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Effect of Yeast Supplementation on the Composition Of Gastrointestinal Microbiota of Broiler Chickens

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Abstract

This study focused on the gut microbiota of broiler chickens fed a diet with yeast included. The study also examines the impact of yeast as a supplement in the diet and its effect on bird faeces. 120-day-old broiler chicks were kept in four treatment groups in three replicates. With T1 being the control, the inclusion level of yeast was T2= 100g, T3=150g, and T4= 200g per 100kg of feed to determine the level of inclusion favourable for the gastrointestinal tract microbiota. The experiment lasted for 7 weeks. Faecal samples were collected from each treatment at different stages (weeks 1, 4, and 7), and all data collected were subjected to a one-way analysis of variance (ANOVA), Using the general linear model of SAS (2002). Differences in treatment means were separated using the Duncan Multiple Range Test. The results obtained showed that the values for raw total coliform count (RTCC) were significantly different (P<0.05) with values obtained highest at T3(46.22±13.89^{a}), and lowest at T1(10.56 ±7.09^{c}). The results obtained based on age showed that Total Bacteria count (TBC) was not significantly different with values obtained highest at week 7 (3.69±0.83), and the lowest at week 4 (3.23±0.79). The result shows that, despite variations in raw counts, the overall total bacterial counts remain relatively consistent across different batches, suggesting stability in bacterial populations. In conclusion, varying levels of yeast inclusion(100g,150g and 200g) in diets led to distinct effects on microbial counts and species proliferation, indicating the role of yeast in modulating the gut microbiota.

Keywords: ***Microbiota, yeast, broiler, faeces***

Description of the problem

The gastrointestinal tract of broiler birds contains a diverse community of microorganisms known as the microbiota. This plays an important role in digestion, immunity, and overall health. This complex microbiota comprises over 600 bacterial species from more than 100 bacterial genera (1). The microbiota consists of various microorganisms, including bacteria, fungi, viruses, and archaea, inhabiting a specific ecological niche, such as the gastrointestinal tract of animals. These microorganisms influence numerous physiological processes and contribute to host health (2). The composition of the gut microbiome in

chickens is influenced by factors such as diet, age, and gut location (3) and can be modified by dietary interventions, such as the use of probiotics and prebiotics (4).

Due to consumer concerns about poultry antibiotic residues in animal products such as meat and eggs, there has been a great effort to completely avoid the usage of antibiotics in livestock production. To achieve that, non-antibiotic alternatives like probiotics, prebiotics, synbiotics, and phytobiotics are being used in poultry feed for improvement of growth and production performance (5). Probiotics (yeasts) have been adopted to mitigate the challenges arising from the withdrawal of antibiotics from feed (6). There are different yeast strains used in poultry nutrition, they include; *Saccharomyces cerevisiae* (*S. cerevisiae*) (7 and 8), *Saccharomyces boulardii* (*S. boulardii*) (9), *Saccharomyces pastorianus* and *Saccharomyces bayanus*, each with its unique characteristics and potential benefits(10). The objective of the study is to investigate the influence of yeast supplementation on the composition and diversity of broiler birds' gastrointestinal microbiota at different levels of inclusion and to evaluate the composition of the gastrointestinal microbiota of the broiler birds.

Materials and method

Experimental site

The Research was conducted at the Teaching and Research farm, Department of Animal Science, Kaduna State University (KASU), Kafanchan Campus, Kaduna State. Kafanchan is located at latitude 9°59'N, and longitude 8°29'E with an elevation of 733m above sea level (Ovimaps, 2022). All the laboratory analyses were carried out at the central diagnostic laboratory, National Veterinary Research Institute (NVRI), Vom.

Experimental birds

One hundred and twenty (120) day-old chicks were acquired from Olam Hatcheries Limited, Kaduna. The study employed a completely randomized design (CRD) to determine the effects of various treatments on broiler chicks. Within each treatment group, three(3) replicate groups of ten(10) birds were housed in compartments. The diets labelled T1 to T4 were administered and manual brooding was done using a charcoal stove for two(2) weeks. Throughout the study, feed and water were provided *ad libitum.* Standard poultry management practices, including vaccination, daily health inspections, mortality checks, cleaning of drinkers, and provision of feed and water, were rigorously upheld. The birds were fed experimental starter diets during the first four weeks, followed by experimental finisher diets for the subsequent three weeks. This regimen was maintained to evaluate the impact of dietary treatments on microbiota of broiler chickens.

