An Assessment of the Gut Bacteriological Flora of Broiler Chickens Fed Synbiotic and Diet-Acidifiers

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Abstract:- **An experiment was conducted to determine the preponderance of both harmful and beneficial bacteria in the guts of broiler chickens fed synbiotic and diet-acidifiers. One hundred and forty four (144) unsexed day old Arbor Acre broiler chicks were purchased and reared for eight (8) weeks. The birds were divided into four groups and replicated thrice with 12 birds per replicate. They were randomly assigned into four different diets in a Completely Randomized Design (CRD). The diets were labelled I, II, III and IV to represent the control, synbiotic diet, diet-acidifiers inclusion, and diet-acidifiers + synbiotic inclusion diet respectively. At the end of the experiment, *E. coli* and *Salmonella species* which are big threat to poultry production were found to be more prominent in the gut of the control birds. Mortality of two (2) birds occurred in this group and none in the rest of the groups. *Lactobacillus species* and other less pathogenic bacteria were found to dominate the gut of birds in the rest of the groups. *E. coli* and *Salmonella species* were completely absent in the duodenum and jejunum of Groups II - IV birds but rather were dominated by *Lactobacillus species.* It was concluded that synbiotic and diet-acidifiers are useful additives that can be adopted in colonizing the gut of broiler chickens with amiable (synbiotic) bacteria, rather than antibiotics usage that are detrimental to gut microbes and of high public health concern.**

Keywords: **Bacteria, synbiotic, diet-acidifiers, guts, broiler chickens**

I. INTRODUCTION

The integrity of the gut of animals is a key factor to effective digestion and assimilation of nutrients in animal production. This organ is indispensable in the uptake of nutrients and their utilization in animal's body. Healthy gut of bird ensures availability of beneficial bacteria, adequate lymphoid follicles for local immunity, effective digestion and assimilation of nutrients [1]. If this organ in any animal is not healthy, the resultant effect will be disadvantageous and inimical to the growth and good performance of such animal. Antibiotics have been used for many years in poultry production to improve gut health and as growth promoter [2]. Presently, antibiotics have been banned in many countries of the world due to the innumerable reports of their resistances in man and animals [3], [4]. Drug resistance is a global public health threat comprising virtually major microbial pathogens and antimicrobial drugs. In just a couple of generations, what once appeared to be miracle medicines have been beaten into ineffectiveness by the bacteria they were once designed to knock out [5]. This problem of antibiotic resistance by highly pathogenic bacteria and drug residues in animal products being consumed by humans have geared up the scientists in experimenting alternatives to synthetic antibiotics in poultry production [6], [7], [8]. Several conducted researches and experiments invariably led to the discovery of prebiotics, probiotics, synbiotics and diet-acidifier inclusion in the feed or their orogastric administration in poultry production. 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. They were first identified and named by Marcel Roberfroid in 1995 [9]. Prebiotics can stimulate intestinal mucosa immunity, perhaps by acting as a non-pathogenic microbial antigen [10]. Probiotics on the other hand are defined as friendly (live), beneficial microorganisms; mainly bacteria that work with the body and help maintain the delicate balance between the beneficial flora and harmful bacteria that is necessary for health and well-being [11]. Probiotic is highly beneficial to animals in that it increases natural resistance to infectious diseases of the intestinal tract, suppression of cancer, reduction in serum cholesterol concentrations, improved digestion and stimulation of gastrointestinal immunity [12], [13], [14]. Probiotic cultures secret acids which lower the pH of the intestine (acidic pH). Acidic pH leads to good enzyme activity, good toxin binding, good absorption of nutrients, reduces *Salmonella* and *E. coli* [15]. Its usage by livestock does not have residue or antibiotic resistance effect on the animals or the consumers of their products [16]. Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism [9]. Diet-acidifiers or gut-acidifiers is a cock-tail of organic acids (e.g. fumaric, citric, formic, lactic, sorbic and propionic acid) or inorganic acids (e.g. hydrochloric, sulfuric and phosphoric acid) which control the growth of pathogenic bacteria and promotes the growth of healthy microflora in the gut of the animal [15]. To this end this study was designed and conducted to examine the population and types of bacteria (beneficial and non-beneficial) present in the gut of broiler chickens fed synbiotic and diet-acidifiers.

