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Synergistic Effects of Saccharomyces Cerevisiae and Inulin on Growth Performance of Broilers

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ABSTRACT

Saccharomyces cerevisiae has been proven to enhance broiler performance by improving nutrient utilization, modulating gut microbiota composition and strengthening immune response. Inulin, a natural fiber derived from plants, has also been shown to promote the growth of beneficial gut bacteria, leading to improved gut health and overall performance in broilers. This study aimed to evaluate the combined effect of *Saccharomyces cerevisiae* and inulin on the growth performance of broilers, to identify an effective supplementation strategy for broiler production. One hundred and twenty-eight Cobb-500 broilers were randomly distributed to four treatments (control (T0) - basal diet; T1 - 0.5% *Saccharomyces cerevisiae* (yeast); T2 - 0.5% inulin; and T3 - 0.5% *Saccharomyces cerevisiae* + 0.5% inulin. Treatments were replicated four times and arranged in two blocks with house side as the blocking factor. Data on feed intake, water intake and live body weight were collected and analyzed in SAS ver. 9.4. Broilers fed combined inulin and yeast had higher ($p < 0.05$) live body weight, weight gain, and feed conversion efficiency. Broilers fed yeast-only and those fed a basal diet had the lowest ($p < 0.05$) live body weight, weight gain and feed conversion efficiency. These findings highlight the potential benefits of synbiotic supplementation in broiler diets to improve feed efficiency, gut microbiota balance, and growth performance, suggesting strong potential as sustainable alternatives to antibiotic growth promoters in broiler production. The inulin used in this study was extracted from Jerusalem artichoke and the one extracted from chicory roots may provide better results.

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INTRODUCTION

Broiler production is the keystone of the global poultry industry as it provides an affordable protein source. According to FAO (2021), the global broiler market was estimated to be worth over 230 United States Dollars in 2022, with the United States, Brazil, and China being the top producers. The global poultry sector is expected to grow as growing populations, rising incomes, and urbanization drive demand for meat and eggs hence the industry is facing unprecedented challenges (Mottet et al., 2017). In the same article, Mottet et al. (2017) assert that the broiler production sector has grown steadily in developing regions like Asia and Africa. Through providing employment opportunities in farming, processing, and feed manufacturing, broiler production has helped uplift rural economies.

Poultry production in Africa is expected to increase significantly in the coming years to meet the continent's food security needs (Abadula et al., 2022). Countries in Sub-Saharan Africa mainly rely on small-scale, semi-commercial, and traditional poultry production systems. On the other hand, most countries in the northern and southern parts of Africa have more industrialized and commercial poultry production systems (Mottet et al., 2017). The poultry industry is vital for the Zimbabwean economy as it contributes significantly to food security and employment creation directly on farms and in allied industries such as feed processing and marketing. In the past two decades, the sector has experienced a rapid rise in the number of backyard broiler producers supplying local markets (Muzari et al., 2020).

Several companies offer commercial feed supply, with poultry feed central to their business. Prices are highly dependent on the production of maize and soya, as well as import costs and exchange rates of antibiotics and other additives for mixes and grower pellets (Scoones et al., 2018). The major limitation of broiler production is the high cost of broiler feed in Zimbabwe, with feed accounting for up to 70% of the total cost of production (Nyoni et al., 2019).

Due to global warming that has led to poor weather patterns, the cost of the major ingredients in broiler feed production has significantly risen in recent years. Poor agricultural practices and limited access to inputs also contribute to the spiking of prices, reducing profit margins for producers.

Some of the technologies that were suggested in previous years to improve the sustainability of broiler production included the discovery of antibiotics in broiler production which led to improved feed efficiency in production and control of infectious pathologies such as coccidia (Engberg et al., 2000). The ethical aspects of intensive broiler farming have come under scrutiny regarding animal welfare as farmers and feed manufacturers began to use antibiotics for rapid weight gain and growth leading to the rise of antibiotic-resistant pathogens (Bessei, 2018).

While the industry continues to grow, technological advancements and sustainable production practices addressing ethical concerns related to animal welfare and consumer safety will be critical to ensure that countries fulfill goal number 12 of the sustainable development goals (SDGs) that were adopted by the United Nations (UN) in 2015. The goal aims to ensure sustainable consumption and production patterns. Developing alternatives to antibiotics in production chains is one step toward this vision (Sampedro et al., 2021). These alternatives include improved management practices and the introduction of phytochemicals, probiotics such as yeast, and prebiotics such as inulin, mannan-oligosaccharides, and fructooligosaccharides used in poultry production. These compounds are an emerging technology that is still being studied for refinement to understand their proper and responsible application by farmers and related sectors of animal production. Current work demonstrates that these compounds can improve feed intake, feed conversion efficiency, and ultimately growth performance in broilers (Mohamed et al., 2023).

Kocher et al. (2004) claim that controlling and maintaining a healthy and diverse gut microflora is the key to successful antibiotic-free animal production systems. Acidifiers, bacteriophages, bio enzymes, phytochemicals, probiotics, prebiotics, and antimicrobial peptides prove to be promising compounds to replace antibiotics in production systems (Rahman et al., 2022). Their findings suggest that antibiotic resistance cannot be eradicated, but its spread can be slowed down by developing other alternatives and using antimicrobials responsibly.

Probiotics prove to work more efficiently when given to animals that were treated with antibiotics before administration. Studies have shown that the administration of probiotics in poultry and swine increased feed intake and daily weight gain (Rahman et al., 2022). Popov et al. (2024) explore the use of Bacillus spp as probiotics on growth performance in broilers. Over the years, researchers have been using different strains of bacteria as probiotics in broiler nutrition. Others have explored non-bacterial probiotics such as yeasts, the most commonly used being *Saccharomyces cerevisiae*.

