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Physiological and meat quality responses to phased and continuous hydrolyzed yeast (*Saccharomyces cerevisiae*) supplementation in densely stocked broiler chickens

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ABSTRACT

The transition toward antibiotic-free broiler production has intensified interest in precision nutritional strategies that enhance bird resilience under intensive rearing conditions. Hydrolyzed yeast (HY, *Saccharomyces cerevisiae*) delivers a complex array of bioactive compounds that support gut function and immune regulation; however, the benefits of HY are likely growth-stage dependent, and continuous supplementation may be economically inefficient. This study evaluated the physiological, gut microbial, and meat quality responses of densely stocked Ross 308 broilers to phase-targeted HY feeding strategies: continuous HY supplementation and HY supplementation restricted to the starter, grower, or finisher phase. Growth performance, haemobiochemical indices, carcass and meat quality, and caecal microbiota composition were assessed. Continuous AGP supplementation yielded the highest ($P < 0.05$) feed intake, body weight gain, and feed efficiency. In comparison, HY feeding strategies did not improve growth performance ($P > 0.05$) relative to antibiotics, but did influence physiological and meat quality outcomes when applied continuously or in a phased manner. Phase-specific HY supplementation induced distinct physiological responses ($P < 0.05$), including changes in nitrogen-related and liver-associated blood indices, with negative impacts on carcass or meat quality. Caecal microbial alpha diversity remained unchanged across treatments ($P > 0.05$), though selective shifts in microbial composition occurred. Overall, few significant differences were detected between phased and continuous HY supplementation across the measured outcomes, suggesting that phased HY supplementation warrants further investigation as a practical nutritional strategy for antibiotic-free intensive broiler production.

1. Introduction

Growing global demand for broiler meat has accelerated the adoption of intensive production systems that maximize output per unit area. However, high stocking density, a common feature of such systems, can impair gut integrity, disrupt immune function, and compromise growth performance (Salois et al., 2016). Because the gastrointestinal tract underpins nutrient utilization and host defense, maintaining gut health is critical for sustainable broiler production (Sumanu et al., 2023). Regulatory restrictions on antibiotic growth promoters (AGPs) have accelerated the transition toward antibiotic-free broiler production systems (Mehdi et al., 2018; Zhen et al., 2023). In this context, maintaining gut function and physiological stability under intensive rearing conditions has emerged as a critical nutritional challenge. Rather than relying on continuous supplementation, there is growing interest in functional feeding strategies that deliver targeted support during periods of heightened physiological demand.

Dietary hydrolyzed yeast (HY) derived from *Saccharomyces cerevisiae* has emerged as a promising functional feed additive in poultry nutrition (Perricone et al., 2022). Produced through controlled yeast cell disruption, HY provides a mixture of bioactive compounds, including peptides, amino acids, and cell wall polysaccharides such as mannan

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oligosaccharides and β-glucans, that support intestinal integrity, metabolic efficiency, and immune function in broilers (Comi et al., 2025; Pratama et al., 2026). Supplementation with HY has been associated with improved intestinal morphology, enhanced immune responsiveness, and better growth performance in poultry (Abbas et al., 2025; Bortoluzzi et al., 2025). However, continuous inclusion of HY may increase feed costs, limiting its routine use as a feed additive that supports antibiotic-free poultry production. Because the physiological benefits of HY are often most pronounced during periods of rapid growth or environmental stress (Hakami et al., 2025; Mazur-Kusnirek et al., 2025), reducing cumulative additive inclusion may have economic benefits; however, these require formal evaluation.

High stocking density is an environmental stressor that can compromise immune function, gut health, and performance in broilers (Sugiharto, 2022), thereby elevating the potential responsiveness to functional feed additives such as HY. Although yeast-derived products are of growing interest, studies directly comparing phase-specific and continuous HY supplementation, particularly with respect to physiology, meat quality, and gut microbiota, are limited. Therefore, the objective of this study was to evaluate the effects of continuous versus phased supplementation with HY on growth performance, physiological responses, meat quality traits, and caecal microbial diversity in densely stocked Ross 308 broiler chickens. The study tested the hypotheses that phased supplementation with HY would elicit physiological, meat quality, and gut microbial responses comparable to those observed with continuous supplementation; and that these responses would occur without compromising growth performance or intestinal health under high stocking density conditions. This study aimed to determine whether phased HY supplementation can elicit biological responses comparable to those achieved with continuous supplementation, thereby informing more economically sustainable nutritional strategies for antibiotic-free broiler production systems.

2. Materials and methods

2.1. Ethical statement

All animal care, handling, and sampling procedures were conducted in accordance with institutional guidelines for the care and use of animals in research. Ethical approval for the study was obtained from the University of Mpumalanga Research Ethics Committee – Animal Sciences (UMP REC-AS; approval no. UMP/Mlambo/1/2024) and the North-West University Animal Production Research Ethics Committee (NWU-ANIMPROD REC; approval no. NWU-00818-24-A5).

2.2. Study site

The experiment was conducted at the Poultry Research Unit of the University of Mpumalanga (UMP), South Africa. Ambient temperatures ranged from 25°C to 32°C during the day and averaged 17°C at night. All experimental procedures were carried out during a single production cycle to minimize environmental variation.

2.3. Feeding strategies

Broiler starter, grower, and finisher diets were formulated to meet the nutritional requirements of Ross 308 birds (Aviagen, 2022). The diets were formulated to be isonitrogenous and isocaloric (Table 1)

Table 1
Gross ingredient and calculated composition (g/kg as fed basis) of experimental diets formulated for the starter, grower, and finisher phases.

	Starter (7 – 14 d)			Grower (15 – 28 d)			Finisher (29 – 42 d)		
Parameter	POSCON	NEGCON	HYS	POSCON	NEGCON	HYG	POSCON	NEGCON	HYF
Yellow corn	440.30	445.30	444.80	481.20	486.20	485.70	543.70	548.70	548.20
Soybean meal (46% CP)	417.10	417.10	417.10	379.40	379.40	379.40	326.70	326.70	326.70
L-Lysine HCl	2.10	2.10	2.10	1.40	1.40	1.40	1.70	1.70	1.70
DL-Methionine (99% CP)	4.20	4.20	4.20	4.50	4.50	4.50	3.50	3.50	3.50
L-Threonine (98% CP)	1.20	1.20	1.20	0.90	0.90	0.90	0.80	0.80	0.80
Limestone	14.00	14.00	14.00	11.60	11.60	11.60	8.70	8.70	8.70
MDCP	21.70	21.70	21.70	17.70	17.70	17.70	14.70	14.70	14.70
Fine salt	2.40	2.40	2.40	2.60	2.60	2.60	2.50	2.50	2.50
Sodium bicarbonate	2.80	2.80	2.80	2.50	2.50	2.50	2.70	2.70	2.70
Poultry VT Premix (15.8% Ca)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soy oil	84.2	84.2	84.2	88.2	88.2	88.2	85.00	85.00	85.00
Zinc bacitracin	5.00	0.00	0.00	5.00	0.00	0.00	5.00	0.00	0.00
Hydrolysed yeast	0.00	0.00	0.50	0.00	0.00	0.50	0.00	0.00	0.50
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Composition (g/kg unless indicated otherwise)									
Crude protein	230			215			195		
Crude fat	107.8			112.3			110.1		
Calcium	9.5			8			6.5		
Av. Phosphorus	5			4.2			3.6		
AME (kcal/kg)	2975			3050			3100		
SID methionine	7.1			7.2			6		
SID lysine	13.2			11.8			10.8		
SID tryptophan	2.6			2.4			2.1		
SID threonine	8.8			7.9			7.2		
SID isoleucine	8.7			8.1			7.3		
SID histidine	5.4			5			4.6		
SID valine	9.3			8.7			7.9		
SID leucine	16.2			15.4			14.3		
SID arginine	14.2			13.2			11.7		
Linoleic acid	55.8			58.1			56.8		
Sodium	1.8			1.8			1.8		
Potassium	10.2			9.5			8.6		
Chloride	2.3			2.3			2.3		