Experimental feed

The major ingredients were sourced from different markets in the Southern part of Kaduna state. The energy source(maize) was obtained from Samaru Market of Zango Local Government Area. Full-fat soya and wheat offal were procured from Kafanchan Market in Jema'a Local Government Area.

Soya-bean cake, yeast, and all other micro-ingredients were purchased at Biotin Feeds, located at BR4, Nnamdi Azikiwe Expressway, Kaduna.

The full-fat soya was pan-roasted to ensure it was suitably prepared for the birds and subsequently taken to a nearby milling station for grinding. The maize was finely ground to an appropriate texture using a designated milling process. The ingredient mixing process was done on a clean floor. After mixing, the feed was packed into labelled bags, distinguished as T1, T2, T3, and T4 for storage. The composition of the broiler starter diets and broiler finisher diet is shown in Table 1 and Table 2, respectively.

Table 1: Percentage composition of broiler starter diets

Ingredients	**T1(control)**	**T2(100g SYC)**	**T3(150g SYC)**	**T4(200g SYC)**
Maize	50.65	50.65	50.65	50.65
Full fat soya	19.0	19.0	19.0	19.0
Soya cake	26.0	26.0	26.0	26.0
Limestone	0.6	0.6	0.6	0.6
Bone meal	3	3	3	3
Salt	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25
Lysine	0.05	0.05	0.05	0.05
Methionine	0.2	0.2	0.2	0.2
Yeast	-	-	-	-
Total	**100**	**100**	**100**	**100**
Calculated Analysis				
Crude protein (%)	23.27	23.27	23.27	23.27
ME (Kcal/kg) (%)	3002.10	3002.10	3002.10	3002.10
Ether extract (%)	7.08	7.08	7.08	7.08
Crude fibre (%)	4.31	4.31	4.31	4.31
Calcium (%)	1.10	1.10	1.10	1.10
Available protein (%)	0.52	0.52	0.52	0.52
Lysine (%)	1.37	1.37	1.37	1.37
Methionine+cyst (%)	0.89	0.89	0.89	0.89

SYC= saccharomyces cerevisiae' T1=control; T2=100g of SYC; T3=150g of SYC; T4=200g of SYC; ME= metabolizable energy; phosphate; met=methionine cyst= cysteine;

Table 2: Percentage composition of broiler finisher diets

Ingredients	T1 (control)	T2(100g SYC)	T3(150g SYC)	T4(200g SYC)
Maize offal	9.0	9.0	9.0	9.0
Maize	50.75	50.75	50.75	50.75
Full fat soya	23.0	23.0	23.0	23.0
Soya cake	13.0	13.0	13.0	13.0
Limestone	0.5	0.5	0.5	0.5
Bone meal	3	3	3	3
Salt	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25
Lysine	0.05	0.05	0.05	0.05
Methionine	0.20	0.20	0.20	0.20
Yeast	-	-	-	-
Total	**100**	**100**	**100**	**100**
Calculated Analysis%				
Crude protein (%)	20.03	20.03	20.03	20.03
ME (Kcal/kg) (%)	3056.95	3056.95	3056.95	3056.95
Ether extract (%)	7.29	7.29	7.29	7.29
Crude fibre (%)	4.40	4.40	4.40	4.40
Calcium (%)	1.05	1.05	1.05	1.05
Available protein (%)	0.52	0.52	0.52	0.52
Lysine (%)	1.13	1.13	1.13	1.13
Methionine+cyst (%)	0.80	0.80	0.80	0.80

SYC= saccharomyces cerevisiae; T1=control; T2=100g of SYC; T3=150g of SYC; T4=200g of SYC; ME= metabolizable energy; phosphate; met=methionine cyst= cysteine;

Samples and data collection

Faecal samples were collected from each replicate in each treatment (T1, T2, T3, and T4), giving a sample size of twelve(12) birds. This was repeated for three(3) growth phases of the birds: week 1, week 4, and week 7, thereby, giving a total of 36 faecal samples collected. During faecal collection, the birds were placed on a tray. After defecating, the faeces were scooped using a spatula and placed in a sample bottle. These samples were packaged in a cooler containing ice packs and transported to the central diagnostic laboratory, National Veterinary Research Institute, NVRI, Vom, for analysis. The experiment spanned seven weeks, allowing for a comprehensive evaluation of the specified parameters and their implications on the broiler chicken microbiota.

