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Influence of Prebiotics, Probiotics or Synbiotics on Performance, Intestinal Mucosal Integrity and Gut Microbiota of Turkey Poults

[*]Omidiwura, B. R. O, Agboola A. F, Olaleye, O. O and Iyayi, E. A.

Department of Animal Nutrition, Department of Animal Science, University of Ibadan, Nigeria
[*]Corresponding author: richardwura@gmail.com; +234-708-207-7886

***Target audience**: Animal scientists and researchers, Feed millers, Turkey farmers*

Abstract

In a four-week experiment, 125 four-week old turkey poults were distributed into five dietary treatment groups: treatment 1 was basal diet while 2, 3, 4 and 5 contained antibiotics, prebiotics, probiotics and synbiotics (combination of prebiotics and probiotics) respectively. Each diet replicated five times in a completely randomised design. At day 56, poults were sacrificed and ileal digesta samples collected for microbial load count and sections from the ileum for histomorphological measurements using standard procedures. Data were analyzed using descriptive statistics and ANOVA at α0.05. The results showed similar feed intake for birds fed experimental diets except those supplemented with synbiotics which was significantly different. The villus height; crypt depth, villus width, villus:crypt ratio except the epithelial cell thickness were significantly influenced. The final weight, weight gain, feed conversion ratio, protein efficiency ratio of the birds and the microbial load were not influenced by the diets. However, the results obtained from the histological indices showed that birds fed prebiotics, probiotics or synbiotics supplementations respectively performed better compared to birds fed antibiotics and basal diets. Therefore, probiotics, prebiotics or synbiotics can be a suitable replacement as growth promoters to conventional antibiotics in turkey poults.

Keywords: Antibacterial growth promoters, Histomorphological measurements, Ileal digesta, Turkey chicken

Description of Problem

The use of small doses of antibiotics for therapeutic purposes and also as antibacterial growth promoters (AGPs) in livestock production have proved effective in the control of diseases and also improved performance (1) by reducing microbial load in the gut, thereby ensuring increased availability of nutrients to the animal (2). Although, the use of dietary antibiotics in feed have been limited or prohibited in many countries especially in Europe, due to the development of drug-resistant bacteria (3) which can be transmitted to the human microbiota (4) and the presence of antibiotic residues in poultry meat and eggs that may have harmful effects on human consumers and also imbalance of normal microflora (5). There is thus a need to seek for viable alternatives capable of increasing the defensive capacity of livestock while avoiding AGPs use and maintaining adequate production levels. Alternatives such as prebiotics, probiotics, synbiotics, organic acids which are added as supplements have been reported to have potential to reduce enteric disease in poultry and subsequent contamination of poultry products (6). They also play a very important role in the balance and multiplication of the beneficial microbial population in the gastrointestinal tract thereby

ensuring digestive health (7) and also improved growth performance and intestinal morphology (8; 9). The state of the intestinal microbiota determines the well being of the host animal; under normal health and nutrition conditions, the main role of the commensal gut microbial community in monogastrics, including birds, is related to its ability to provide an effective health barrier against invading pathogens (10). The use of nutraceuticals such as probiotics and prebiotics may prove beneficial in modulating intestinal microbiota, enhancing immune response and also protecting the intestinal integrity thus, improving performance of poultry birds (11). However, there is limited information on the influence of these feed additives on the intestinal mucosa integrity and gut microbiota of turkey poults as current knowledge have been primarily extrapolated from broiler chicken studies. It was therefore the objective of this study to evaluate the effect of prebiotics, probiotics or synbiotics on the performance, the intestinal mucosal integrity and gut microbiota of turkey poults.

Materials and Methods

Experimental site

This experiment was carried out at the Poultry unit of the Teaching and Research Farm, University of Ibadan, Oyo State in the South –West zone of Nigeria which is within the tropical rain forest region.