Basal diets: NEGCON = a basal diet without antibiotic growth promoter (AGP) or HY; POSCON = a basal diet with AGP.
Hydrolyzed yeast supplementation strategy: a basal diet containing 0.05% HY as an alternative to AGP fed either during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF).

according to the recommendations of Aviagen (2022), as follows: 1. a diet with neither AGP nor HY, fed from the starter phase through to the finisher phase [NEGCON]; 2. a diet containing AGP only, fed continuously [POSCON]; 3. a diet containing 0.05% HY as an alternative to AGP, fed in the starter phase only for a week [HYS]; 4. a diet containing 0.05% HY as an alternative to AGP, fed in the grower phase only for two weeks [HYG]; 5. a diet containing 0.05% HY as an alternative to AGP, fed in the finisher phase only for two weeks [HYF]; and 6. a diet containing 0.05% HY as an alternative to AGP, fed continuously [HYC]. The HY inclusion level (0.05%) was selected based on previous work by Maina et al. (2024), which reported physiological benefits at this inclusion level. During the non-supplemented phases in the HYS, HYG, and HYF groups, AGP was not administered. The dietary HY, Maxi-Nutrio, was manufactured by CBS Bio-platforms (Ontario, Canada) and included at 0.5 g/kg. Maxi-Nutrio® is a commercial hydrolyzed yeast product containing approximately 15% free nucleotides, with the remaining fraction comprising yeast-derived constituents and carrier materials supplied as part of the commercial formulation. The AGP used in this study was a commercial zinc bacitracin premix (Pucheng Lifecome Biochemistry Co., Ltd.) containing 15% active zinc bacitracin. The premix was included at 5.0 g/kg in the finished feed, providing a final active zinc bacitracin concentration of 750 mg/kg in the complete diet. Feed ingredients were sourced from Simple Grow Agric Services (Pty) Ltd (Centurion, Pretoria, South Africa) and Nutroteq (Pty) Ltd. (Centurion, Gauteng, South Africa).

2.4. Experimental design and bird management

The study employed a completely randomized design. A total of 900 one-week-old, unsexed Ross 308 broiler chicks were randomly allocated to six dietary treatments, each replicated six times. A total of 25 birds were allocated to each pen (1.2 m^2) to evaluate the effects of HY under high-stocking-density conditions. Based on the final average body weight at market age (approximately 2.0 kg/bird), the birds were reared at a stocking density of approximately 41.7 kg live weight/m^2. According to the South African Poultry Association (SAPA, 2012), conventional broiler production systems typically operate at a maximum stocking density of approximately 30 kg live weight/m^2. The stocking density imposed in the present study, therefore, exceeded industry recommendations and was deliberately selected to create a moderate environmental challenge for evaluating the efficacy of HY supplementation strategies. Birds remained in their assigned pens and followed the same feeding strategy throughout the experimental period.

Birds were housed in floor pens bedded with clean wood shavings and managed under commercial broiler production practices. Birds had continuous access to fresh water and were fed *ad libitum* throughout the experimental period. A routine vaccination program appropriate for broiler chickens was implemented in accordance with local veterinary recommendations. Birds were monitored daily for general health status, behavior, and mortality. To maintain a constant stocking density throughout the experimental period, bird mortality was monitored daily, and mortalities were immediately replaced with age-matched birds from the same source flock. The treatment allocation, timing, and body weight of each mortality and replacement bird were recorded. Mortality and replacement numbers for each treatment have now been reported in the Results section. Feed intake was measured on a pen basis; therefore, feed consumed by replacement birds was included in the total pen feed intake. However, replacement birds were introduced solely to maintain stocking density and were excluded from all performance measurements. Consequently, body weight gain was calculated using only the original experimental birds, and replacement birds did not contribute to BWG calculations. Mortality-adjusted feed conversion ratio was calculated as: Mortality-adjusted FCR = Average pen feed intake (kg) ÷ average body weight gain (kg) of the original experimental birds. No therapeutic antibiotics were administered during the experimental period, except those included in the positive control diet. Data collection began at 7 days of age, following a six-day adaptation period during which birds acclimated to the housing conditions and experimental management practices.

2.5. Growth performance

Body weight and feed intake were recorded on a pen basis at the beginning of the trial and at the end of each production phase (starter (7 – 14 days), grower (15 – 28 days), and finisher (29 – 42 days). Birds were weighed collectively per pen using a calibrated digital scale to determine body weight gain (BWG). Feed intake (FI) was calculated as the difference between feed offered and feed refused for each pen during the corresponding period. Feed conversion ratio (FCR) was calculated as the ratio of feed intake to body weight gain (g feed/g gain) for each pen. Daily mortality was recorded, and the weights of deceased birds were used to correct the FCR values.

2.6. Blood parameters, visceral morphometry, and meat quality

On day 40, two birds per pen (n = 12 birds per dietary treatment), with body weights close to the pen mean, were randomly selected for blood collection. Birds were fasted for approximately 8 h prior to sampling, and blood was collected via brachial vein puncture using sterile syringes. Samples were divided into two portions: one collected into EDTA-coated tubes for haematological analysis and the other into plain tubes for serum separation. Haematological parameters, including haematocrit, thrombocyte count, heterophil count, red blood cell count, total white blood cell count, and differential leukocyte counts, were determined using an automated veterinary haematology analyser (IDEXX ProCyte One; IDEXX Laboratories, Johannesburg, South Africa), which employs fluorescence flow cytometry and laser-based optical analysis for cell identification and enumeration. Serum samples were obtained by centrifugation at 3,000 × g for 10 min and stored at -20°C until analysis. Serum biochemical parameters, including total protein, albumin, globulin, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, total bilirubin, calcium, and phosphorus, were quantified using Chem 16 CLIP reagent slides (IDEXX Laboratories, Johannesburg, South Africa) on an IDEXX Catalyst One chemistry analyser. Analyses were performed according to the manufacturer's instructions using dry-slide chemistry.