Prebiotics are hydrolyzed into monosaccharides or disaccharides; these carbohydrates act as substrates for the fermentation of probiotics in the gut. This increases the biomass and colonization in the host. They are said to increase the production of acetate, propionate, and butyrate, short-chain fatty acids that contribute to the health of the intestinal environment. Butyrate, with its anti-inflammatory properties, reduces the incidence of enteric disease (Pourabedin et al., 2015).

Saccharomyces cerevisiae, a probiotic, and Inulin - a prebiotic, are well-known feed additives that improve gut health and nutrient digestion. While feeding costs remain the largest expense in broiler production, this research aims to identify a dietary strategy that could help enhance performance, reducing reliance on antibiotic growth promoters (AGPs) and broiler production costs. The combination of minimum levels

of *Saccharomyces cerevisiae* and inulin may provide a more comprehensive approach to improving broiler production.

MATERIAL and METHOD

Experimental Site

The experiment was conducted at the University of Zimbabwe's Department of Livestock Sciences bio-assay laboratory located in Mount Pleasant, Harare. The site is bound by the coordinates 17.7824° S, 31.0546° E. It is located on a fairly sloppy terrain with an average wind speed of 16km/h and mean temperatures ranging from 18 to 27.5°C in summer. Ambient temperature inside the rearing pens were controlled with the aid of 250 W infrared lamps.

Animal Ethics

All procedures were conducted in accordance with the Bioassay Laboratory at the University of Zimbabwe, Department of Livestock Sciences following guidelines provided in the Zimbabwe Scientific Animal Act, Chapter 19:12, 1963, License number L624 (Scientific Experiments on Animals Act, 1963; 03-2025-14).

Inulin powder extracted from Jerusalem artichoke was sourced from Willow Wellness™ in South Africa and *Saccharomyces cerevisiae* from Lassafre Zimbabwe™. Feed was provided as mash to ensure proper homogeneity during mixing. Broiler concentrates were mixed with crushed maize in the ratio of 2:3 for starter-grower and 3:7 for grower-finisher to create 2-phase basal diets for the trial. Feed samples were analyzed using Near-infrared reflectance spectroscopy (NIRS) - FOSS NIRS™ DS2500 L, using the broiler feed ground calibration from Cargill™ (Table 1).

Table 1. Nutritional Composition of Diets

Nutrient	Treatment, Feeding phase & Nutrient level (%)							
	Control		T1		T2		T3	
	Ph1	Ph2	Ph1	Ph2	Ph1	Ph2	Ph1	Ph2
Protein	20.60	17.30	21.10	17.50	21.10	17.00	20.90	17.50
Moisture	9.30	10.10	9.00	9.80	10.00	10.30	9.10	10.00
Starch	39.3	42.10	39.0	40.60	39.30	42.00	39.10	42.70
Fat	2.80	3.50	2.60	3.20	3.00	3.20	2.80	3.30
Fibre	3.20	4.00	3.50	4.30	3.60	4.50	3.40	4.30

Ph1: Two phase starter-grower (Stargro®), Ph2: two phase grower-finisher (Grofin®)

Battery cages with a floor area of 1m^2 and recommended stocking density of 10 birds/m^2 were used for the experiment. The cages were cleaned with Biogen Super® (containing Glutaraldehyde, Quaternary Ammonium Compound, Phosphoric Acid

and Non-ionic Surfactant) two weeks before the experiment and disinfected with Biopharm® (containing tar acids and surfactants) and a footbath containing Virukill® (containing Poly Dimethyl Ammonium Chloride) was placed at the entrance as a biosecurity measure.

Chickens were fed *ad libitum* at 1100 hr every day over the trial period. Fresh, portable water was supplied every day with a stress mix containing vitamins in the first week of life. A total of 64 chickens identified with the aid of tags were weighed weekly. Birds were vaccinated against Newcastle Disease and IBD. The vaccination protocol was strictly followed from d 7 during which no other medication was offered to the broilers until d 35.

Bedding was placed and heated 24 hr before receiving the chickens, 250W infrared lamps were used as a heat and light source. Chickens were received, checked for deformities, weighed and labeled using tags on day one and then allocated to their respective treatment groups. Four chickens per cage were labeled with their treatment number and a letter. These 64 chickens would then be weighed each week for LBW and BWG determination.

House temperature was recorded using a mercury thermometer. A 2-phase broiler starter mash and clean potable water were provided *ad libitum* from tray feeders and 3L water fonts. Crop fill was checked after 24 hr. From d 17, chickens were fed a diet with mixed broiler starter and broiler grower in the ratio 2:1 respectively until d 20. Broiler grower was provided from d 21 to 35.

One hundred and twenty-eight Cobb 500 broilers were randomly distributed into 4 treatment groups of 8, with 4 replicates each arranged in 2 blocks with house side as the blocking factor (RCBD). The trial ran for a period of 5 weeks. A 2 × 2 factorial design was used for the assessment of each additive's main effect (Inulin – at 0 & 0.5% and *Saccharomyces cerevisiae* - 0 & 0.5%) as well as their potential interaction.

