



Effect of Yeast and Organic Acid (Biotronic® Top Forte) on the Gut Microbiota of Broiler Chickens

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Abstract

*This study was conducted at the Teaching and Research Farm, Faculty of Agriculture, Kaduna State University to determine the effects of yeast and organic acid (Biotronic® top forte) as feed additives on the gut microbiota of broiler chickens. One hundred and twenty day-old-broiler chicks obtained from a reputable hatchery were used. The birds were randomly shared into four treatments, each containing three replicates: T1 (control), T2 (yeast), T3 (organic acid) and T4 (yeast and organic acid). Six faecal samples were collected from each treatment (2 chickens/replicate) at the end of a six-week experimental period. Data were analysed using ANOVA in the Statistical Package for Social Sciences (SPSS). The results showed that there was a significant ($p < 0.05$) difference between T1 and T4 for the Total Bacterial Count (TBC), with T1 (5.12 ± 4.46 logCFU/g) having the highest value and T4 (4.74 ± 4.91 logCFU/g) the lowest. The results obtained for Total Coliform Count (TCC) showed no significant ($p > 0.05$) difference among treatments. The results obtained for Total Non-Coliform Count (TNCC) showed a significant ($p < 0.05$) difference between T1 and T2 with T1 (5.11 ± 4.46 logCFU/g) having the highest value and T2 (4.87 ± 4.10 logCFU/g) showing lowest value, suggesting that supplementing the feed with yeast can reduce TNCC. It was also observed that *E. coli* was not detected in T3 and T4, treatments containing organic acid, suggesting that organic acid may have bactericidal properties against *E. coli*. It was concluded from the study that supplementing broiler feed with yeast and organic acid as growth promoters does not negatively affect the gut microbiota of broiler chickens.*

Key words: Broiler chicken, gut microbiota, yeast, organic acid

Introduction

Broiler chickens are the most common source of meat globally, contributing significantly to the meat industry (Bennett *et al.*, 2018). Chickens contribute about 27% of the world's meat supply (FAO, 2019). With the increase in consumer demand, there is increasing need to provide healthier meat products. Therefore, the health and productivity of broiler chickens needs to be prioritized and optimised. Globally, the population of broiler chickens was estimated to be 23 billion, with an annual consumption rate of 86 million metric tons, while the total national population of broiler chickens in Nigeria was estimated to be 180 million with an annual consumption rate of 1.5 million metric tons (FAO, 2019). To efficiently produce healthier meat products, it is

important to understand and optimise the health of the gut microbiota, which contributes to overall health, nutrient absorption, and immune system development in broiler chickens (Wei *et al.*, 2013).

The gut microbiota is the complex community of microorganisms that inhabit the gastrointestinal tract of animals (Wei *et al.*, 2013). It is made up of a variety of bacteria, archaea, fungi, and other microorganisms which are responsible for various functions, such as optimising nutrient absorption and strengthening of the immune system in the host animal (Adebiyi *et al.*, 2012). The microorganisms residing in the gastrointestinal tract are essential for enhancing the health and productivity of chickens. The composition of gut

microbiota exhibits regional variations, with the cecum displaying the greatest microbial diversity (Yan *et al.*, 2017). The gut microbiota plays a vital role in optimizing nutrient absorption, inhibiting pathogen establishment, enhancing growth performance, and detoxifying mycotoxins present in the feed (Slizewska and Piotrowska, 2014). Monitoring the gut microbiota in chickens is critical, as certain microbes, such as *Salmonella* and *Campylobacter*, are responsible for zoonotic diseases in humans (Davies and Wray, 1996). The initial colonization of the gut by these microorganisms is significant, as it can influence the intestinal morphology, physiology, and the host's vulnerability to infections. Bacteria species are the most abundant group of microbes in the gut. The bacterial concentration in the small intestine is about 1×10^5 CFU/g and it increases to about 1×10^{11} CFU/g in the caeca (Gabriel *et al.*, 2006). The bacterial species are also divided into the coliform and non-coliform bacteria. Coliform bacteria are rod-shaped Gram-negative non-spore forming and motile or nonmotile bacteria capable of lactose fermentation with the production of acid and gas at incubation temperature of 35–37°C (Daoliang and Shuangyin, 2019). They are frequently used as indicator of sanitary quality of foods and water. Coliforms are not usually indicted as causes of serious illness, but their presence is used to indicate the presence of other pathogenic organisms of faecal origin. Such pathogens include disease-causing bacteria, viruses, or protozoa and many multicellular parasites. Common coliform bacteria include *Escherichia coli* and *Klebsiella pneumoniae* (Daoliang and Shuangyin, 2019). Non-coliform bacteria encompass all bacterial types that fall outside the coliform classification. These bacteria are not specifically linked to faecal contamination and do not function as indicators for the presence of potentially pathogenic bacteria (Ray and Bhunia, 2007). Non-coliform bacteria inhabit a diverse array of