At 42 days of age, feed was withdrawn from all experimental broilers for 12 h to allow evacuation of the crop. Birds were humanely slaughtered by electrical stunning followed immediately by exsanguination. Immediately after slaughter, carcasses were eviscerated and dissected to determine the weights of major carcass parts (breast, thigh, drumstick, and wing) and internal organs (proventriculus, ventriculus, spleen, liver, small intestine, and caeca). All measurements were expressed as g/100 g of hot carcass weight (HCW). Breast muscle pH was measured 24 h postmortem using a Corning Model 4 pH–temperature meter (Corning Glass Works, Medfield, MA, USA) fitted with a spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland). Meat colour parameters [lightness (L*), redness (a*), and yellowness (b*)] were read twice on freshly cut breast muscle surfaces using a Minolta colourimeter (Spectrophotometer CM-2500c, Konica Minolta, Osaka, Japan) with a 20-mm aperture and 45°/0° geometry. Colour measurements were subsequently used to calculate hue angle and chroma values. The following formulae were used to calculate hue angle and chroma values: Chroma = $\sqrt{(a^2 + b^2)}$ and Hue angle = $\tan^{-1}\left(\frac{b*}{a*}\right)$

Shear force (N) of breast meat was determined by shearing raw samples perpendicular to the direction of muscle fibers using a Meullenet–Owen's razor shear blade (A/MORS) mounted on a texture analyzer (TA.XT Plus, Stable Micro Systems, Surrey, UK). Cooking loss was determined according to the method described by Pang et al. (2020). Water-holding capacity of breast meat was assessed based on the amount of fluid released under applied pressure to the freshly

excised *pectoralis major* muscle, following the procedure described by Mararenі and Mnisi (2020). Drip loss was determined using the method described by Yang et al. (2025).

2.7. Gut microbiome

At slaughter, caecal contents were collected aseptically from three birds per experimental unit, with each bird analysed individually (no pooling). Samples were placed into sterile 50-mL Falcon tubes, snap-frozen in liquid nitrogen, and stored at −80°C until further analysis. Genomic DNA was extracted using the Zymo Research DNA Extraction Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The full-length bacterial 16S rRNA gene was amplified using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR amplification was performed using a high-fidelity polymerase under the following conditions: initial denaturation at 95°C for 3 min; 25–30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 90 s; followed by a final extension at 72°C for 5 min. Reaction volume was 25 μL containing standard buffer, dNTPs, primers (0.2–0.4 μM), polymerase, and approximately 20–50 ng template DNA. Amplicon libraries were prepared using the PacBio SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences, Menlo Park, CA, USA) according to the manufacturer's protocol and purified using AMPure PB beads. Sequencing was performed on the PacBio Sequel II platform to generate high-fidelity (HiFi) reads. Raw circular consensus sequencing (CCS) reads were generated on the Sequel II platform and processed using PacBio SMRT Link (version 10.2) with default quality-filtering parameters to produce HiFi reads. Reads were demultiplexed and further processed using QIIME 2 and DADA2 for denoising, quality filtering, and chimera removal using the consensus method. Only high-quality HiFi reads were retained for downstream analysis. After quality control, HiFi read counts ranged from 148 to 97,178 reads per sample, representing the final dataset used for downstream statistical and microbiome analyses. Taxonomic assignment was performed using the SILVA database (version 138) with a confidence threshold of 70%. Alpha diversity was calculated using Shannon and Chao1 indices, and group comparisons were assessed using the Kruskal–Wallis test. Beta diversity was evaluated using Bray–Curtis dissimilarity and both weighted and unweighted UniFrac distance matrices. Principal coordinates analysis (PCoA) was used for ordination, and differences between treatment groups were tested using PERMANOVA with 999 permutations in the vegan package in R.

Blood and caecal samples were collected only at the end of the experimental period (day 40 and slaughter, respectively). Consequently, the study evaluated endpoint physiological and microbial responses following different hydrolysed yeast supplementation schedules rather than longitudinal changes during the starter, grower, and finisher phases.

2.8. Statistical analyses

The experimental unit for growth performance was the pen. For bird-level data (blood parameters, carcass traits, meat quality, visceral morphometry, and microbiome analyses), measurements were averaged within each pen before statistical analysis, with the pen considered the experimental unit. Prior to analysis, all data were tested for homogeneity of variance and normality using Levene's test and the NORMAL option of the PROC UNIVARIATE procedure in SAS (SAS Institute Inc., Cary, NC, USA), respectively. Cross-sectional data were analysed using the general linear models procedure of SAS, with feeding strategy included as the fixed effect. Statistical significance was declared at $P < 0.05$, and least squares means were separated using Tukey's honestly significant difference (HSD) test.

For caecal microbiota analyses, alpha diversity indices, including Shannon diversity, Pielou's evenness, and Simpson's index, were calculated to assess within-sample diversity. Differences in alpha diversity among dietary treatments were evaluated using the Kruskal–Wallis test. Beta diversity was evaluated using Bray-Curtis and weighted UniFrac distance matrices. Principal coordinates analysis (PCoA) was used to visualize differences in microbial community structure among dietary treatments. Differences in microbial community composition among dietary treatments were evaluated using permutational multivariate analysis of variance (PERMANOVA) based on the Bray-Curtis distance matrix with 999 permutations. All microbiome-related statistical analyses and visualizations were conducted using QIIME2, SAS, and R software (Reproducible Omics Workflow, Version 0.1; Idowu, 2026), with the *rstatix, FSA, vegan, ggpubr, kableExtra,* and *ggplot2* packages.

3. Results

3.1. Mortality

No significant differences in mortality rate ($P > 0.05$) were observed across all feeding strategies. The mean mortality rates were 6.01%, 1.28%, 4.46%, 4.46%, 3.36%, and 2.28% for NEGCON, POSCON, HYS, HYG, HYF, and HYC, respectively.

3.2. Growth performance

The growth performance responses of broilers to different feeding strategies are presented in Table 2. During the starter phase, feed intake was higher ($P < 0.05$) in birds receiving the NEGCON diet than in those receiving the HYF diet, with statistical similarities to diets HYS, HYG, and HYC. From the grower phase onward, feed intake increased ($P < 0.05$) in birds fed the POSCON diet when compared with the NEGCON. Birds receiving the POSCON diet showed the highest overall weight gains. However, during the starter phase, BWG in the POSCON group did not differ ($P > 0.05$) from that observed in the HYF and HYC groups. In the grower phase, BWG in the POSCON group was not significantly different from those of the HYS, HYG, and HYF groups ($P > 0.05$), whereas in the finisher phase, no significant differences ($P > 0.05$) were observed between the POSCON group and birds receiving HYS, HYG, and HYC diets. The FCR differed among treatments, with birds fed the POSCON diet recording the lowest overall FCR ($P < 0.05$) compared to the other dietary treatments.