Table 2. Dietary treatment descriptions

Treatment	**Diet composition**
Control (T0)	Basal diet without Inulin or *S. cerevisiae*
Treatment 1 (T1)	Basal diet + 0.5% Inulin
Treatment 2 (T2)	Basal diet + 0.5% *S. cerevisiae*
Treatment 3 (T3)	Basal diet + 0.5% Inulin + 0.5% *S. cerevisiae*

Broilers were fed once per day for 5 weeks and data were collected on feed offered and left over each day. At the end of each week, live body weight was measured for each bird on a SF-400 digital scale – degree of accuracy ±3g and maximum capacity of 10kg.

Average feed intake was calculated daily per cage, using the following equation;

$$Feed\ intake = \frac{FO-LO}{\mathrm{BC}} \quad (1)$$

Where FO was the feed offered to the birds daily while LO was the leftover feed and BC the total number of birds per cage.

Live body weight was measured weekly using SF-400 digital scale – degree of accuracy ±3g and maximum capacity of 10kg. The following equation was used to calculate the average daily gain;

$$Body\ weight\ gain = \frac{FW-SW}{d} \quad (2)$$

Where FW was the finishing weight per week, SW was the starting weight per week and d, the age of the birds in weeks.

The feed conversion ratio (FCR), also calculated weekly with the following equation;

$$Feed\ Conversion\ Ratio = \frac{BWG}{TF} \quad (3)$$

Where BWG was body weight gain per week and TF was the total feed given. Mortality was recorded as it occurred, noting the possible causes of death and conducting post-mortems, where necessary.

$$Mortality\ rate = \frac{DB \times 100}{\mathrm{B}} \quad (4)$$

Where DB was the number of dead birds per cage and B is the number of birds per cage.

Warm carcass and eviscerated carcass weight were measured at slaughter and cold dressed mass was measured 24 hr after refrigeration.

During slaughter, the tagged birds were euthanized humanely and plucked manually. The abdominal area was disinfected with 70% ethanol and an incision was made just below the sternum. Paired pouches at the ileocecal junction, separating the small and large intestines, were removed using a razor blade and placed in well labeled zip-lock bags (Di Marcantonio et al., 2022). Due to limited resources, some test were carried out a day after slaughter, and during this time the samples were frozen at -80°C.

Laboratory Analysis

Nutrient agar (HiMedia®) was used to test-grow bacteria for total bacterial counts (TBC). It was prepared by dissolving 28g of agar powder in 1L of distilled water followed by autoclaving at 121°C for 15 minutes. The frozen samples were thawed and 1g of the cecal contents transferred into 9ml of sterile distilled water. This was followed by 5 serial dilutions. Petri dishes were labeled and 1ml of the appropriate dilutions were added to the Petri dishes then 20ml of the agar was poured over the dilutions. Samples were incubated for 48 hr at 37°C.

MacConkey agar (Babio®) was used to incubate bacteria for total coliform counts (TCC). It was prepared by dissolving 50g of agar powder in 1L of distilled water then autoclaved as previously described for TBC. The frozen samples were thawed and 1g

of the cecal contents were transferred into 9ml of sterile distilled water followed by 5 serial dilutions. Petri dishes were labeled and 1ml of the appropriate dilutions were added to the Petri dishes then 20ml of the agar was poured over the dilutions. Samples were incubated in aerobic conditions for 48 hr at 37°C.

De Man, Rogosa, and Sharpe (MRS) (HiMedia®) agar was used to grow Lactobacillus. It was prepared by suspending 66.73g of dehydrated MRS agar powder in 1L of distilled water then boiling until the powder was completely dissolved. The solution was then sterilized by autoclaving as previously described. The frozen samples were thawed and 1g of the cecal contents were transferred into 9ml of sterile distilled water, diluted 5 times, dishes labeled and 1ml of the appropriate dilutions added to the Petri dishes then 20ml of the agar was poured over the dilutions. Samples were incubated in a CO_2 incubator (anaerobic) for 48 hr at 37°C.

EMB agar was used to grow E. coli, 35.96g of EMB agar powder (HiMedia®) was suspended in 1000ml of purified/distilled water. The mixture was stirred until a uniform suspension was achieved. The solution was heated to boiling to completely dissolve the medium. It was then sterilized by autoclaving at 121°C for 15 minutes. After autoclaving, the medium was cooled to 45–50°C, and then gently shaken to oxidize the methylene blue and suspend the flocculent precipitate. The frozen samples were thawed and 1g of the cecal contents were transferred into 9ml of sterile distilled water, diluted 5 times, 1ml of the diluted samples was then pippeted on pre-poured agar and gently spread using an L shaped glass spreader to cover the surface of the agar. The plates were incubated at 37°C for 48 hr under aerobic conditions.

Statistical Analysis

Data were entered and edits performed in MS Excel and then exported to SAS ver 9.4 (2017) for analysis. Data were then tested for normality using the Kolmogorov-Smirnov test, Kurtosis and skewness data and related parameter. Data were subjected to ANOVA with repeated measures using the Proc GLM of SAS with house temperature as a random variable. Means were generated by the LSMEANS and compared using the PDIFF options of SAS. Significance was considered at the 0.05 level of probability. The following model was used;

$$Y_{ijkl} = \mu + InW_i + T_j + W_k + B_l + (T\times W)_{jk} + (T\times B)_{jl} + (T\times W\times B)_{jkl} + \varepsilon_{ijklm},$$

where Y_{ijklm} = response variable, μ = overall mean, InW_i = initial weight as a covariate, T_j = effect of the j^{th} treatment (i = 0%, 0.5% inulin, 0.5% *S. cerevisiae*, and 0.5% inulin + 0.5% *S. cerevisiae*), W_k = effect of the k^{th} week (k = 1, 2….6), B_l = effect of the l^{th} block (l = 1 and 2) and ε_{ijklm} = random error. Data for carcass weight were log_{10} transformed in SAS.