environments such as water, soil, and the skin or gastrointestinal tracts of both humans and animals. Notable examples of non-coliform bacteria include species from the genera *Bacillus*, *Streptococcus*, *Pseudomonas*, and *Staphylococcus*. While the presence of non-coliform bacteria does not inherently suggest the existence of pathogenic bacteria, it may still hold significance in evaluating the overall microbiological quality of a given sample (Ray and Bhunia, 2007). Imbalance in the gut microbiota (dysbiosis) can lead to disease conditions and a drop in overall broiler performance (Adebiyi *et al.*, 2012). In our endeavour to improve the gut microbial community, there has been growing interest in the use of dietary supplements. This includes the use of yeast and organic acids.

Organic acids are a class of compounds that contain one or more carboxyl groups (COOH) (Shahidi *et al.*, 2014). Organic acids are commonly found in various natural sources such as fruits, vegetables, and fermented products. They play key roles in metabolism, cellular processes, and as preservatives in food and feed industries (De Lange *et al.*, 2010). Examples include acetic acid, citric acids, lactic acid, mallic acid, propanoic acids, and butyric acid (Shahidi *et al.*, 2014). Each organic acid has its own unique chemical and physiological properties including acidity, solubility, and antimicrobial effects (De Lange *et al.*, 2010). They have antimicrobial properties and can selectively inhibit the growth of pathogens while promoting the growth of beneficial bacteria in the gut (Agbai *et al.*, 2020). For example, organic acids such as butyric acid can promote the growth of butyrate-producing bacteria, which is beneficial for gut health (Agbai *et al.*, 2020). Some organic acids are purified and sold under various company brands such as Biotronic® topforte which is a company-made organic acid composed of selected organic acids, alongside their salts with phytochemicals and the unique Biomin

Permeabilizing Complex (Agbai *et al.*, 2020).

Yeast is a single-celled fungi which can ferment sugars into alcohol and carbon dioxide through anaerobic respiration (Kurtzman and Fell, 2011). It is commonly employed in processes such as baking, brewing, and used as a probiotic in animal feeds (Kurtzman and Fell, 2011). According to Kabir *et al.*, (2004), yeast ensures a normal intestinal microflora condition through competitive exclusion and antagonism. It alters metabolism by enhancing digestive enzyme activity and reducing bacterial enzyme activity and ammonia production; it also improves digestion and strengthens the immune system (Kabir *et al.*, 2004). These combined effects underpin the significance of yeast in promoting growth performance and overall health in broiler chickens. This study explores the effect of using yeast and organic acid, as feed additives, on the gut microbiota of broiler chickens.

Materials and methods

Study Area

This research was carried out at the Poultry unit, Teaching and Research Farm, Faculty of Agriculture, Kaduna State University (KASU), Kafanchan, Kaduna State. Kafanchan is located between Latitudes 9°33'30" and 9°36'30"N, and Longitude

8°16'0" and 8°20'0"E with an elevation of 739 m above sea level (Mande *et al.*, 2023) and an average yearly temperature of 25.3°C (Averages, 2019).

Experimental Chickens and Diets

A total of 120-day-old commercial broiler chicks, purchased from Olam Agri Holdings in Kaduna were used for the experiment. These chicks were randomly divided into four different dietary treatments: T1 (control), T2 (300g yeast/100kg diet), T3 (300g organic acid/100kg diet), and T4 (300g yeast + 300g organic acid/100kg diet). Table 1 gives the composition of the basal diet. Each of these treatments was made up of 3 replicates, with ten chicks per replicate. The birds were kept in a deep litter house which was properly cleaned, washed, and disinfected a week before the arrival of the chicks. The temperature in the environment during the brooding period started at 35°C when the birds were one day old and progressively lowered to 25°C by day 21. Feed and water were made available *ad libitum* through trough feeders and a bell drinker. The birds were vaccinated for Newcastle disease and infectious bronchitis on the sixth day of age, followed by a vaccination for Gumboro on the twelfth day. Table 1 gives the composition of the experimental diet.