3.3. Blood parameters, visceral morphometry, and meat quality

Blood biochemical and haematological responses to the different feeding strategies are presented in Tables 3 and 4. Supplementation with HY during the starter phase resulted in higher ($P < 0.05$) blood urea nitrogen concentrations than the other dietary treatments. Similarly, HY supplementation during the grower phase was associated with significantly higher ($P < 0.05$) serum albumin, alanine aminotransferase, and total bilirubin concentrations relative to the remaining feeding strategies (Table 4). Birds receiving HY during the finisher phase exhibited higher ($P < 0.05$) thrombocyte and monocyte counts compared with the other experimental groups (Table 4). Heterophil relative percentage was significantly higher ($P < 0.05$) in birds fed the NEGCON diet as well as those receiving phased or continuous HY supplementation, in comparison with those of the POSCON group. Visceral morphometry results are summarized in Table 5. Ileal length differed among feeding strategies, with birds fed the POSCON diet recording longer ilea ($P < 0.05$) than those fed HY-based diets. Meat quality traits are presented in Table 6. Drip loss was higher ($P < 0.05$) in birds supplemented with HY during the grower phase, with values comparable ($P > 0.05$) to those observed in the NEGCON group. Shear force differed among treatments, with higher ($P < 0.05$) values recorded in birds fed the POSCON diet and in those receiving continuous HY supplementation.

Table 2
Effects of hydrolyzed yeast supplementation strategies on the growth performance (all parameters are expressed in grams, except for FCR) of Ross 308 broilers.

Parameters[2]	Supplementation strategy[1]						SEM
	NEGCON	POSCON	HYS	HYG	HYF	HYC	
Feed intake S	412.5^{a}	358.6^{ab}	351.7^{ab}	387.7^{ab}	338.0^{b}	349.7^{ab}	15.83
Feed intake G	1150.0^{b}	1199.0^{a}	1129.0^{b}	1165.0^{b}	1164.0^{b}	1129.0^{b}	13.61
Feed intake F	1735.0^{b}	1867.0^{a}	1724.0^{b}	1747.0^{b}	1741.0^{b}	1741.0^{b}	27.27
Overall FI	3297.0^{b}	3425.0^{a}	3205.0^{b}	3300.0^{b}	3243.0^{b}	3240.0^{b}	31.28
BWG S	174.0^{b}	191.7^{a}	153.3^{c}	170.3^{b}	182.0^{ab}	175.5^{ab}	3.89
BWG G	799.2^{b}	881.3^{a}	816.7^{ab}	831.7^{ab}	835.6^{ab}	792.1^{b}	16.85
BWG F	854.9^{b}	988.7^{a}	920.4^{ab}	896.9^{ab}	862.3^{b}	895.3^{ab}	28.44
Overall BWG	1828.0^{b}	2062.0^{a}	1890.0^{b}	1899.0^{b}	1880.0^{b}	1863.0^{b}	27.71
FCR S	2.375^{a}	1.862^{b}	2.313^{a}	2.283^{ab}	1.859^{b}	1.996^{ab}	0.10
FCR G	1.440	1.363	1.383	1.402	1.396	1.431	0.02
FCR F	2.038^{a}	1.895^{b}	1.878^{b}	1.952^{b}	2.022^{a}	1.962^{b}	0.04
Overall FCR	1.806^{a}	1.663^{b}	1.696^{a}	1.738^{a}	1.726^{a}	1.726^{a}	0.02

^{abc} Means with different superscripts are different ($P < 0.05$). NEGCON = a basal diet without antibiotic growth promoter (AGP) or HY fed continuously; POSCON = a basal diet with AGP fed continuously.
Sample size = 900 birds; Experimental unit = 25 birds/1.2 m^2.
[1] Supplementation strategy: a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF).
[2] Parameters: S = Starter, G = Grower; and F = Finisher. BWG = Body weight gain; FCR = Feed conversion ratio.

Table 3
Effects of hydrolyzed yeast feeding strategies on blood serum parameters of Ross 308 broilers.

Parameters	Supplementation strategy[1]						SEM
	NEGCON	POSCON	HYS	HYG	HYF	HYC	
GLU mmol/L	15.8	20.7	18.7	22.3	16.8	17.8	2.92
BUN mg/dL	2.8^{b}	2.2^{b}	4.8^{a}	3.8^{b}	3.3^{b}	2.3^{b}	0.50
PHOS mg/dL	14.6	13.7	13.4	14.3	13.4	14.1	0.59
CA mg/dL	10.2	10.2	10.7	10.4	10.1	10.3	0.25
TP g/dL	5.2	5.1	5.6	6.1	5.5	4.8	0.30
ALB g/dL	1.9^{b}	1.8^{b}	2.1^{b}	2.3^{a}	1.9^{b}	2.0^{b}	0.09
GLOB g/dL	3.3	3.2	3.5	3.8	3.6	2.9	0.23
ALBGLOB	0.58	0.56	0.6	0.61	0.53	0.70	0.31
ALT U/L	57.0^{b}	21.2^{b}	57.7^{b}	179.3^{a}	36.8^{b}	54.3^{b}	21.10
ALKP U/L	289.8	311.1	348.2	224.1	257.2	258.9	74.56
TBIL mg/dL	1.4^{b}	1.1^{b}	1.7^{b}	2.8^{a}	1.6^{b}	1.4^{b}	0.29
CHOL mg/dL	112.1	107.8	121.3	111.0	102.3	112.3	5.93
AMYL U/L	315.8	358.0	373.0	369.5	360.5	358.5	22.20
LIPA U/L	107.2	138.5	85.0	213.8	158.6	140.1	45.54

^{abc} Means with different superscripts are different ($P < 0.05$). NEGCON = a basal diet without antibiotic growth promoter (AGP) or HY fed continuously; POSCON = a basal diet with AGP fed continuously.
Sample size = 72 birds; Experimental unit = 25 birds/1.2 m^2.
[1] Supplementation strategy: a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF). GLU, glucose; BUN, blood urea nitrogen; PHOS, phosphorus; CA, calcium; TP, total protein; ALB, albumin; GLOB, globulin; ALB/GLOB, albumin-to-globulin ratio; ALT, alanine aminotransferase; ALKP, alkaline phosphatase; TBIL, total bilirubin; CHOL, total cholesterol; AMYL, amylase; and LIPA, lipase.

3.4. Gut microbiome

Alpha diversity indices and measures of beta diversity were not affected by feeding strategies ($P > 0.05$; Table 7, Fig. 1). The analysis of variation (ANOVA) revealed that feeding strategies affected only the species *Tidjanibacter inops* (syn. *Alistipes inops*) (Fig. 4), while no other taxa showed significant differences among treatments. Across all feeding strategies, the most abundant phyla were Bacillota A (44.9%), Bacillota (38.7%), Bacteroidota (11.4%), Synergistota (11.0%), and Actinomycetota (2.7%) (Fig. 2). Bacillota A, Bacillota, Bacteroidota, and Actinomycetota were detected across all feeding strategies, whereas Synergistota were observed only in birds receiving continuous HY supplementation. At the class level, Clostridia, Bacilli, Bacteroidia, Actinomycetes, and Vampirovibrionia were the most abundant, accounting for 44.9%, 38.7%, 11.4%, 3.0%, and 1.8% of the total community, respectively. All classes were present across feeding strategies, except Actinomycetes, which were detected only in birds fed in a phased HY regimen.