II. MATERIALS AND METHODS

The experiment was conducted with one hundred and forty four (144) unsexed Arbor acre day old broiler chicks at the Livestock Unit of the Teaching and Research Farm of the

Federal University of Technology, Akure (FUTA). The experiment lasted for eight (8) weeks. All chicks were weighed individually upon arrival at the experimental site and continued weekly till 8th week of age using Kerro® digital electronic top pan balance with 1g accuracy. The birds were served feed and water *ad libitum* and vaccinated against Gumboro disease, Newcastle disease and fowl pox disease. No antibiotic was administered to the broiler chickens throughout the period of the experiment.

A. Experimental design and layout

There were four groups and each group was replicated thrice to give a total number of 12 replicates. All the broiler chicks were randomly assigned at the quantity of 12 birds per replicate in a completely randomized design (CRD).

B. Experimental diets

Four types of diets were formulated namely, diet 1 which served as control (zero inclusion of synbiotic and diet-acidifier), diet II having synbiotic (TGI®) inclusion (prebiotic as Mannan Oligosaccharide and Probiotics as *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum, Streptococcus faecium and Bifidobacterium bifidus*), diet III had buffered feed acidifier (H-Plus® containing formic acid and propionic acid) while diet IV had both synbiotic and buffered feed acidifier combined (Table 1).

C. Bacteriological examination

The bacteriological examination of the experimental broiler chickens was conducted in the laboratory of Animal Production and Health of the Federal University of Technology Akure. Two broiler chickens were humanely sacrificed and intestinal samples for bacteriology were collected with the aid of sterile swab from the duodenum (small intestine) and jejunum (large intestine) as described by [17]. Bacteriological samples were taken when the birds were 5 days old (baseline), middle of the experiment (28 days old) and at the end of the experiment (56 days old). Data generated were expressed as mean values of the replicates in the groups.

TABLE 1 COMPOSITION OF EXPERIMENTAL DIETS; STARTER AND FINISHER PHASES (%)

Ingredients	**Diet 1**		**Diet 2**		**Diet 3**		**Diet 4**	
	ST	FI	ST	FI	ST	FI	ST	FI
Maize	60	56	60	56	60	56	60	56
Soya bean 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
Total	100	100	100	100	100	100	100	100

Calculated analysis of the experimental diets							
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 as recommended by the manufacturer. *****Each 2.5kg of broiler Vitamin and Mineral premix contains; Vitamin A-12,000,000IU, Vitamin D_3-3,000,000IU, Vitamin E-30,000mg, Vitamin K_3-2500mg, Folic Acid-1,000mg, Niacin-40,000mg, Calpan-10,000mg, vitamin B_2-500mg, Vitamin B_{12}-20mg, Vitamin B1-2,000mg, Vitamin B_6-3500mg, Biotin-80mg, Antioxidant-125,000mg, Cobalt-250mg, Selenium-250mg, Iodine-1,200mg, Iron-40,000mg, Manganese-70,000mg, Copper-8000mg, Zinc-60,000mg, Choline Chloride- 200,000mg. ST = Starter and FI = Finisher, CP= Crude protein, ME= Metabolizable Energy, DM= Dry Matter, EE= Ether Extract, CF= Crude Fibre, Ca= Calcium and P= Phosphorus.

III. RESULTS AND DISCUSSION

The results from the bacteriological examination of the guts of the experimental broiler chickens are presented in Table 2, 3, 4, 5 and 6. The general characteristics of the bacterial isolates from the experimental broiler chickens (Table 2) depicted the presence of varieties of entero-bacteria. *Salmonella spp., E. coli* and *Lactobacillus spp.* were found in

the gut of birds in all the Groups (Table 3) on day five of the experiment. The population of bacteria at the middle of the experiment (week 4) showed the presence of *Lactobacillus spp*. in the gut of birds in all the groups (I – IV) while *Salmonella spp.* and *E. coli* were completely absent in the gut of birds in group III and IV (Table 4). *Lactobacillus spp.* and *Streptococcus faecalis* were present in the gut of the broiler chickens in all the groups. At the end of the experiment (week eight), *Salmonella spp.* and *E. coli* were completely absent in Groups II – IV while the two bacteria were found in the duodenum of group I broiler chickens (Table 5).