Statistical analysis

All data collected were subjected to a one-way analysis of variance (ANOVA), using

the general linear model of SAS (2002). Differences in treatment means were separated using Duncan Multiple Range Test.

Results and Discussion

Effects of yeast supplementation on the gut microbiota of broiler birds

The effects of yeast supplementation on the gut microbiota of broiler birds are presented in Table 3 below.

Table 3: Effects of yeast supplementation on the gut microbiota of broiler birds

	Treatments				
Parameters (CFU/g)	**T1(control)**	**T2(100g SYC)**	**T3(150g SYC)**	**T4(200g SYC)**	**LOS**
RTBC $\times 10^{-4}$	55.00±22.85	84.67±25.70	86.00±23.97	55.33±20.71	NS
TBC $\times 10^{6}$	4.63 ± 1.24^{a}	1.70 ± 0.20^{b}	4.14 ± 0.92^{ab}	3.56 ± 0.88^{ab}	*
RTCC $\times 10^{-4}$	10.56 ± 7.09^{c}	40.44 ± 22.61^{a}	46.22 ± 13.89^{a}	16.11 ± 6.71^{b}	*
TCC $\times 10^{6}$	2. 56±0.85	1.73±0.86	3.60±1.13	2.56±0.87	NS

RTBC= Raw count Total Bacteria count; TBC= Total Bacteria count; RTCC= Raw count Total Coliform count; TCC= Total Coliform count; LOS= Level of significance; NS= No significance; T1=control; T2=100g of SYC; T3=150g of SYC; T4=200g of SYC;. *=P<0.05

The results showed values obtained from the laboratory for Raw total bacteria count (RTBC), Total Bacteria count (TBC), Raw count total coliform count (RTCC), Total Coliform Count (TCC). Values for Raw total bacteria count (RTBC) had no significant effect. Total Bacteria count (TBC) was significantly different amongst treatment and was highest in T1(4.63 ± 1.24^{a}) which was the control and lowest in T2 (1.70 ± 0.20^{b}), at 100g level of inclusion. Lower count in T2 suggest an inhibitory effect or less favourable conditions for microbial growth due to the yeast concentration used. Variation in yeast levels could alter competitive interactions among microbes, leading to differential growth rates and consequently, varying total bacterial counts. This is supported by the study done by (11).

The result for RTCC was significantly different (P<0.05) with values obtained highest at T3(46.22 ± 13.89^{a}), 150g level of inclusion and lowest at T1(10.56 ± 7.09^{c}), the control. This is probably because higher yeast concentration in T3 will favour specific microbial species' growth or alter the gut environment to support higher coliform counts. Different yeast levels could trigger

specific microbial dynamics, impacting coliform counts differently across treatments. Total Coliform Count had no significant difference across treatments. However, values were obtained highest in T3 (3.60±1.13), while the lowest value was obtained in T2 (1.73±0.86). Yeast supplementation in T3 will most likely favour certain microbial strains that contribute to higher coliform count which indicates a potential issue with the gut health and the general well-being of the bird, whereas yeast concentration in T2 tends not to support such growth which is consistent with the findings by (12).