The most abundant orders were Lactobacillales (38.8%), Peptostreptococcales (36.0%), Bacteroidales (11.3%), Oscillospirales (5.3%), and Lachnospirales (3.7%). At the family level, Peptostreptococcaceae (36.0%), Lactobacillaceae (31.3%), Enterococcaceae (9.1%), Streptococcaceae (6.7%), and Bacteroidaceae (6.7%) predominated (Fig. 3). Peptostreptococcaceae, Lactobacillaceae, Streptococcaceae, and Bacteroidaceae were detected across all feeding strategies, whereas Enterococcaceae were absent in birds receiving continuous HY supplementation.

At the genus level, *Romboutsia* (33.6%), *Paraprevotella* (16.0%), *Ligilactobacillus* (13.5%), *Limosilactobacillus* (12.0%), and *Lactobacillus* (9.8%) were the most abundant taxa. *Romboutsia, Ligilactobacillus, Limosilactobacillus*, and *Lactobacillus* were detected across all feeding strategies, whereas *Paraprevotella* was observed primarily in the HYS and HYC groups. At the species level, *Romboutsia ilealis* (36.0%), *Ligilactobacillus aviarius* (10.3%), *L. aviarius* B (7.8%), *Limosilactobacillus vaginalis* (6.9%), and *L. aviarius* C (6.9%) were most abundant. *L. aviarius, L. aviarius* B, and *L. aviarius* C were not detected in birds receiving the HYF and HYC feeding strategies.

Table 4
Effects of hydrolyzed yeast supplementation strategy on haematological parameters in Ross 308 broilers.

Parameters	Supplementation strategy[1]						SEM
	NEGCON	POSCON	HYS	HYG	HYF	HYC	
White cell count (10^9/L)	21.4	15.6	17.1	22.8	24.2	21.5	2.50
Thrombocyte (10^9/L)	27.4[b]	19.9[b]	27.8[b]	29.5[b]	43.6[a]	29.6[b]	3.90
Haematocrit (%)	37.4	36.5	37.1	36.0	35.2	35.0	0.01
Heterophil (%)	80.5[a]	64.5[b]	72.1[a]	78.6[a]	75.9[a]	75.0[a]	0.03
Lymphocyte (%)	9.8	17.8	13.8	10.4	10.3	12.2	0.03
Monocyte (%)	9.2	10.8	12.3	9.8	12.7	11.6	0.02
Eosinophil (%)	3.5	5.2	2.5	3.0	1.8	4.5	0.04
Heterophil (10^9/L)	17.5	9.5	12.3	18.3	18.6	16.4	2.40
Lymphocyte (10^9/L)	2.0	2.7	2.4	2.1	2.4	2.5	0.33
Monocyte (10^9/L)	1.8[b]	1.7[b]	2.1[b]	2.2[b]	3.0[a]	2.5[b]	0.29
Eosinophil (10^9/L)	0.8	0.9	0.4	0.4	0.4	0.6	0.31
H:L ratio	8.3	3.8	5.4	8.2	8.1	7.3	0.03

[abc] Means with different superscripts are different ($P < 0.05$). NEGCON = a basal diet without antibiotic growth promoter (AGP) or HY fed continuously; POSCON = a basal diet with AGP fed continuously.
Sample size = 72 birds; Experimental unit = 25 birds/1.2 m^2.
[1] Supplementation strategy: a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF). H:L = Heterophil:Lymphocyte.

Table 5
Effects of hydrolyzed yeast supplementation strategy on internal organ sizes and lengths of Ross 308 broilers.

Parameters	Supplementation strategy[1]						SEM
	NEGCON	POSCON	HYS	HYG	HYF	HYC	
Organ size (g/100 g)							
Proventriculus	0.6	0.6	0.6	0.6	0.5	0.6	0.02
Ventriculus	2.1	2.0	2.2	2.0	2.0	2.1	0.09
Spleen	0.2	0.1	0.2	0.2	0.2	0.2	0.01
Liver	2.4	2.3	2.4	2.3	2.4	2.0	0.11
Pancreas	0.2	0.2	0.2	0.2	0.2	0.2	0.03
Duodenum	1.1	1.0	1.0	1.0	0.9	1.0	0.06
Jejunum	1.6	1.6	1.7	1.8	1.7	1.8	0.12
Ileum	1.4	1.4	1.3	1.3	1.3	1.2	0.07
Caeca	0.7	0.73	0.7	0.7	0.6	0.7	0.04
Length (cm)							
Duodenum	41.7	39.6	38.1	41.4	38.7	40.0	1.91
Jejunum	78.7	76.5	83.4	82.7	77.2	81.8	3.85
Ileum	80.1[b]	88.8[a]	79.1[b]	84.2[b]	86.9[b]	84.3[b]	2.11
Caeca	23.7	25.2	22.2	23.4	24.6	23.4	1.55

[abc] Means with different superscripts are different ($P < 0.05$). NEGCON = a basal diet without antibiotic growth promoter (AGP) or HY fed continuously; POSCON = a basal diet with AGP fed continuously.
Sample size = 72 birds; Experimental unit = 25 birds/1.2 m^2.
[1] Supplementation strategy: a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF).

4. Discussion

4.1. Growth performance

This study provides insight into the growth performance responses of densely stocked broilers to different HY feeding strategies. Phased HY supplementation resulted in growth performance outcomes statistically similar to those observed with continuous HY inclusion. This finding supports the concept that targeted supplementation during specific production phases may be effective in eliciting physiological responses similar to those achieved with continuous exposure, despite reduced cumulative inclusion. The functional benefits of HY are largely attributed to its composite bioactive fractions, including β-glucans, yeast-derived peptides, amino acids, and mannan oligosaccharides, which support gut integrity and immune modulation rather than directly stimulating growth (Kiarie et al., 2013; Spring et al., 2000). Similar findings have been reported in previous studies evaluating the effect of yeast-derived feed additives in broiler chickens (Sampath et al., 2021). The lack of consistent growth performance with HY supplementation relative to antibiotics may be attributed to the fact that antibiotics suppress pathogenic bacteria, enhance villus architecture, and improve nutrient absorption efficiency, thereby conferring performance advantages in broilers exposed to chronic stressors (Sethiya, 2016). In contrast, HY primarily exerts indirect effects by reducing intestinal immune activation, supporting epithelial turnover, and modulating immune responses (Awaad et al., 2019). These effects could be insufficient to overcome the compounded stress linked with prolonged high stocking density. Notably, none of the feeding strategies enabled Ross 308 broilers to attain the expected market body weight range at 42 days of age (2.3–3.0 kg; Aviagen, 2022). This may, in part, be explained by the elevated starter-phase FCR values observed in the present study (1.9–2.4), which exceeded Ross 308 performance objectives. The reduced feed efficiency likely reflects the combined effects of the deliberately imposed high-stocking-density challenge, with birds attaining an average final live weight of 2.0 kg ± 82.4, resulting in an estimated stocking density of approximately 41.7 kg live weight/m^2 at market age. This stocking density exceeded the conventional industry recommendation of approximately 30 kg live weight/m^2 (SAPA, 2012) and, together with relatively high ambient temperatures (up to 32°C), likely contributed to the observed reduction in feed efficiency. High ambient temperatures may have contributed additional physiological stress under the high-density challenge conditions. These conditions

Table 6
Effects of hydrolyzed yeast supplementation strategy on carcass and meat quality traits of Ross 308 broiler chickens.