TABLE 2 GENERAL CHARACTERISTICS OF ISOLATED BACTERIA FROM THE DUODENUM AND JEJUNUM OF BROILER CHICKENS FED SYNBIOTIC AND DIET-ACIDIFIERS

Tests	**Number of Isolates**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Gram staining	-	+	+	+	+	+	-	-	-	+	-	+
Morphology of cells	R	S	S	R	S	S	R	R	R	R	R	R
Motility	+	-	-	-	-	-	+	+	+	-	+	+
Catalase	+	+	+	-	+	-	-	+	+	-	+	+
Oxidase	-	-	-	-	-	-	-	+	-	-	-	-
Spores	-	-	-	-	-	-	-	-	-	-	-	+
Indole	+	-	-	-	-	-	-	-	+	-	-	+
Coagulase	-	-	-	-	+	-	-	-	-	-	-	-
Sugar fermentation												
Fructose	AG	A	A	-	A	-	-	A	-	-	-	AG
Manitol	AG	A	A	-	A	A	-	A	A	A	-	-
Sucrose	-	A	A	A	A	A	-	A	-	A	A	A
Galactose	AG	A	AG	-	AG	A	A	-	A	A	-	AG
Lactose	AG	A	A	A	A	A	A	A	A	A	-	-
Glucose	AG	A	A	A	A	A	AG	A	AG	A	AG	A
Oxidation fermentation	OF	OF	-/F	-/F	-/F	-/F	-/F	O/-	-/F	-/F	-/F	OF

Key: Probable organisms: 1= *Enterobacter aerogenes*, 2= *Micrococcus leteus*, 3= *Staphylococcus epidermidis*, 4=*Lactobacillus spp*. 5= *Staphylococcus aureus*, 6= *Streptococcus faecalis*, 7= *Salmonella spp*. 8= *Pseudomonas aureginosa*, 9= *E. coli*, 10= *Actinomyces spp*. 11= *Proteus vulgaris*, 12= *Bacillus spp*. - = Negative, += Positive, R= Rod, S= Sphere, A= Acid, G= Gas, O= Oxidation and F= Fermentation.

TABLE 3 BACTERIAL ISOLATES FROM THE DUODENUM AND JEJUNUM OF THE EXPERIMENTAL BROILER CHICKENS FED SYNBIOTIC AND DIET-ACIDIFIERS ON DAY 5 OF THE EXPERIMENT

Groups	**Duodenum**	**Jejunum**
G I	*Lactobacillus spp., Salmonella spp.,* and *E. coli.*	*Lactobacillus spp., Salmonella spp., E. coli, Staphylococcus aureus* and *Streptococcus faecalis.*
G II	*Staphylococcus aureus, Salmonella spp. and Lactobacillus spp.,*	*Lactobacillus spp., Salmonella spp., E. coli* and *Staphylococcus aureus.*
G III	*Lactobacillus spp., Salmonella spp., E. coli and Staphylococcus aureus.*	*Lactobacillus spp., Salmonella spp., E. coli Staphylococcus aureus and Streptococcus faecalis.*
G IV	*Lactobacillus spp., Salmonella spp.* and *E. coli.*	*Lactobacillus spp., Salmonella spp.* and *E. coli.*

Where G = Group**. NB:** *Salmonella spp., E. coli* and *Lactobacillus spp.* were found in the gut of birds in all the groups

TABLE 4 BACTERIAL ISOLATES FROM THE DUODENUM AND JEJUNUM OF BROILER CHICKENS FED SYNBIOTIC AND DIET-ACIDIFIERS AT WEEK FOUR

Groups	**Duodenum**	**Jejunum**
G I	*Lactobacillus spp., Salmonella spp., E. coli, Staphylococcus aureus, Streptococcus faecalis, Micrococcus leteus and Staphylococcus epidermidis.*	*Lactobacillus spp., Serratia marcesces, Staphylococcus aureus, Streptococcus faecalis, Micrococcus leteus and Staphylococcus epidermidis*
G II	*Lactobacillus spp., Salmonella spp., E. coli. Staphylococcus aureus, Streptococcus faecalis and Micrococcus leteus.*	*Lactobacillus spp., Salmonella spp., E. coli, Staphylococcus aureus, Streptococcus faecalis, Micrococcus leteus and Enterobacter aerogenes.*
G III	*Actinomyces spp., Lactobacillus spp., Staphylococcus aureus and Streptococcus faecalis*	*Lactobacillus spp., Actinomyces spp., Staphylococcus aureus and Streptococcus faecalis,*
G IV	*Lactobacillus spp., Staphylococcus aureus, Streptococcus faecalis, Micrococcus leteus, Staphylococcus epidermidis, Actinomyces spp. and Serratia marcesces.*	*Lactobacillus spp., Staphylococcus aureus, Streptococcus faecalis, Micrococcus leteus, Staphylococcus epidermidis and Enterobacter aerogens.*

Where G = Group. *NB: Lactobacillus spp.* were present in the gut of birds in all the groups *while Salmonella spp., and E. coli* were completely absent in the gut of birds in group III and IV.