RESULTS and DISCUSSION

The ability of inulin to support beneficial gut microbiota may have led to increased appetite. Treatment, week, as well as house temperature, had a significant effect ($p < 0.05$) on both voluntary feed intake and water intake. Treatment × week, treatment × block as well as treatment × week × block interactions were observed ($p < 0.05$) on VFI. Treatment × block interaction, however, did not influence ($p > 0.05$) water intake (Table 3).

Table 3. Levels of significance for all parameters studied on the growth performance of broiler chickens fed inulin and S. cerevisiae based diets

	Parameters				
Factors	**LBW**	**VFI**	**WI**	**FCE**	**BWG**
Initial weight	NS	NS	NS	*	NS
Treatment	***	***	***	***	***
Block	**	NS	*	***	**
Week	***	***	***	***	***
House temperature	***	***	***	NS	NS
Treatment × Week	***	*	***	***	***
Treatment × Block	**	*	NS	***	**
Treatment × Block × Week	***	***	**	***	***
F values	*831.16*	*480.80*	*647.86*	*383.36*	*185.55*

p< 0.0001 (***), p<0.01 (**), p<0.05 (*). LBW: Live body weight, VFI: Voluntary feed intake, WI: Water intake, BWG: Body weight gain, FCE: Feed conversion efficiency.

The study revealed that broilers supplemented with combined inulin and *Saccharomyces cerevisiae* had higher voluntary feed intake than the control group. Birds in Treatment 2, fed a diet with 0.5% inulin, had a significantly higher ($p < 0.05$) VFI than birds in the other treatments. The control group had the highest water intake, (87.3±0.69ml). The lowest values for both water intake (199.2±1.48ml) and VFI (86.8±0.69g) were in the *Saccharomyces cerevisiae*-only group (Figure 1), these findings contrast with those of (Hossain et al., 2025) who found that addition of *Saccharomyces cerevisiae* improved performance parameters in broilers. Dos Santos et al. (2021), however, showed that the addition of yeast cell walls did not affect performance parameters in broilers.

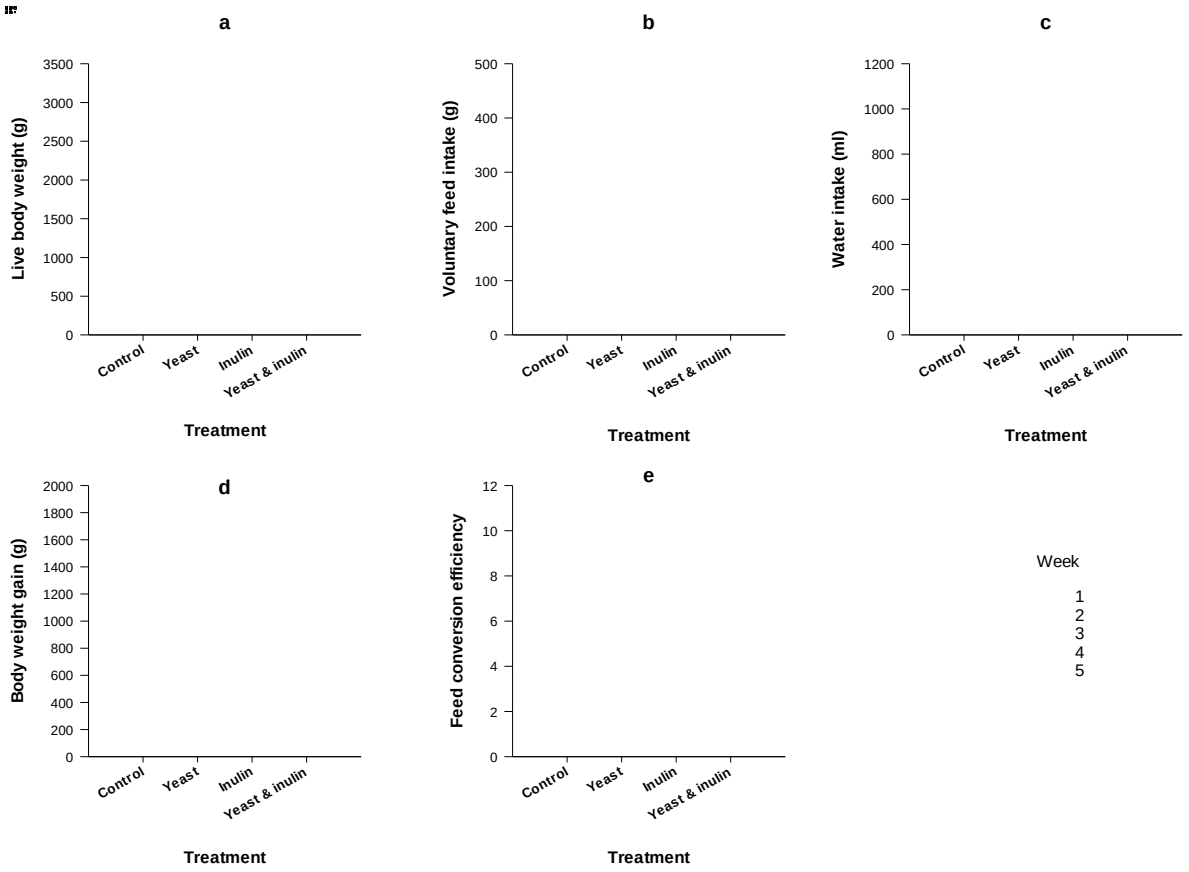

Figure 1. Treatment × week interaction on broiler growth performance

Water intake followed a similar trend consistent with feed intake, as water is essential for digestion, nutrient absorption, and metabolism. The birds that consumed more feed generally had higher water intake to facilitate digestion and thermoregulation. The results indicate variations in feed and water consumption between blocks, which in turn influenced growth performance.