Parameters	Supplementation strategy[1]						SEM
	NEGCON	POSCON	HYS	HYG	HYF	HYC	
HCW (g)	1644.0	1669.0	1585.0	1685.0	1620.0	1593.0	35.57
Dressing (%)	71.8	70.9	74.0	76.8	72.2	70.7	1.60
Drumstick (g/100 g)	5.2	5.0	5.2	5.5	4.9	5.0	0.14
Thigh (g/100 g)	6.9	6.2	6.4	6.8	6.4	6.0	0.25
Wing (g/100 g)	3.9	3.9	4.0	4.1	3.9	3.9	0.09
Breast (g/100 g)	15.7	15.1	16.0	16.8	15.6	15.6	0.47
pH	6.0	5.7	6.0	5.9	5.9	5.9	0.12
*L**	46.6	49.7	47.4	49.0	47.7	50.0	1.50
*a**	3.0	3.5	4.0	3.3	3.5	3.4	0.36
*b**	7.3	7.4	7.6	7.3	7.7	7.8	0.50
Chroma	7.9	8.2	8.7	8.0	8.5	8.6	0.55
Hue	1.2	1.1	1.1	1.1	1.1	1.2	0.04
WHC (%)	83.2	88.7	80.3	87.2	82.5	86.5	3.02
Cooking loss (%)	9.0	11.6	9.0	9.5	10.0	10.0	0.93
Drip loss (%)	3.2^{a}	2.8^{b}	2.2^{b}	4.3^{a}	3.6^{b}	2.3^{b}	0.41
Shear force (N)	4.2^{b}	6.3^{a}	4.7^{b}	3.8^{b}	4.8^{b}	5.3^{a}	0.38

[abc] Means with different superscripts are different ($P < 0.05$). NEGCON = a basal diet without antibiotic growth promoter (AGP) or HY fed continuously; POSCON = a basal diet with AGP fed continuously.
Sample size = 72 birds; Experimental unit = 25 birds/1.2 m^2.
[1] Supplementation strategy: a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF). HCW = Hot Carcass Weight; WHC = Water Holding Capacity.

Table 7
Influence of hydrolyzed yeast supplementation strategy on gut microbiome alpha diversity.

	Observed ASVs	Mean Shannon	Mean Simpson	Mean Pielou Evenness
NEGCON	42.00	2.04	0.75	0.55
POSCON	46.33	1.96	0.75	0.52
Supplementation strategy[1]				
HYS	40.33	1.83	0.67	0.50
HYG	34.33	2.23	0.82	0.63
HYF	28.67	1.58	0.59	0.46
HYC	31.00	1.81	0.70	0.53
P-value	0.53	0.66	0.60	0.51

NEGCON = a basal diet without antibiotic growth promoter (AGP) or HY fed continuously; POSCON = a basal diet with AGP fed continuously.
[1] Supplementation strategy: a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF). ASVs = amplicon sequence variants. Sample size = 108 birds; Experimental unit = 25 birds/1.2 m^2.

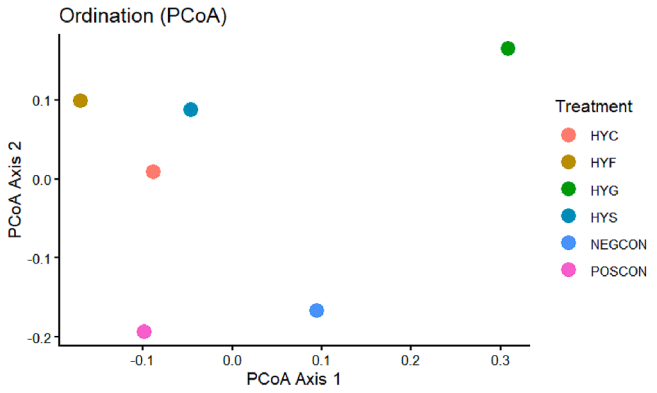

Fig. 1. Principal coordinates analysis (PCoA) based on a Bray–Curtis distance matrix illustrating microbial community structure across dietary treatments. Differences among groups were assessed using PERMANOVA. R^2 represents the proportion of variance explained (effect size) by treatment (R^2 = 0.34; P = 0.12). Pairwise comparisons were not statistically significant. Treatment groups included: a basal diet without AGP or HY (NEGCON) fed continuously, a basal diet with AGP (POSCON) fed continuously, and a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF). Sample size = 108 birds.

were intentionally imposed to simulate intensive commercial production environments and evaluate the efficacy of HY supplementation strategies under physiological challenge. Consistent with this challenge model, body weights throughout the trial remained below Ross 308 breed targets, indicating compromised growth efficiency across treatments. The recorded stocking densities at the end of the starter and grower phases were 6.32 kg live weight/m^2 and 23.5 kg live weight/m^2. High stocking density, particularly when coupled with elevated environmental temperatures, has been shown to restrict feeder access, increase physiological stress, impair litter and air quality, and deteriorate pen microclimatic conditions, ultimately reducing feed utilization efficiency and growth performance in broiler chickens (Dozier et al., 2005). This outcome underscores the dominant role of high stocking density as a growth-limiting factor and shows the difficulty of sustaining efficient performance under these conditions, even when functional feed additives are applied (Ghaseminejad & Salari, 2024). Nonetheless, these findings indicate that phased HY supplementation can maintain comparable growth performance as continuous supplementation, but neither strategy is sufficient to alleviate the performance constraints imposed on birds by high stocking density.

4.2. Serum biochemistry

Variations in blood biochemical indices observed at the end of the trial indicate that the timing of HY supplementation increased selected markers of protein metabolism and liver-associated function. The higher endpoint blood urea nitrogen (BUN) observed in chickens supplemented with HY during the starter phase suggests alterations in nitrogen metabolism during early development, associated with the supplementation schedule. Given that the starter phase is characterized by elevated metabolic demand and rapid tissue accretion, these endpoint findings may reflect early developmental processes (Lawrence-Azua et al., 2018). Increased BUN concentrations in broiler chickens are commonly associated with increased amino acid turnover and changes in protein utilization, especially when dietary interventions enhance metabolic activity (He et al., 2021). In contrast, supplementation with HY during the grower phase was associated with increases in serum concentrations