TABLE 5 BACTERIAL ISOLATES FROM THE DUODENUM AND JEJUNUM OF BROILER CHICKENS FED SYNBIOTIC AND DIET-ACIDIFIERS AT WEEK EIGHT

Groups	**Duodenum**	**Jejunum**
G I	*Enterobacter aerogenes*, Actinomyces *spp., Lactobacillus spp., Salmonella spp., E. coli, Staphylococcus aureus, Streptococcus faecalis, Micrococcus leteus*, Staphylococcus *epidermidis Baccilus spp.* and *Proteus vulgaris*,	*Enterobacter aerogenes, Lactobacillus spp., Staphylococcus aureus, Streptococcus faecalis, Micrococcus leteus, Staphylococcus epidermidis and , Baccillus spp.and Pseudomonas aeruginosa*
G II	*Lactobacillus spp., Micrococcus leteus, Staphylococcus aureus, Streptococcus faecalis* and *Pseudomonas aeruginosa*.	*Enterobacter aerogenes, Lactobacillus spp., Staphylococcus aureus, Streptococcus faecalis, Micrococcus leteus* and *Pseudomonas aeruginosa*.
G III	*Staphylococcus aureus, Micrococcus leteus, Lactobacillus spp, Staphylococcus epidermidis, Enterobacter aerogenes.*	*Streptococcus faecalis, Staphylococcus aureus, Lactobacillus spp.,* Actinomyces spp., *Micrococcus leteus, Enterobacter aerogenes* and *Bacillus spp.*
G IV	*Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus leteus, Streptococcus faecalis, Lactobacillus spp* and *Enterobacter aerogenes.*	*Lactobacillus spp., Staphylococcus aureus, Micrococcus leteus, Streptococcus faecalis, Enterobacter aerogenes* and *Staphylococcus epidermidis*

Where G = Group**. NB:** *Lactobacillus spp.* and *Streptococcus faecalis* were present in the gut of broilers in all the groups. *Salmonella spp*. and E. coli were completely absent in Groups II – IV while the two bacteria were found in the duodenum of birds in group I.

TABLE 6 TOTAL BACTERIAL COUNT IN THE DUODENUM AND JEJUNUM OF BROILER CHICKENS FED SYNBIOTIC AND DIET-ACIDIFIERS (CFU/ML X 10^4)

Groups	**Day five**		**Week four**		**Week eight**	
	D	J	D	J	D	J
G I	4	9	12	56	20	21
G II	9	8	9	15	19	22
G III	8	15	10	28	22	26
G IV	4	5	9	11	30	34

NB: G = Group, D = Duodenum and J = Jejunum

TABLE 7 MORTALITY RATES (%) OF BROILER CHICKENS FED SYNBIOTIC AND DIET-ACIDIFIERS

Groups	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	Total
G I	-	-	-	-	0.69	-	-	0.69	1.39
G II	-	-	-	-	-	-	-	-	-
G III	-	-	-	-	-	-	-	-	-
G IV	-	-	-	-	-	-	-	-	-
Grand Total	-	-	-	-	0.69	-	-	0.69	1.39