Block 1 had better air circulation than Block 2 and recorded lower water intake (Table 4). The lower water consumption could be attributed to the cooler micro-environment resulting from better ventilation. When airflow is adequate, birds experience less heat stress and tend to drink less water. Improved air circulation may have contributed to lower respiratory discomfort, reducing the need for excessive water intake.

Table 4. Comparison of means for blocks 1 and 2

	Parameters				
Block	**LBW**	**VFI**	**WI**	**BWG**	**FCE**
1	603.6±4.23^{b}	88.8±0.54^{a}	206.2±1.16^{b}	326.4±1.78^{a}	1.8±0.02^{b}
2	622.3±3.63^{a}	89.2±0.47^{a}	209.7±1.00^{a}	318.3±1.52^{b}	2.1±0.02^{a}
p-value	*0.0008*	*0.5350*	*0.0241*	*0.0005*	*<.0001*

$^{a, b}$ values in the same column with different superscripts differ significantly ($p < 0.05$). LBW: Live body weight, VFI: Voluntary feed intake, WI: Water intake, BWG: Body weight gain, FCE: Feed conversion efficiency.

Orakpoghenor et al. (2021) states that water and feed intake are interdependent and a reduction in water intake can lead to reduced feed intake as well. The findings of this study agree with this statement as water intake was significantly higher ($p = 0.0008$) in block 2 and the VFI was numerically higher although not significant. A slightly warmer environment may have influenced the increased water intake in block 2 due to less air circulation, which could have prompted birds to consume more water for improved thermoregulation. Live body weight (LBW), body weight gain, and feed conversion efficiency (FCE) were also significantly higher ($p <.0001$) in block 2. Block did not affect VFI ($p = 0.5350$).

There were interactions ($p < 0.05$) for treatment × week (Figure 1), treatment × block as well as treatment × week × block on the body weight gain and FCE in addition to the significant individual effects ($p < 0.05$) of treatment, block, and week. FCE was significantly lower ($p > 0.05$) in T3 (0.5% *Saccharomyces cerevisiae* + 0.5% inulin). Body weight gain was highest ($p < 0.05$) in treatment 3 (352.4±2.60g), followed by treatment 2 (327.1±2.25g) then treatment 1 (305.0±2.25g) and the control group (304.9±2.25g) which were not significantly different ($p < 0.05$ (Figure 1d)). The presence of inulin likely provided fermentable fiber that supported beneficial bacteria proliferation, enhancing the efficiency of nutrient digestion and subsequent uptake. Combined with *Saccharomyces cerevisiae*, this might have resulted in better FCE and higher carcass weight observed (Figure 2). Birds fed the synergistic treatment consumed more feed, providing them with sufficient nutrients to support greater muscle development and overall growth. Our findings corroborate the research by Acharya et al. (2024) on effects of synbiotics on growth performance.

The combination of inulin and *Saccharomyces cerevisiae* had the highest ($p < 0.05$) live body weight among all treatment groups followed by inulin-only (627.6±5.36g), control (589.1±5.35g) and lastly *Saccharomyces cerevisiae* only (583.2±5.36g). This suggests a synergistic effect, where inulin provides a fermentable substrate that enhances the proliferation of beneficial gut bacteria (Keser and Bilal, 2009), while *Saccharomyces cerevisiae* improves intestinal health and nutrient absorption (Alkalbani et al., 2022). The combined effects led to better feed efficiency and higher weight gain. This aligns with studies by Song et al. (2022) and Mohammed et al. (2018), which prove that, depending on the dosage of synbiotics included in the diet, they significantly increase body weight and the average weight gain.

All factors studied had significant effects $p < 0.05$ on live body weight. There were interactions ($p < 0.05$) between treatment and week, treatment and block and also a three-way interaction between treatment, week and block. Live body weight significantly increased weekly across all treatments.