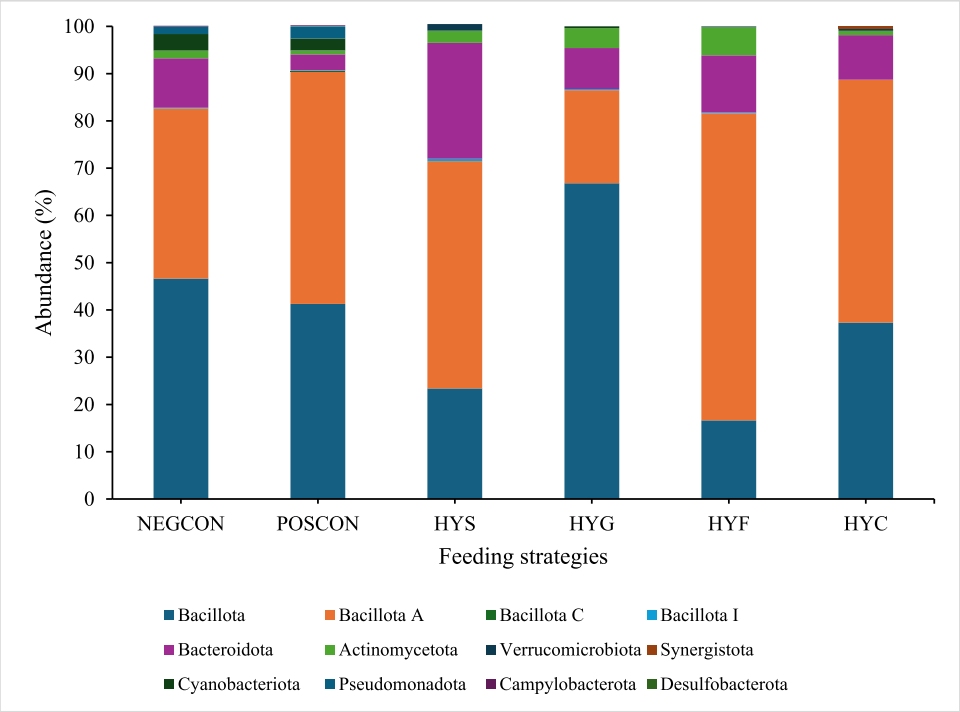

Fig. 2. Effects of diets containing hydrolyzed yeast on the relative abundance (%) of phyla in caeca digesta samples of Ross 308 broilers. Feeding strategies: a basal diet without AGP or HY (NEGCON) fed continuously, a basal diet with AGP (POSCON) fed continuously, and a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF). Sample size = 108 birds.

of alanine aminotransferase (ALT), total bilirubin, and albumin. Increased albumin concentrations may suggest alterations in nutritional or metabolic status and hepatic protein synthesis (Yalcinkaya et al., 2008). Elevations in ALT reflect a shift in hepatic metabolic activity; when values are within an established physiological range, they are considered a metabolic adaptation rather than pathological liver damage (Al-Baadani et al., 2018). In the present study, ALT concentrations were above the reported reference intervals for Ross 308 broilers; this response may reflect hepatic impairment (Zálešáková et al., 2025). Variations in total bilirubin concentration may be attributed to changes in erythrocyte turnover or hepatic processing of hemoglobin metabolites, which could be linked to altered metabolic demand (Lumeij, 2008; Meluzzi et al., 1992). Birds receiving the POSCON diet had lower values for these biochemical indices; this could reflect the stabilizing effects of AGPs on gut function and nutrient utilization under high stocking density conditions. Nevertheless, birds supplemented with HY during the finisher phase showed a decrease in serum cholesterol concentrations, though this was not statistically significant. Furthermore, the absence of longitudinal measurements precludes direct conclusions regarding physiological changes during this phase

4.3. Haematology

The haematological responses observed in this study indicated the influence of phase-specific HY supplementation in modifying physiological responses. Broiler chickens supplemented with HY during the finisher phase exhibited higher monocyte and thrombocyte counts, whereas the NEGCON birds had an elevated heterophil count. However, an increase in circulating heterophils, especially when accompanied by changes in the heterophil-to-lymphocyte (H:L) ratio, is often linked to physiological stress mediated by corticosterone release (Gross & Siegel, 1983; Maxwell & Robertson, 1998). Monocytes contribute to inflammatory responses and immune surveillance; therefore, an increase in circulating monocytes could indicate alterations in immune surveillance (Lumeij, 2008). This response may be associated with the influence of HY supplementation during the finisher phase, which was a metabolically demanding period. Importantly, all the haematological parameters obtained during the study did not differ across experimental groups. Generally, elevated thrombocyte and monocyte counts in HY-supplemented broilers may indicate alterations in physiological status and immune responses. However, further functional assessments are required to confirm the impact of HY on broiler immune competence.

4.4. Meat quality parameters

The carcass traits and meat quality attributes observed across the feeding strategies showed that supplementation with HY, regardless of timing, did not adversely affect overall meat quality. The absence of treatment effects on muscle pH, carcass yield, colour, cooking loss, and water-holding capacity suggests that these traits remained stable during the study (Geng et al., 2016). These findings are partly consistent with previous reports by Zhang et al. (2005), who stated that dietary yeast supplementation enhances meat quality and carcass yield in broilers. Birds receiving phased HY supplementation exhibited lower shear force values compared with those fed continuous HY supplementation or the POSCON diet. Shear force is an important indicator of meat tenderness; this response, therefore, suggests that phase-specific supplementation may influence meat tenderness. Meat tenderness and water-holding capacity are determined by post-mortem proteolysis and muscle fiber characteristics, which can be indirectly influenced by nutritional strategies that affect overall growth rate and bird metabolism (Hossain et al., 2025; Thao et al., 2025). During the grower phase, an increase in drip loss was observed in birds fed with HY, despite the decrease in shear force values. Drip loss reflects the retention of water in muscle tissue, and this is often influenced by fiber structure and protein integrity (Nastoh et al., 2026). The concurrent increase in drip loss and reduction in shear force could be attributed to changes in muscle