Where WK = Week and G= Group

On day 5 of the experiment (baseline), *E. coli*, *Salmonella spp*. and *lactobacillus spp.* were the most dominant bacteria isolated from the guts of birds in all the groups. The population of *E. coli*, *Salmonella* were still rampant in group I and II at the middle of the experiment (week four) though with absence of *E. coli* and *Salmonella* in Group III and IV. The absence of *E. coli* and *Salmonella* in Group IV agrees with the findings of [18], who reported low population of *Salmonella spp*. in the gut of broiler chickens fed diet containing probiotic. At the end of the experiment as shown in Table 5, *E. coli* and *Salmonella species* were found only in the gut of birds in Group I that had no probiotic, synbiotic or diet-acidifier in their diet. *E. coli* and *Salmonella species* were completely absent in Groups II to IV which actually corroborates the findings of [19] that probiotics are useful biotechnological product that prevents the growth of intestinal infectious bacteria like *Salmonella spp*. and *E. coli*. The guts of the birds in groups III and IV were dominated by *Lactobacillus species.* This could be attributed to the synergistic effect exhibited by the synbiotic and diet acidifier (combination) in the diet of birds in these groups. This agrees with the reports of [20], [21] that diet supplemented with synbiotic and diet-acidifiers were capable of reducing the colonization of the gut of broiler chickens with *Salmonella spp*. The report of this study also agrees with [22] that probiotics, prebiotics and synbiotics are very useful in the maintenance of intestinal flora equilibrium. The competitive exclusion mechanism actually played a significant role in eliminating the pathogenic microorganisms in the gut as reported by [15]. It can be seen from Table 6 that Group III had the highest bacterial load of 15 x 10^4 (cfu/mL) in the jejunum while Group IV and I (control) had the lowest bacterial load of 4 x 10^4 (cfu/mL) in their duodenum on day 5 of the experiment. The bacterial load of the duodenum and jejunum of each of the Groups at the middle of the experiment was observed to be highest in the jejunum of the birds in Group I (56 x 10^4 cfu/mL) which were more of pathogenic type as shown in Table 6. Group III had a moderate load of 10 x 10^4 (cfu/mL) and 28 x 10^4 (cfu/mL) in duodenum and jejunum respectively. This range agrees with the findings of [14] as sufficient load of *L. acidophilus* to improve the gut health of broiler chickens. There was a general increase in the number of bacteria in both the duodenum and jejunum of the birds in all the treatments compared to the baseline bacterial population. At the end of the experiment (8^{th} week) as shown in Table 6, bacterial load (count) in the duodenum and jejunum of the experimental broiler chickens reached the largest population in Group IV (30 x 10^4 and 34 x 10^4 cfu/mL respectively), followed by Group III (22 x 10^4 and 26 x 10^4 cfu/mL) and Group II (19 x 10^4 and 22 x 10^4 cfu/mL). The least bacterial load was recorded in the jejunum of birds in Group I (21 x 10^4 cfu/mL). The heaviest beneficial bacterial load recorded in group IV could be attributed to the synergistic effect of both organic acids and synbiotic. The lowest number of beneficial bacterial load recorded in Group I was not a big surprise because the group received neither synbiotic nor organic acids. They were left to nature for gut colonization by entero-bacteria. However, more of the pathogenic bacteria (*E. coli* and *Salmonella species*) were found mainly in the duodenum of the Control Group. The presence of *E. coli* and *Salmonella species* in the duodenum of the control group could be attributed to the mortalities of birds recorded only in this group (Table 7) as some authors have reported these bacteria to be pathogenic to poultry birds [23], [24]. According to [25], probiotics are feed additives that are very useful in lowering chick mortality and this may be the cogent reason why no mortality was recorded from the birds in groups II - IV. According to the report of [26], prebiotics has significant advantage on the immunity of broiler chickens which will eventually be translated to high livability percentage. *E. coli* and *Salmonella species* were both absent in the duodenum and jejunum of the experimental birds in Groups II – IV. The bacteria found were more of the beneficial *Lactobacilli* species. The reduction in bacterial load count reduces the disease threat and this has a knock-on benefit of zero mortality in this group and this agrees with the report of [27] that diet-acidifiers are preferred alternative to antibiotic administration with good performance in the rearing of broiler chickens. High population of *Salmonella* and *Escherichia* numbers in the intestine destroy the villi (intestinal lining) that are required for nutrient and water absorption necessary for growth as observed in birds in group I. According to [28], reduction in pathogen numbers allows the villi to develop fully. This will result in improved feed conversion ratio (FCR), which helps compensate for the removal of antibiotic growth promoters. The absence of *Salmonella* and *E. coli* noticed in the guts of broiler chickens in groups II – IV (Table 5) in this study also agrees with the report of [29] that organic acids are very efficient against *Salmonella spp.* by preventing their growth in gastro-intestinal

tract through alteration of the pH and making the medium acidic. According to the report of [7], [30], probiotics and synbiotic have great protective potential against enteropathic disorder and hence can improve the gut health of broiler chickens. Absence of *Salmonella* in the gut will definitely prevent food (meat) contamination as this organism is one of the pathogens responsible for direct food contamination, a precursor to zoonoses [22].

IV. CONCLUSION

The inclusion of synbiotic and diet-acidifiers in the diets of broiler chickens favoured the proliferation of the gut with beneficial bacteria and also helped in elimination of the harmful ones (like *Salmonella spp* and *E. coli*) through competitive exclusion mechanis-0pm. This could be attributable for the zero mortality recorded in the group of birds fed synbiotic (II), diet-acidifier (III) and synbiotic + diet-acidifier (IV). The highest population of beneficial bacteria (*Lactobacillus spp, Bacillus spp., Streptococcus spp etc*) were found in the group of birds given the combination (synergy) of synbiotic and diet-acidifiers (IV).

Recommendation

Based on the results obtained from this study, synbiotic and diet-acidifiers are hereby recommended for usage in the rearing of broiler chickens as replacement for antibiotic growth promoters. They have the potentials to colonize the gut with beneficial bacteria and prevent mortality of birds or bring it to the scientifically permissible level (less than 10 %) as experienced in this study.

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