Experimental diets and management of the turkey poults

One hundred and twenty-five one-day-old unsexed turkey poults (Nicholas strain) were sourced from a reputable commercial hatchery. The turkey poults were fed on the basal diet to stabilise them for a period of four weeks after which they were weighed, tagged and sorted by body weight into five dietary treatments and five replicates of five birds each in a completely randomised design. Experimental diets and clean water were given *ad libitum* during the study period that lasted for 8 weeks. The basal diet was a corn-soya bean meal diet formulated to meet the nutrient requirements (12) for starter (8 to 28 days) and grower (29 to 56 days) turkeys. Diet 1 was the negative control (basal without antibiotic); diet 2 was the Positive control containing amoxicillin trihydrate and colistin sulphate added at the rate of 200g/tonne feed; diet 3 contained the basal diet with mannanoligosaccharides at the rate of 500g/tonne; diet 4 was the basal diet with *Bacillus subtilis* (probiotics) also added at the rate of 500g/tonne and diet 5 had the basal diet plus synbiotics (prebiotics + probiotics used in diets 3 and 4 respectively) at the rate of 500g/tone. The gross compositions of the experimental diets (starter and grower) are presented in Tables 1 and 2.

Data and sample collection

Performance indices

Performance parameters such as; feed intake, body weight gain, feed conversion ratio, protein intake and protein efficiency, were calculated. Feed intake was calculated as difference between amounts of feed given and left over. The birds were weighed at the end of the grower phase and values were used to calculate body weight gain, feed conversion ratio, protein intake and protein efficiency ratio.

Organ weight

At day 56, one bird per replicate was sacrificed through cervical dislocation and eviscerated. The liver, lungs, heart, gizzard, spleen, bursa of fabricus and pancreas were harvested and weighed.

Gut morphology

From the birds sacrificed and eviscerated, the transverse and longitudinal sections of the ileal tissue were cut and tissues from each group of birds in replicate were preserved in

pre-labelled polyvinyl bottle filled with 10% formalin and later processed for histological examination according to the methods of (13).

Gut histomorphometry

The slides of tissues of the gastrointestinal tract were examined under the light microscope at x 400 magnification and villus height, villus width, crypt depth and epithelial thickness were measured with the aid of the graticle in micrometers (µm) and the multiplication factor of 0.209293.

Microbial count

Digesta was collected from the ileum of each bird for microbial count and stored in a sterile container and refrigerated at 4ºC (14).

The culture media were prepared 24 hours before collected samples were poured into petri-dishes. To examine the count of Lactobacilli (Man Rogosa Sharpe agar, incubated anaerobically 48h); Total bacteria count (nutrient agar, incubated aerobically 24h); *Escherichia coli* (Eosin methyl blue agar and Salmonella (Salmonella shigella Agar, incubated aerobically 24h) were used. One millilitre of the digesta was added to a 9ml pre-reduced salt medium in other tubes. The suspension was prepared from 10^{-1} dilution and serial dilutions were done (10^{-2}-10^{-5}), then serial dilution at 10^{-3} and 10^{-5} was used to culture the media. From the dilution, 0.1 ml of the sample was plated onto the appropriate medium for enumeration of bacteria. Discrete colonies on plates were counted using a colony counter and counts estimated in logarithm number of bacteria per 1-g sample ($\log_{10}$ Cfu/g).

Chemical and statistical analysis

The proximate compositions of diets used were determined by the methods of (15). Data obtained were analysed using ANOVA of SAS (16) and significant level of $P< 0.05$. Mean differences were compared using Duncan Multiple Range Test.

Results and Discussions

The results on performance are as shown on Table 3. The experimental diets fed had effect ($P< 0.05$) on the feed intake and protein intake of the birds. Similar feed intake was recorded for birds on the negative control (1421.52g/poult), positive control (1452.52g/poult), prebiotics (1354.36g/poult) or probiotics (1402.64g/poult) experimental diets but significantly higher ($P< 0.05$) than birds supplemented with synbiotics (1287.16 g/poult) but still similar to those fed prebiotics. Similar trends were observed in the protein intake of birds on the dietary treatments, the protein intake of birds fed the basal diet only (NC) was highest (368.17g/poult) and was significantly ($P< 0.05$) different from the birds supplemented with synbiotics (333.37g/poult) which was the lowest. No significant differences were observed in the live weight, final weight, weight gain, feed conversion ratio and protein efficiency ratio of birds on experimental diets.