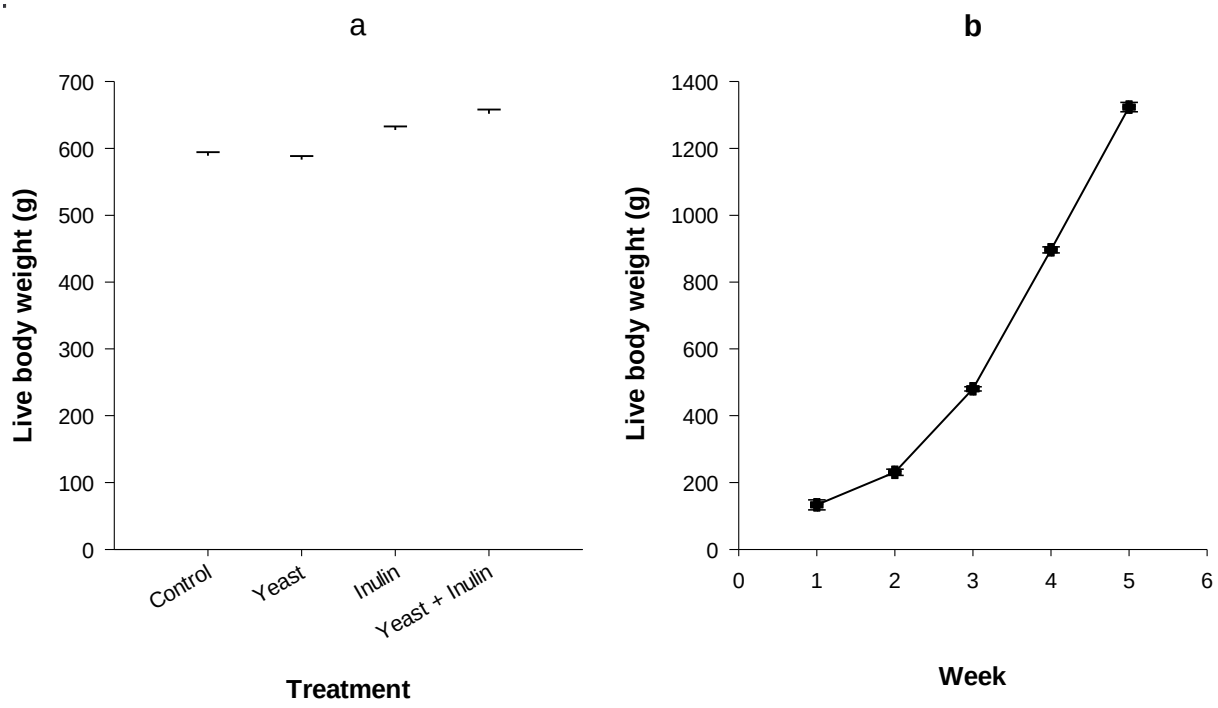

Figure 2. Effect of treatment and week on the live body weight of broiler chickens fed inulin and Saccharomyces cerevisiae

This study found that broilers supplemented with inulin had higher live body weights than those on a basal diet. Similar findings have been reported in a study by Guaragni et al. (2020), where inulin supplementation increased body weight gain by improving feed utilization efficiency.

Nabizadeh (2012) showed that inulin did not affect final body weight compared to the control group. The study also reported that the effectiveness of inulin may be affected by different factors such as the source, inclusion level, and type of diet, animals being used, animal management, and environmental stress.

In our study, broilers in the control group showed the lowest ($p < .0001$) live body weight (Figure 1a), indicating that standard diets may, in fact, not fully support optimal growth. The difference in weight gain between the control and supplemented groups highlights the role of gut microbiota modulation in improving growth performance.

Compared to the control group, broilers fed diets containing *Saccharomyces cerevisiae* did not have increased live body weight. The yeast's probiotic properties, including the competitive exclusion of harmful bacteria, immune system enhancement, and production of beneficial metabolites, did not contribute to their growth performance. A report by Osita et al. (2020) confirms that the inclusion of *Saccharomyces cerevisiae* alone in broiler diets did not affect body weight, daily gain, feed intake, feed conversion (Figure 1) and carcass characteristics.

Carcass weight directly influences profitability and meat yield (Abdullah and Matarneh, 2010). There were significant differences in carcass weight among the treatment groups, with the highest values ($p < 0.05$) recorded in birds receiving the synergistic combination of *Saccharomyces cerevisiae* and inulin, followed by those fed inulin alone (Figure 3a).

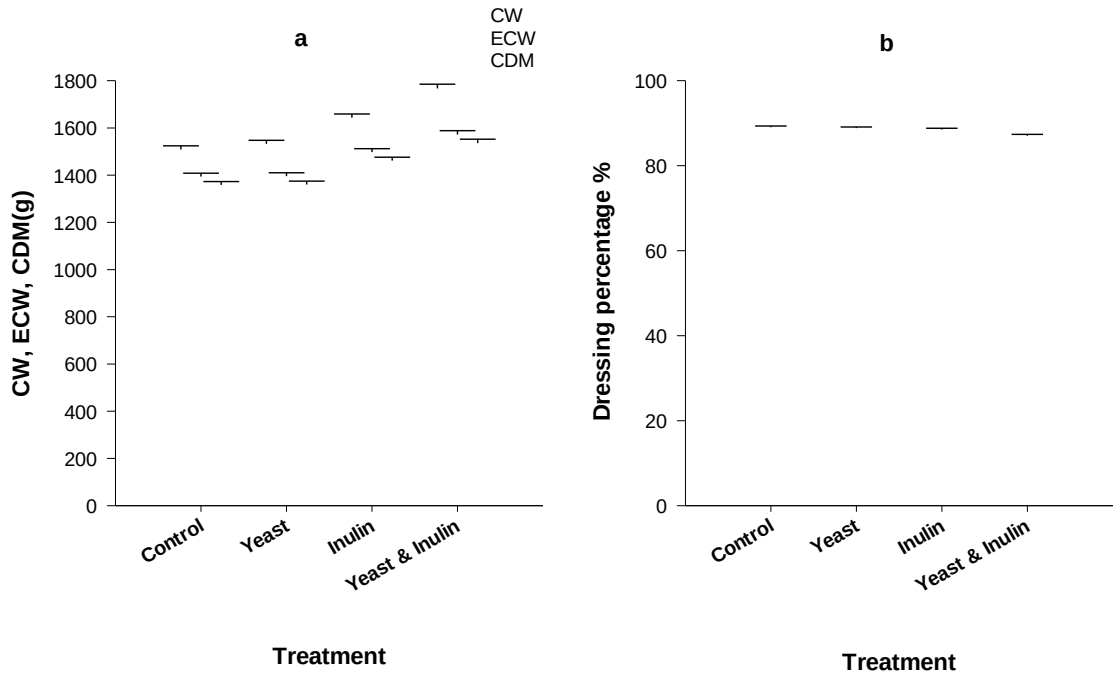

Figure 3. Carcass weight (CW), eviscerated carcass weight (ECW), and cold dressed mass (CDM) and dressing percentage for each treatment

Treatment, and the interaction between treatment × block influenced ($p < 0.05$) carcass weight, eviscerated carcass weight, cold dressed mass and dressing percentage. Block only influenced ($p < 0.05$) carcass weight. Treatment 3 had the highest ($p < .0001$) carcass weight followed by treatment 2 with a mean of 1767.6±17.89g. Carcass weight for treatment 1 and the control group were not significantly different ($p > 0.05$ (Figure 3a)).