Table 1. Composition of experimental Diets (starter and finisher)

Ingredient	Starter (kg)	Finisher (kg)
Maize	56.25	56.00
Maize Offal	-	9.00
Groundnut Cake	11.00	12.00
Soybean Cake	28.30	18.60
Limestone	0.50	0.50
Bone meal	2.90	3.00
Salt	0.25	0.25
Premix	0.25	0.25
Lysine	0.25	0.20
Methionine	0.30	0.20
Total	100	100
CP	23.02	20.01
Ether Extract	4.69	4.45
CF	4.25	4.44
Lysine	1.36	1.08
Methionine + Cysteine	0.96	0.85

Data Collection and Sampling

The birds were fed with the experimental diet for six weeks during from mid-August to end of September after which the faecal samples were collected for laboratory analysis. For each treatment, two samples were collected from each replicate, giving a total of 24 samples. During sample collection, the birds were carefully placed on a clean tray and left to defecate. Each faecal dropping was quickly but carefully sampled using a spatula and placed in a labelled sample bottle. After collection, the samples were packaged in an ice pack and sent for laboratory analysis.

Laboratory analysis

Enumeration of Bacteria from Fecal Sample using the spread plate method

Ten mL of Sterile Distilled Water (SDW) was dispensed into the first test tube while 9mL of SDW was dispensed into 5 labelled test tubes. One gram (1g) of the faecal sample was homogenized and inoculated into 10mL of the labelled sterile distilled water tube and a tenfold serial dilution was carried out from 10^{-1} to 10^{-6} . The mixture in the first test tube was vortexed to ensure an even distribution of bacteria. One millilitre (1ml) of the mixture was aseptically transferred to 10^{-2} dilution tube. The tube 10^{-2} was mixed and 1mL was transferred to tube 10^{-3} . This was repeated until the 10^{-6} dilution tube was reached. Using a sterile micropipette, 100 μ L (0.1mL) was aspirated from the 10^{-3} and 10^{-4} dilution tubes and dispensed into the appropriately labelled prepared MacConkey Agar (MCA) and Plate Count Agar (PCA). The inoculum was spread over the agar surface with a sterile spreader. The spreader was moved back and forth in a straight line while rotating the plate on a turntable for approximately 10 seconds. **NB:** The surface of the agar was allowed to dry on the bench for 10 minutes and the plates were inverted. The inoculated plates were incubated at 37°C for 24-48 hours. After an appropriate incubation period, the colonies were counted on the

plates of the dilution showing discrete colonies in the countable range of 30-300. The number of viable cells were calculated in the original sample by accounting for all dilutions, using the formula:

$$\text{CFU/g} = \frac{\text{Number of colonies counted} \times \text{dilution factor}}{\text{volume of culture plated}}$$

Isolation and Identification of Bacterial Isolates

Pure colonies of the bacterial isolates were purified by sub culturing onto Blood agar, MacConkey agar, and Nutrient agar. The inoculated plates were incubated at 37°C for 24 hours. The plates were read macroscopically for morphological characteristic (size, colour, edge, consistency, surface of elevation). The pure colonies were Gram stained and biochemical tests were carried out (catalase, oxidase, indole, TSIA, urase, citrate, glucose, arabinose, dulcitol, lactose, sucrose, mannitol, mannose, Cellobiose, melibiose, sorbitol, rhamnose, trehalose, xylose).

Statistical analysis

The data for Total Bacterial Count (TBC), Total Coliform Count (TCC), Total Non-Coliform Count (TNCC), and individual bacterial isolates in each treatment was analysed using one-way ANOVA in the Statistical Package for Social Sciences (SPSS). The laboratory values for TBC, TCC, and TNCC were first log-transformed before performing the analysis. P-values < 0.05 were considered significant.

Results and Discussion

Table 2 shows the effects of yeast, organic acid (Biotronic® topforte), and yeast in combination with organic acid (Biotronic® topforte) on the gut microbiota of broiler chickens. The results show values obtained from the laboratory for Total Bacterial Count (TBC), Total Coliform Count (TCC), and Total Non-Coliform Count (TNCC). The values for TBC, shows a significant

($P < 0.05$) difference between T1 and T4 and was highest in T1 (5.12 ± 4.46) and lowest in T4 (4.74 ± 4.91). This is likely because of supplementation of the feed with yeast and organic acid, consistent with the study by Wei *et al.*, (2013) which stated that the gut microbiota of broiler chickens is densely populated by bacterial organisms (as seen in T1), and the collective action of yeast and organic acid reduces the bacterial load in the gut microbiota. It is also consistent with the study by Li *et al.*, (2017) which states that yeast as a prebiotic has the effect of stimulating the growth of beneficial bacteria in the gut microbiota, improving nutrient digestion, enhancing gut barrier function, and modulating immune responses. It also possesses anti-inflammatory and antimicrobial properties, helping to establish a balanced gut microbial community. Agbai *et al.*, (2020) stated in a study that Biotronic® topforte contains essential oils, organic acids, and their salts, which in combination with yeast influences the gut microbiota of broiler birds as follows: organic acids help create a favourable gut environment by reducing pH levels and inhibiting the growth of

pathogens, while essential oils have antimicrobial properties that can promote the growth of beneficial bacteria.