The results of the present study showed that the inclusion of probiotics, prebiotics and synbiotics in corn-soya bean meal based diets did not improve feed conversion ratio (FCR), body weight gain, and protein efficiency ratio but had an effect on feed intake and protein intake at the grower phase. This result is in agreement with the findings of (17), that the inclusion of a multi-strain DFM (Lactobacilli, Bacillus, *Saccharomyces cerevisiae*) significantly influenced feed intake. According to the observations of (18), it was reported that the inclusion of prebiotics and probiotics in diet improved feed intake of broilers which is still in line with the results obtained in this study. Evaluating the use of different growth promoters in broilers, (19) found no effect of using these growth promoters on the final weight, weight gain, and feed intake of broiler

chickens from 22 to 43 days of age. Similar results were also observed by (20), who found no effect of using inulin and probiotics on the final weight, weight gain, feed intake and feed conversion of broilers in the period of 1-42 days of age. Likewise, (21) observed no significant differences in performance variables of broilers aged from 1 to 42 days when fed different antibiotics and probiotics. In probiotics, (22) demonstrated that the level of enzyme production that probably enhances digestibility and absorption of nutrients in the gut (23) differs between different bacteria species and even between strains of the same species. Therefore, it is very difficult to draw a parallel between studies and also directly compare different studies using different growth promoters because the efficiency of a growth promoter depends on several factors such as: age, host species, strains, dosage of administration, diets, storage conditions, environment, mode of action among others.

As presented in Table 4, the supplementation of prebiotics, probiotics and synbiotics had no significant effect on the relative weight of liver, lungs, heart, spleen, pancreas, bursa of fabricius and gizzard of turkey poults. The results obtained in this study were similar to the findings of (8) that weights of liver, gizzard, and bursa of fabricius were not affected by the inclusion of probiotics and prebiotics in broilers' diets. The results in this study also averred the observations of (24) that the relative weights of the gizzard, heart, liver and pancreas were unaffected by the supplementation of probiotic and symbiotic in the diets of turkey poults. (25) also agreed to the fact that dietary mannanoligosaccharides and Lactobacilli spp. had no effect on gizzard weight of broilers.

The results of the ileal histomorphometry of turkey poults fed diets supplemented with prebiotics, probiotics or synbiotics are represented in Table 5. Significantly higher villus height was observed in birds fed diet supplemented with antibiotics (positive control, PC) (1335.70µm) than those fed basal diet without supplementation (negative control) (780.70µm). The villus height of birds fed the probiotics (1233.20µm), prebiotics (1113.70µm) or synbiotics (10.73.50µm) were not different from one another and were as well similar to birds on the antibiotics supplemented diet (1335.70µm). The villus height of birds fed negative control (780.70µm) was similar ($P > 0.05$) to those on prebiotics (1113.70µm) or synbiotics supplemented diets (1073.50 µm) but significantly ($P < 0.05$) different from the positive control (1335.40µm) or probiotics group (1233.20µm).

There were significant ($P < 0.05$) differences in the villus width of turkey poults on the dietary treatments. The villus width recorded in birds fed antibiotics supplemented diet (217.54µm), though similar to those on synbiotics supplemented diets (198.24µm), was significantly ($P < 0.05$) higher than birds fed the prebiotics (155.65µm), probiotics (170.67 µm) and no supplementation (163051µm) groups which were similar ($P > 0.05$).