The dressing percentage was significantly higher ($p < 0.05$) in T0. In T1 and T2, there was no noticeable difference in dressing percentage. T3 had the lowest dressing percentage (87.1±0.33%) among all treatments (Figure 3b).

Published data on the effect of *Saccharomyces cerevisiae* on broiler performance shows conflicting results. Lin et al. (2023) showed that supplementing *Saccharomyces cerevisiae* aided digestion and gut health leading to improved average weight gain, live body weight and feed efficiency, however, it does not provide direct energy sources like carbohydrates or fats.

Broilers that were fed *Saccharomyces cerevisiae*-only were not significantly different ($p > 0.05$) to the control group on all parameters including carcass weight and live body weight, these findings correspond to those of (Okasha et al., 2023).

The absence of an additional substrate to support microbial fermentation could have limited its overall impact on nutrient absorption and growth. Birds in the control group had the lowest voluntary feed intake, which likely contributed to lower carcass weight. The absence of growth-promoting additives in their diet resulted in suboptimal nutrient utilization and muscle development.

Treatment significantly affected ($p < 0.05$) on total bacterial count, coliforms, *Escherichia Coli*, and *Lactobacillus* spp. Treatment did not affect ($p > 0.05$) the *Salmonella* spp populations in the gut. Total bacterial counts were highest ($201\times10^4\pm32.43\times10^3$ cfu/g) in the synergy group (*Saccharomyces cerevisiae* + inulin), followed by the *Saccharomyces cerevisiae*-only group ($183\times10^4\pm32.43\times10^3$ cfu/g), while the control group exhibited the lowest counts ($66.7\times10^3\pm32.43\times10^3$ cfu/g). The inulin-only group was not significantly different ($p > 0.05$) from the control group (Figure 4).

The results indicate significant variations in microbial populations among the treatment groups, highlighting the effects of *Saccharomyces cerevisiae*, inulin, and their combination on gut microbiota balance. Combining inulin and *Saccharomyces cerevisiae* created a favorable gut environment. The proliferation of Lactobacillus in the gut led to improved digestion and absorption of essential nutrients needed for muscle growth. In contrast, the *Saccharomyces cerevisiae*-only and control groups exhibited the lowest ($p > 0.05$) carcass weights and *Lactobacillus* populations.

The combination of Saccharomyces cerevisiae and inulin provided an optimal environment for microbial proliferation. Inulin acted as a substrate for beneficial bacteria, while Saccharomyces cerevisiae enhanced microbial activity. The synergy between these two dietary components resulted in an overall increase in *Lactobacillus* spp. and *E. coli.* Yeasts provide essential nutrients that support bacterial colonization (Carvalho et al., 2020a). The presence of *Saccharomyces cerevisiae* alone stimulated bacterial growth because of its fermenting properties. Birds in the control group, which did not receive any supplementation, had the lowest TBCs (Figure 4a). This suggests that the absence of prebiotic or probiotic interventions resulted in a less diverse microbial environment, potentially negatively affecting digestion and nutrient absorption, contributing to a higher total bacterial count.