The result for TCC shows no significant ($P > 0.05$) differences among the treatments. This suggests that yeast and organic acid do not have a significant effect on the prevalence of coliform bacteria, which is contrary to the work of Li *et al.*, (2017) who observed that yeast have the ability to reduce coliform bacteria population in the gut. This disparity in results could be because of differences in feed composition, as Li *et al.*, (2017) included *Bacillus subtilis* in the experimental diets.

The result for TNCC shows a significant ($P < 0.05$) difference between T1 and T2, with T1 (5.11 ± 4.46) having the highest value and T2 (4.87 ± 4.10) having the lowest value for number of non-coliform bacteria. This result shows that the absence of yeast or organic acid in T1 may result in denser population of bacteria in the gut microbiota as observed by (Wei *et al.*, 2013).

Table 2: Total bacterial count, total coliform count, and total non-coliform count in faecal samples of broiler chickens fed

	T1 (control)	T2 (Yeast)	T3 (Organic acid)	T4 (organic acid and yeast)
TBC	$5.12^a \pm 4.46$	$4.88^{ab} \pm 4.10$	$5.03^{ab} \pm 4.36$	$4.74^b \pm 4.91$
TCC	3.66 ± 3.41	3.00 ± 2.78	3.86 ± 3.30	3.94 ± 3.75
TNCC	$5.11^a \pm 4.46$	$4.87^b \pm 4.10$	$4.99^{ab} \pm 4.33$	$4.94^{ab} \pm 4.05$

Key: TBC (Total Bacterial Count), TCC (Total Coliform Count), TNCC (Total Non-Coliform count). Values were transformed logarithmically before analysis.

Table 3 shows the effects of yeast, organic acid (Biotronic® topforte), and the combination of both in a diet on the bacterial isolates present in the gut microbiota of broiler chickens. The result shows Total *Klebsiella aerogenes* Count (TKaC), Total *Micrococcus spp* Count (TMiC), Total *Bacillus spp* Count (TBac), Total Coagulase-negative Staphylococci

Count (TCoNSC), Total *E. coli* Count (TEcC), Total *Streptococcus faecalis* Count (TSfcC), Total *Pseudomonas aeruginosa* Count (TPaC) and Total *Proteus mirabilis* Count (TPmC). The values for TKaC, shows no significant ($p > 0.05$) difference across all four treatments. However, values obtained shows that T1 (control) has the highest number of *K. aerogenes* bacteria

and T2 (yeast) has the lowest number of the bacteria. *K. aerogenes* is an opportunistic bacterium which is capable of causing infection in the respiratory tract and even in the nervous system, which means that it may be harmful to the broiler chicken; *K. aerogenes* was observed to be highest in T1 (control) and lowest in T2 (yeast) which suggests that organic acid may play a role in lowering the bacterial population. This is in line with the study of Wei *et al.* (2013) which states that the gut microbiota of broiler birds is densely populated with bacteria and other microbes, both beneficial and non-beneficial, but the presence of yeast reduces the number of non-beneficial ones, usually the coliforms bacteria in the gut microbiome of broiler chickens.

The values for TMiC showed no significant ($P > 0.05$) differences across the treatments. The Micrococcus bacteria is beneficial, as it helps in nutrients cycling in the body. This study shows that yeast and organic acid and their combination do not lead to a significant deviation of the population of micrococcus bacteria, in line with the study by Ezema (2007) who reported that yeast does not antagonise beneficial microbes, such as the micrococcus, in the gut microbiota of broiler chickens.

The values for TBacC showed no significant difference across all four treatments. *Bacillus spp.* provides competitive exclusion to pathogenic bacteria in the gut microbiome. This supports the work of Agbai *et al.*, (2020) and Li *et al.*, (2017) who also observed that feeding yeast to broilers do not lead to a reduction in Bacillus population.

The values of TCoNSC also showed no significant difference across all the four treatments. Coagulase-negative staphylococci is a commensal bacterium, which means its presence in the gut microbiome is important and beneficial to the animal (Silva *et al.*, 2022). As observed in this study, yeast and organic acid do not

cause a significant deviation in the population of Coagulase-negative staphylococci.