The dietary treatments influenced the crypt depth; the birds on the antibiotics supplemented diet (141.53µm) had statistically similar crypt depth with birds fed synbiotics supplemented diet (152.55µm) but were significantly higher than those on the prebiotics (112.70µm) and probiotics (120.71µm) supplemented diets but lower than the crypt depth of birds fed diet without supplementation (170.53µm). There were significant ($P < 0.05$) differences observed in the villus to crypt depth ratio of birds on dietary treatments. The villus: crypt ratio of birds fed antibiotics supplemented diet (9.84) was not different from those on the probiotics (10.37) and prebiotics supplemented diets (9.99) but were significantly ($P < 0.05$) higher than the villus: crypt ratio of birds on

synbiotics supplemented diets (7.12). The least ratio was observed in birds fed diet without supplementation (5.51). The epithelial cell thickness was not influenced by the experimental diets.

According to (26), the health status of the gastrointestinal tract of an animal is a true reflection of the intestinal morphology, which also affects proper nutrients absorption and immune response. In the present study, results on histomorphometry showed that birds fed the antibiotics supplemented diet had the highest villus height but was not different from the dietary groups. Although birds fed diet without supplementation had the shortest villus height, they also had the longest crypt depth and was significantly different from the prebiotic, probiotic or the antibiotics groups which were similar. From literature, longer villi are associated with activation of cell mitosis (23) while shortening of villi and deeper crypts lead to poor nutrient absorption, increased secretion in the gastrointestinal tract and reduced performance (27). The short villus height and increase in the crypt depth of the birds fed diet without supplementation likely indicates poor absorption of nutrients and increases in the poults' susceptibility for pathogens which will subsequently impair the birds' growth (28). According to (29), a large luminal area with villus height and mature enterocytes provides maximum absorption and digestion capacity and it is essential to animal development.

In the present study, supplementation with *Bacillus subtilis* in turkey poults resulted in increased villus height, villus height to crypt ratio and also decreased crypt depth in the ileum at day 56. The results obtained in the present study are consistent with the findings of (23) who reported increased villus height and villus height to crypt depth ratio in the duodenum of birds supplemented with *Bacillus subtilis var. Natto.* It is also in agreement with the findings of (30) and (31) who both reported that supplementation with probiotics significantly influenced villus height of turkey poults and broilers respectively. However, a higher villus height to crypt depth ratio results in a decreased turnover of the intestinal mucosa (32). A slower turnover rate of the intestinal epithelium results in a lower maintenance requirement, which may lead to a higher growth rate or growth efficiency of the animal (33) because less energy will be used by the gut, thus allowing for the partitioning of more resources toward production and growth (34). In addition, a deeper crypt may indicate faster tissue turnover to permit renewal of the villus, which indicates that the host's intestinal response mechanism is trying to compensate for normal sloughing or atrophy of villi due to inflammation from pathogens and their toxins (35). In contrast, (36) reported that increase in crypt depth could be beneficial to the host as it may represent an increase in the number of proliferating stem cells which could increase the number of mucin producing goblet cells, which are important constituent of innate defence system.

The results on Table 6 show the influence of different dietary supplementations on the microbial population in the ileal section of turkey poults. Diets had no significant effect on total bacteria count, Salmonella, *Escherichia coli* and lactic acid bacteria at the ileal section of turkey poults.

The results on microbial load showed that total bacteria count, Salmonella, *E.coli* as well as lactic acid bacteria (LAB) concentrations were not affected by the dietary treatments. This was not expected but was in agreement with (37) who also observed no difference in the microbial population of Salmonella, *E.coli,* Streptococcus, LAB by supplementing probiotics at graded levels of inclusion (200, 400, 600 mg/kg) in broilers feed. In the findings of (38), Coliforms, *E.coli* as well as Lactobacilli and Enterococci concentrations were not affected by dietary supplementations of mannanoligosaccharide and/or Bacitracin

methylene disalicylate in turkey poults at 6 weeks. The reasons for these results could be because turkeys are raised for a longer period of time unlike broilers that are raised for 6-8 weeks, it might take a longer time for the microbial population to be fully established in their gut.