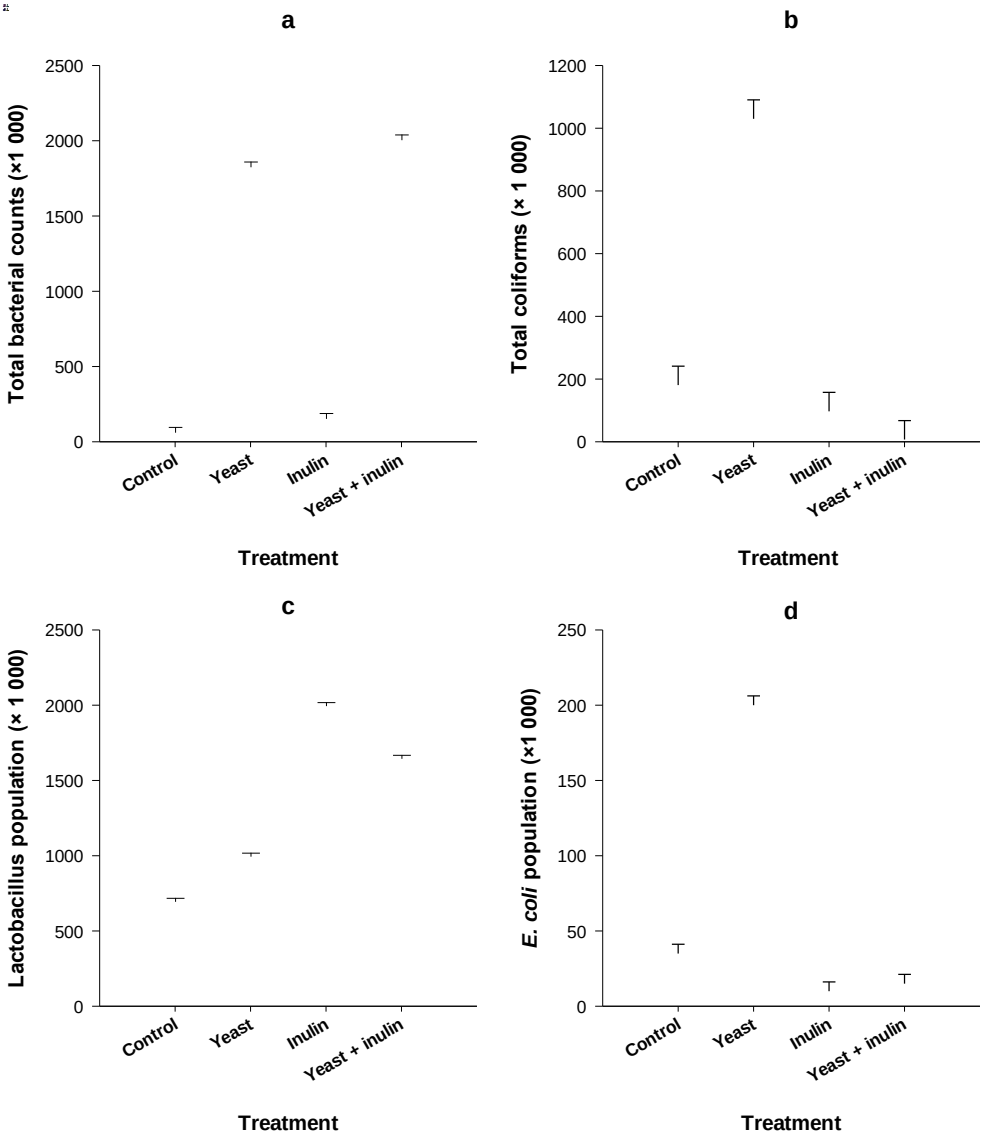

Figure 4. Effects of *Saccharomyces cerevisiae* and inulin on broiler cecal microbial population

Coliform bacteria are anaerobic, Gram-negative, non-spore-forming rods that actively ferment lactose (Britton et al., 2021). Total coliform populations were highest ($p < 0.05$) in the *Saccharomyces cerevisiae* group followed by the control group, while the inulin and synergy groups had the lowest ($p < 0.05$) counts (Figure 4).

Saccharomyces cerevisiae's fermentation activity may have indirectly created conditions that allowed coliforms to thrive. The absence of prebiotics to selectively promote beneficial bacteria may have led to increased coliform growth. The control group had relatively high coliform levels, due to the absence of dietary interventions that could help suppress pathogenic bacteria. Inulin, known for promoting beneficial bacteria like *Lactobacillus*, may have contributed to competitive exclusion, reducing coliform populations (Bucław, 2016). The synergy group showed even lower coliform counts,

suggesting that the combination of *Saccharomyces cerevisiae* and inulin created optimal microbial balance, limiting the detrimental proliferation of coliforms.

The combination of *Saccharomyces cerevisiae* and inulin further supported *Lactobacillus* growth, though not to the same extent as inulin alone. While *Saccharomyces cerevisiae* contributes to a healthier gut environment, it does not directly provide fermentable substrates like inulin (Carvalho et al., 2020a). This may explain the slightly lower *Lactobacillus* levels compared to the inulin-only group (Figure 4c). Carvalho et al. (2020b) stress that some *Lactobacillus* spp like *L. fermentum* compete with yeast for nutrients, inhibiting the fermentation process and consequently the growth of yeast cells. This may explain why birds that were fed the *Saccharomyces cerevisiae*-only diet performed comparatively poorly and with moderate *Lactobacillus* populations. The control group had the lowest *Lactobacillus* counts, indicating that supplementation is necessary to enhance beneficial bacterial populations.

The increase in *E. coli* populations in the *Saccharomyces cerevisiae*-only group (Figure 4d) may be due to microbial competition. While *Saccharomyces cerevisiae* supports general gut health, it may not have been sufficient to suppress *E. coli* proliferation without the presence of a prebiotic. The control group had relatively higher *E. coli* levels, likely due to the absence of dietary interventions to modulate gut microbiota. Without competitive exclusion from beneficial bacteria, *E. coli* was able to thrive.

Inulin supplementation suppressed *E. coli* populations. Ding et al. (2020) observed that inulin supplementation significantly reduces *E. coli* growth. Prebiotics promote the growth of beneficial bacteria such as *Lactobacillus*, which produce antimicrobial compounds that inhibit *E. coli* proliferation (Nabizadeh, 2012) with the synergy group exhibiting a similar effect in the current study.

CONCLUSION and RECOMENDATIONS

The results of the current study showed positive effects of combined inulin and *Saccharomyces cerevisiae* at 0.5% inclusion level on growth performance, caecum microbial population and carcass output in broiler chickens. These findings highlight the potential benefits of synbiotic supplementation in broiler diets to improve feed efficiency, gut microbiota balance, and growth performance, supporting their potential as sustainable alternatives to antibiotic growth promoters in broiler production. Future research should explore different prebiotic-probiotic combinations, long-term effects on gut health, and application in commercial poultry production systems to further improve broiler performance. Inclusion of the synbiotic was at 0.5%, and the performance outcome might be higher with an increase in the inclusion level.

Conflict of Interests statement

The authors have declared that there are no competing interests.

Author's contributions

NCS conceptualized and executed the study. TM supervised the entire experiment and was responsible for reviewing the manuscript. NCS, SCC, and EPM helped with the fieldwork, laboratory analyses, and drafting of the manuscript. Each author has reviewed and approved of this manuscript.

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