The effect of age on the gut microbiota of broilers is presented in Table 4

Table 4: Effects of Age on the gut microbiota of broiler birds

Parameters	Week 1	Week 4	Week 7	LOS
RTBC (10^{-4})	128.83±17.42^{a}	76.17±16.02^{b}	5.75±1.02^{c}	*
TBC (CFU/g) $\times 10^{6}$	3.61±0.87±0.87	3.23±0.79	3.69±0.83	NS
RTCC (10^{-4})	66.08±16.18^{a}	17.08±5.71^{b}	1.83±0.74^{c}	*
TCC (CFU/g) $\times 10^{6}$	3.88±0.88^{a}	2.55±0.70^{ab}	1.42±0.69	*

RTBC= Raw count TBC, TBC= Total Bacteria count RTCC= Raw count TCC, TCC= Total Coliform Count, LOS= Level of significant, NS= Not significant, *= P<0.05

The results showed values obtained from the laboratory for Raw total bacteria count (RTBC), Total Bacteria count (TBC), Raw total coliform count (RTCC), Total Coliform Count (TCC). The values for Raw total bacteria count (RTBC) were significantly different and were obtained highest at week 1(128.83±17.42^{a}) while lowest value was obtained at week 7 (5.75±1.02^{c}). Variability in environmental conditions, hygiene practices, or management across different batches/weeks could also impact microbial diversity and abundance based on a study by (13) Total Bacteria count (TBC) were not significantly different with values obtained highest at week 7 (3.69±0.83), and the lowest was at week 4 (3.23±0.79). The result shows that, despite variations in raw counts, the overall total bacterial counts remain relatively consistent across different batches, suggesting stability in bacterial populations. The result for raw count total coliform count (RTCC) was significantly different (??<0.05) with values obtained highest at week 1 (66.08±16.18^{a}), and lowest at week 7 (1.83±0.74^{c}). Total Coliform Count was significantly different across the weeks; the highest value was obtained in week 1(3.88±0.88^{a}) while the lowest value was obtained in week 7 (1.42±0.69). Based on a study by (14) states that age likely impacts specific coliform populations differently

which is likely due to changes in diet, bird health, or management practices.

Correlation between parameters

The correlation between raw total bacterial count, raw total coliform count, total bacterial count, and total coliform count is presented in Table 5

Table 5: Correlation between parameters

	RTBC	TBC	RTCC	TCC
RTBC	*	-0.341*	0.674**	0.279^{NS}
TBC		*	-0.191^{NS}	-0.017^{NS}
RTCC			*	0.317^{NS}
TCC				*

RTBC= Raw count TBC, TBC= Total Bacteria count, RTCC= Raw count TCC, TCC= Total Coliform Count, NS= Not significant, *= Significant at 0.05, ** = Significant at 0.01 percent,

The correlation between RTBC and TBC (-0.341x) indicates a moderate negative relationship. This suggests that as the raw count total bacteria count increases, the total bacteria count decreases, though this correlation is significant ($p<0.05$). This is consistent with the findings of (15), who reported that the inverse relationship might be due to various microbial species' interactions or competition within the ecosystem. There appears to be a moderate positive correlation (0.674**) between RTBC and RTCC. When the raw count total bacteria count increases, the raw count total coliform count also tends to increase significantly at a 0.01 level. This positive correlation likely signifies a symbiotic relationship between certain bacterial species or a shared ecological niche favouring their simultaneous proliferation as observed by (16). This will likely affect the microbial load in the gut because the gut environment seems to be conducive for the growth of bacteria. An excessive population of coliform will however create health problems for the birds. . The lack of significant correlation (0.279NS) between RTBC and TCC suggests no strong relationship between raw count total bacteria count and total coliform count (17), observed and reported that the lack of association could be attributed to the complex interplay of various microbial species with distinct responses to environmental conditions or their relatively independent growth patterns. The correlations between TBC and RTCC (-0.191NS) and TBC and TCC (-0.017NS) both show no significant relationships between total bacteria count and either raw count total coliform count or total coliform count. This lack of correlation could be due to the diverse nature of microbial communities and their differential responses to changes in the environment, rendering no consistent pattern between these parameters.

Bacterial isolates

The various bacterial isolates identified in

different treatments are presented in table 6

Table 6: Bacterial isolates identified.