Conclusions and Applications

The results obtained from the present study showed that

1. Birds fed dietary supplementations (probiotics, prebiotics or synbiotics) and the positive control (antibiotics) performed better in term of performance when compared to the negative control (basal diet without supplementation).
2. The results also indicated an improvement in the gut integrity of poults with dietary supplementation of the aforementioned feed additives with increasing villus height and villus to crypt ratio, thereby increasing the surface area of the villi for nutrients absorption.
3. Based on the results obtained, the present study showed similarity in parameters measured for poults on antibiotic and other dietary supplementations (probiotics, prebiotics or synbiotics).
4. To avoid the continuous development of drug-resistant bacteria from the over-use of antibiotics as growth promoter, it is suggested that probiotics, prebiotics or synbiotics can serve as viable replacement to the use of antibiotics to improve birds' performance thereby reducing cost of production and prevent the transmission of such drug-resistant bacteria to humans.
5. It is also suggested that to increase beneficial bacteria such as Lactobacilli and other lactic acid bacteria, probiotics, prebiotics or synbiotics should be administered early in poults' life.

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Table 1: Gross composition of experimental diet (starter phase, 0-28 days)

Ingredients g/kg	Basal diet
Maize	435.00
Soyabean Meal	483.00
Fish Meal	25.00
Soya Oil	20.00
Dicalcium phosphate	16.00
Premixes	2.50
Limestone	14.00
Methionine	1.00
Lysine	1.00
Salt	2.50
Total	1000.00
Calculated analysis	
Crudeprotein (g/kg)	269.0
Energy ME, kcal/kg	2809.38
Fat g/kg	35.43
Crude fibre g/kg	43.55
Calcium g/kg	10.32
Total P, g/kg	7.79
Non-phytate P g/kg	4.36

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.

Table 2: Gross composition of experimental diet (Grower phase, 29-56days)

Ingredients (g/kg)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Maize	456.00	456.00	456.00	456.00	456.00
Soyabean Meal	467.00	466.80	466.50	466.50	466.00
Fish Meal	18.00	18.00	18.00	18.00	18.00
Soya Oil	29.00	29.00	29.00	29.00	29.00
Dicalcium Phosphate	15.00	15.00	15.00	15.00	15.00
Premixes	2.50	2.50	2.50	2.50	2.50
Limestone	8.00	8.00	8.00	8.00	8.00
Methionine	1.00	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00	1.00
Salt	2.50	2.50	2.50	2.50	2.50
Antibiotics	0.00	0.20	0.00	0.00	0.00
Prebiotics	0.00	0.00	0.50	0.00	0.50
Probiotics	0.00	0.00	0.00	0.50	0.50
Total	1000	1000	1000	1000	1000
Calculated nutrients					
Crude protein g/kg	259.48	259.39	259.26	259.26	259.26
Energy ME, kcal/kg	2901.67	2901.22	2900.55	2900.55	2900.55
Fat g/kg	35.39	35.38	35.37	35.37	35.37
Crude fibre g/kg	42.84	42.83	42.81	42.81	42.81
Calcium g/kg	7.79	7.79	7.79	7.798	7.79
Total phosphorus g/kg	7.35	7.35	7.35	7.35	7.35
Non-phytate P, g/kg	4.14	4.14	4.14	4.14	4.14

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.

Diet 1 – basal diet, diet 2 – basal diet + antibiotics (amoxicillin trihydrate and colistin sulphate); diet 3 – basal diet + mannanoligosaccharides (prebiotics); diet 4 – basal diet + probiotics (*Bacillus subtilis*); diet 5 – basal diet + synbiotics (probiotic and prebiotic).

