Andreas, B. Y. (2019). Potency of probiotics *Bifidobacterium spp*. and *Lactobacillus casei* to improve growth performance and business analysis in organic laying hens. *Veterinary World*, 12(6), 860–867.

22. Agboola, A. F., Aroniyo, I., Suberu, S. A and Adeyemi, W. T. (2014). Dietary supplementation of probiotics and synbiotics on intestinal microbial populations and gut morphology of turkey poults. *African Journal of Livestock Extension,* 14, 13-20.

23. Omidiwura, B. R. O., Agboola, A. F., Olaleye, O. O and Iyayi, E. A. (2018). Influence of Prebiotics, Probiotics or Synbiotics on Performance, Intestinal Mucosal Integrity and Gut Microbiota of Turkey Poults. *Nigerian J. Anim. Sci.,* 20(2), 64-75.

24. Garcia, V., Catala-Gregory, P., Hermandaz, F., Megias, M.D. and Madrid, J. (2007). Effect of formic acid and plant extracts on growth, nutrient digestibility, intestinal mucosa morphology and meat yield of broilers. *Journal of Applied Poultry Research Winter,* 16(4), 555-562.

25. Seeley, R. R., Stephens, T. D., and Tate, P. (2008). In Anatomy and Physiology. 8th Ed., Pub McGraw Hill, New York. USA pg 874-876

26. Mescher, A L. (2010). In Junqueira's Basic Histology (Text and Atlas). Int. Ed. McGraw Hill Pub. Singapore. Pg 271.