Treatment	Names of bacterial found
1	*Proteus mirabilis, E. coli, Bacillus* species
2	*Proteus mirabilis, E. coli, Micrococcus spp, Coagulase negative Staphylococcus*
3	*Proteus mirabilis, E. coli, Streptococcus spp, Micrococcus spp*
4	*Proteus mirabilis, E. coli, Streptococcus spp*

Treatment 1 shows the presence of *Proteus mirabilis, E. coli, and Bacillus* species. The composition likely reflects the specific environmental conditions or selective pressures that favour the proliferation of these species. The presence of these bacteria creates a balance in the gut microbiota of the birds. This agrees with the result of (18). The study also showed that in Treatment 2, *Proteus mirabilis* and *E. coli* are consistent with Treatment 1, but with the addition of *Micrococcus spp and Coagulase Negative Staphylococcus (*CoNS). The additional presence of *Micrococcus spp* and CoNS likely indicates an alteration in environmental factors or an introduction of new elements that promote these species' growth alongside the previously observed ones. Treatment 3 shares *Proteus mirabilis and E. coli* with the previous treatments but introduces *Streptococcus spp and Micrococcus spp*. The appearance of *Streptococcus spp* in this study suggests a different set of conditions that likely favours the proliferation of this species, potentially involving alterations in nutrient availability or changes in the microbial community structure within the environment. The study also showed that Treatment 4 had a composition akin to Treatment 3, comprising of *Proteus mirabilis, E. coli, and Streptococcus spp*. This consistency between Treatments 3 and 4 suggests the presence of these specific species which could be resilient to certain changes in the experimental conditions, indicating a robust microbial composition under those circumstances (17).

The presence of *Proteus mirabilis* and *E. Coli* in all four treatments could be due to the variability of microbiota in young broilers and influence factors such as diet and hygiene as noted by (19).

Bacterial isolates present in different age groups

The bacterial isolates present at weeks 1, 4, and 7 are present in Table 7

This study shows that Week 1 is composed of *Proteus mirabilis, Escherichia coli, Bacillus species, and Micrococcus spp*. The presence of these bacterial species might relate to the initial microbial composition introduced or developed within Batch 1, influenced by the source of materials or initial conditions (18). Batch 2 showed a more diverse composition, including Bacillus species, CoNS, *Proteus*

Table 7: Bacterial isolates present in different age groups.

AGE	Names of bacterial found
1Wk	*Proteus mirabilis, Escherichia coli, Bacillus* species, *Micrococcus* spp
4Wk	*Bacillus species, CoNS, Proteus mirabilis, CoNS, E. co li, Bacillus species, Micrococcus spp, Streptococcus spp*
7Wk	*Escherichia coli, CoNS, Bacillus spp, Streptococcus spp., Bacillus spp, Streptococcus spp., CoNS*

Wk=Week, CoNS=Coagulase negative Staphylococci

mirabilis, E. coli, Micrococcus spp, and Streptococcus spp. The increased diversity compared to Batch 1 suggests potential alterations in environmental conditions, nutrient availability, or interactions among the introduced species, promoting the proliferation of a broader range of microbes (20). Batch 3 shows *Escherichia coli*, CoNS, *Bacillus spp, Streptococcus spp*. The variability in this batch, including the repeated presence of *Bacillus spp and Streptococcus spp*, likely indicates their resilience to the experimental conditions or their intrinsic capability to thrive within the provided environment (21) The differences in bacterial isolates across batches could be attributed to variations in initial microbial compositions, the introduction of diverse materials, or fluctuations in environmental parameters such as temperature, pH, and nutrient availability, all of which significantly influence microbial growth and survival (21).

Conclusion

From this study, it is concluded that:

1. Varying levels of yeast inclusion (100g,150g and 200g) in diets led to distinct effects on microbial counts and species proliferation, indicating a role for yeast in modulating gut microbiota. However, SYC added at 150g level of inclusion is concluded to be the preferred level of inclusion for optimal bird performance.
2. These results suggest the potential of yeast as a natural growth promoter in poultry diets for enhanced performance. This is shown in week 1 where yeast affected the microbiota modulation in the gut of the birds.

Recommendations

- Based on the study, it is recommended that supplementation of yeast in broiler feed at 150g per 100kg is optimal for modulating the gastrointestinal tract of broiler chicken
- Conducting extended trials spanning different developmental stages to provide comprehensive insights into the sustained effects of yeast on microbiota and performance.
- Further research should focus on refining the levels of yeast inclusion to maximize its beneficial impact on broiler bird growth and gut health.

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