Table 3: Performance of turkey poults (grower phase) fed experimental diets

Parameters	Treatments					SEM	P-value
	NC	PC	NC + Prebiotics	NC + Probiotics	NC + Synbiotics		
Initial weight (g/poult)	265.08	273.56	271.64	271.02	269.72	6.90	0.99
Final weight (g/poult)	862.92	907.88	896.57	900.80	884.87	19.12	0.96
Weight gain (g/poult)	597.84	634.32	624.93	629.78	615.15	13.38	0.93
Feed intake (g/poult)	1421.52[a]	1452.52[a]	1354.36[ab]	1402.64[a]	1287.16[b]	18.86	0.03
Feed conversion ratio	2.38	2.29	2.19	2.26	2.10	0.05	0.38
Protein intake (g/poult)	368.17[a]	376.20[a]	350.78[ab]	363.28[a]	333.37[b]	4.88	0.03
Protein efficiency ratio	1.63	1.68	1.78	1.73	1.84	0.04	0.35

[a, b,] : Means with different superscripts on the same row are significantly different (P< 0.05)
values are means of 5 replicates of 5 birds each
NC= Negative Control, PC= Positive Control
SEM= Standard error of mean

Table 4: Relative Visceral Organs Weights (g/100g live weight) of Poults fed Experimental Diets

Parameter	TREATMENTS						
	NC	PC	NC + Prebiotics	NC + Probiotics	NC + Synbiotics	SEM	P-value
Liver	1.78	1.75	1.76	1.99	1.95	0.05	0.29
Lungs	0.53	0.49	0.55	0.65	0.55	0.03	0.43
Heart	0.49	0.55	0.49	0.51	0.55	0.01	0.65
Spleen	0.11	0.10	0.10	0.10	0.15	0.01	0.62
Bursa of Fabricius	0.16	0.17	0.20	0.20	0.18	0.01	0.87
Pancreas	0.32	0.27	0.26	0.29	0.30	0.01	0.65
Gizzard	5.66	4.86	5.08	5.96	5.17	0.20	0.43

Means with different superscripts on the same row are significantly different (P< 0.05)
values are means of 5 replicates of 1 bird each
NC= Negative Control, PC= Positive Control
SEM= Standard error of mean

Table 5: Ileal Histomorphometry of Turkey poults fed Supplemented Diets

Parameter	TREATMENTS						
	NC	PC	NC + Prebiotics	NC + Probiotics	NC + Synbiotics	SEM	P-value
Villus Height (µm)	780.70^{b}	1335.70^{a}	1113.70^{ab}	1233.20^{a}	1073.50^{ab}	59.06	0.0224
Villus Width (µm)	163.51^{c}	217.54^{a}	155.65^{c}	170.67^{bc}	198.24^{ab}	6.30	0.0017
Crypt Depth (µm)	170.53^{a}	141.53^{b}	112.70^{c}	120.71^{c}	152.55^{b}	6.69	0.0259
Epithelial cell(µm)	47.69	60.79	58.04	55.41	61.87	2.49	0.4142
Villus:Crypt ratio	5.51^{c}	9.84^{a}	9.99^{a}	10.37^{a}	7.12^{b}	0.55	0.0058

$^{a, b, c}$: Means with different superscripts on the same row are significantly different (P<0.05)
values are means of 5 replicates of 1 bird each
NC= Negative Control, PC= Positive Control

Table 6: Ileal Microbial Counts (log_{10} cfu/g) of Turkey Poults on Experimental Diets

Microflora (log_{10} cfu/g)	**TREATMENTS**						
	NC	PC	NC + Prebiotics	NC + Probiotics	NC + Synbiotics	SEM	P-value
TBC	5.4	6.2	4.98	6.44	4.17	0.49	0.62
Salmonellae	4.0	2.5	1.4	2.64	2.64	0.66	0.85
E. Coli	4.1	6.9	5.38	5.73	5.39	0.56	0.68
LAB	4.1	4.2	4.34	4.27	5.62	0.69	0.96

Means with different superscripts on the same row are significantly different (P< 0.05)
values are means of 5 replicates of 1 bird each
NC= Negative Control, PC= Positive Control, CFU= Colony forming unit
TBC= Total bacteria count, LAB= Lactic acid bacteria, E. coli= *Escherichia Coli*
SEM= Standard error of mean