The values for TEcC showed significant differences between T1 (5.27 ± 4.94) and T3 (0.00 ± 0.00) and T4 (0.00 ± 0.00). *E. coli* was not detected in treatments which contained organic acids. This is supported by the work of Emami *et al.*, (2017) and Lopez *et al.*, (2012) who also observed that supplementing broiler diets with organic acids resulted in significant reduction in *E. coli* counts. Organic acid exhibits its bactericidal activity on *E. coli* by disrupting the structural integrity of the cell membrane, causing the bacteria to lyse (Kovanda *et al.*, 2019). *E. coli* is mostly considered a pathogenic bacterium, which causes discomfort, illness, and even death in broilers (Emami *et al.*, 2017). Therefore, the ability of organic acid to control *E. coli* will likely lead to improvement in the health of broilers.

The values of TSfC showed no significant difference across all four treatments. *Streptococcus faecalis* is a pathogenic bacterium which causes infection in animals. It suggests that yeast and organic acid have no significant effect on *Streptococcus faecalis*. This contradicts the work of Kovanda *et al.*, (2019) who observed that organic acid significantly reduced the bacteria load of *Streptococcus spp.* This contradiction may have been as a result of difference in experimental designs; Kovanda *et al.*, (2019) used *in vitro* investigation while this study used *in vivo*. In *in vivo* experiments, there are other complex biological factors that may interfere with the bactericidal activity of the organic acid on *Streptococcus*, such as release of interfering organic compounds from other microbes such as bacteriocin.

The values of TPmC shows no significant difference across all four treatments. It suggests that yeast and organic acid have no significant effect on *Proteus mirabilis*. *P. mirabilis* is a gram-negative pathogenic

bacterium that has been linked to urinary tract infections in humans and can be present as a food borne pathogen within poultry products, including broilers (Yu *et al.*, 2021). *P. mirabilis* was only detected in T3 which looks like an anomaly and may be due to sample contamination at the point of sample collection or in the laboratory. However, Burns *et al.*, (2021) and

Stanojević-Nikolić *et al.*, (2016) had observed that *P. mirabilis* population is significantly reduced by organic acids like lactic acid, whose mode of action is likely due to the disruption of structural components of the bacterial cell wall, causing fluid from the surrounding to enter and lyse the cell due to osmotic pressure.

Table 3: Bacterial isolates LogCFU/gram in faecal samples of broiler chickens fed

	T1 (Control)	T2 (Yeast)	T3 (Organic acid)	T4 (Yeast and organic acid)	LOS
TkaC	5.57 ± 5.34	0.00 ± 0.00	4.57 ± 4.57	5.20 ± 4.83	NS
TMiC	4.72 ± 4.53	5.23 ± 5.05	0.00 ± 0.00	0.00 ± 0.00	NS
TBacC	5.32 ± 5.26	0.00 ± 0.00	5.35 ± 5.15	5.56 ± 5.56	NS
TCoNSC	5.49 ± 5.15	5.55 ± 5.23	4.11 ± 4.11	5.35 ± 4.91	NS
TEcC	5.27 ^a ± 4.94	4.79 ^{ab} ± 4.66	0.00 ^b ± 0.00	0.00 ^b ± 0.00	*
TSfC	4.76 ± 4.76	5.00 ± 4.68	5.13 ± 4.72	4.48 ± 4.30	NS
TPmC	0.00 ± 0.00	0.00 ± 0.00	4.51 ± 4.78	0.00 ± 0.00	NS

Key: SF = *Streptococcus faecalis*, EC = *E. coli*, Bac = *Bacillus* species, Ka = *Klebsiella aerogenes*, Pm = *Proteus mirabilis* Mic = *Micrococcus spp*, CoNS = Coagulase-negative Staphylococci, CFU/g = Colony forming unit per gram, T = Total, C = Count. Values are given as mean ± standard error.

Conclusion

It was observed from this study that supplementing broiler diets with yeast and organic acid (Biotronic® topforte) does not cause an unhealthy gut microbiota when used as feed additive for broilers. Yeast and organic acid have been shown, in this experiment, to down regulate *E. coli* in the gut.

Recommendation

1. Farmers should be encouraged to adopt the use of yeast and organic acid in broiler farming as feed additives to improve growth performance.
2. More elaborate research is required to delve into the microbiota of specific sections of the gastrointestinal tract of broilers and how it is affected by the use of feed additives such as yeast and organic acid.

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