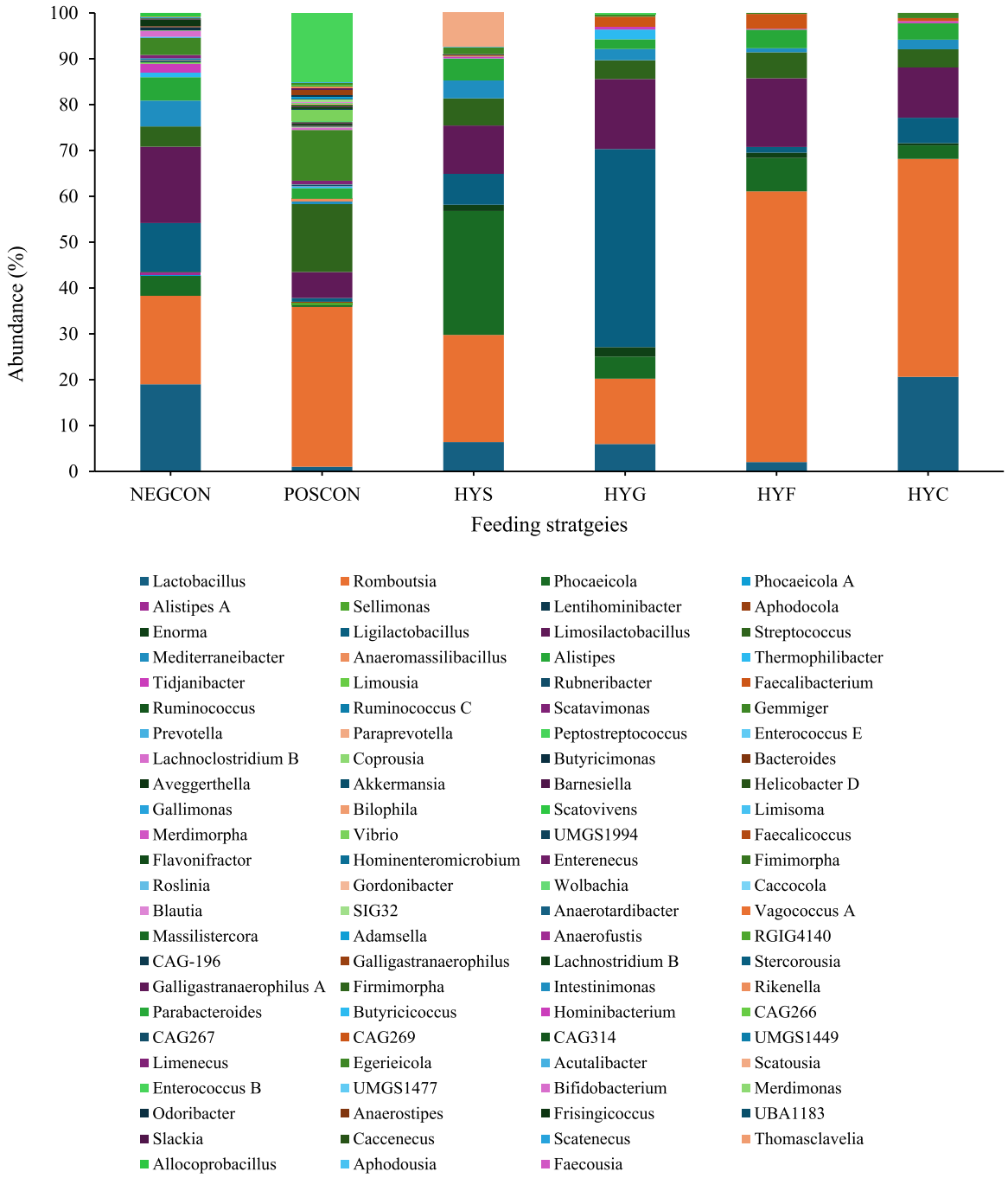

Fig. 3. Effects of diets containing hydrolyzed yeast on the relative abundance (%) of genera in caeca digesta samples of Ross 308 broilers. Feeding strategies: a basal diet without AGP or HY (NEGCON) fed continuously, a basal diet with AGP (POSCON) fed continuously, and a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF). Sample size = 108 birds.

remodeling rather than a compromise in meat quality. Nonetheless, further investigation is required to determine the influence of HY on muscle characteristics, as this was not directly investigated in this study.

4.5. *Visceral morphometry*

The gastrointestinal tract is crucial in the digestion and absorption of nutrients, with the small intestine being vital for the uptake of nutrients and minerals in poultry (Zanaty et al., 2025). Broilers fed the POSCON diet exhibited longer ilea than those receiving continuous or phased HY supplementation. This could be because AGPs are known to influence gut morphology by preventing intestinal inflammation and microbial load, thereby increasing absorptive surface area and enhancing epithelial development (Mehdi et al., 2018). In contrast, HY supplementation did not alter ileal length, suggesting that its primary mode of action may not involve gross morphological adaptation of the intestine. Yeast-derived feed additives, including HY, primarily support gut health via bioactive components such as mannan oligosaccharides and β-glucans, which enhance epithelial barrier function and modulate the microbiota rather than altering gross intestinal dimensions (Zi et al., 2025). The lack of significant differences in the weights of other visceral organs may suggest that continuous or phased HY

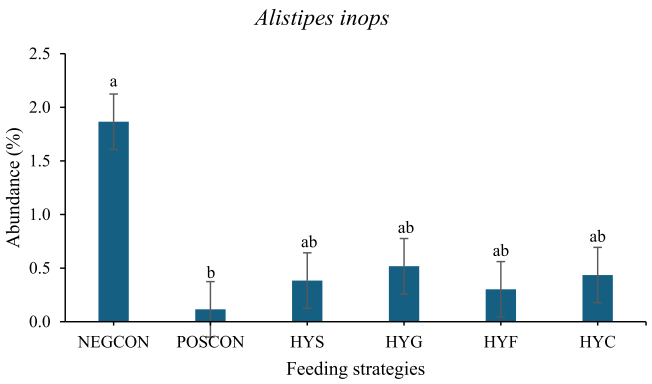

Fig. 4. Effects of diets containing hydrolyzed yeast on the relative abundance (%) of *Alistipes inops* species in caeca digesta samples of Ross 308 broilers. ^{abc} Means with different superscripts are different ($P < 0.05$). Feeding strategies: a basal diet without AGP or HY (NEGCON) fed continuously, a basal diet with AGP (POSCON) fed continuously, and a basal diet containing 0.05% HY as an alternative to AGP fed either continuously (HYC), during the starter phase only (HYS), during the grower phase only (HYG), or during the finisher phase only (HYF). Sample size = 108 birds.

supplementation did not adversely affect the normal organ development. Collectively, these findings suggest that while ileal morphology was influenced by AGP supplementation at high stocking density, HY-based feeding strategies were not associated with marked changes in intestinal dimensions under the evaluated conditions.

4.6. Gut microbiome

The present study demonstrated that feeding strategies had a limited effect on the overall caecal microbial community structure, with no differences observed in alpha or beta diversity indices. However, differential abundance analysis revealed that feeding strategy influenced the relative abundance of *Alistipes inops* (syn. *Tidjanibacter inops*), indicating a targeted rather than community-wide microbial response. *Alistipes inops* belongs to the family Rikenellaceae and was first described from human fecal isolates (Shkoporov et al., 2015). Its detection within monogastric gastrointestinal microbiomes has been reported in subsequent studies (Ovi et al., 2025). Its reduced abundance in birds fed the POSCON diet may reflect the selective pressure exerted by AGPs, which are known to suppress specific bacterial taxa. In contrast, HY supplementation, similar to the NEGCON diet, did not exert comparable selective pressure on this species.

Across all feeding strategies, Bacillota A, Bacillota, and Bacteroidota accounted for most of the microbial community, collectively representing more than 90% of total relative abundance. This compositional profile is consistent with previous reports describing the dominant phyla in the healthy broiler caecum (Pan and Yu, 2014). Members of Bacillota and Bacteroidota play central roles in maintaining intestinal homeostasis by mediating nutrient fermentation, gut barrier function, and immune modulation (Wilde et al., 2024). At lower taxonomic levels, genera such as *Romboutsia, Ligilactobacillus, Limosilactobacillus*, and *Lactobacillus* were among the most abundant across feeding strategies. These taxa are commonly associated with carbohydrate metabolism and the production of short-chain fatty acids, including butyrate, acetate, and propionate, which contribute to intestinal integrity and host metabolic regulation (Gao et al., 2024). The statistically indifferent distribution of these beneficial genera across phased and continuous HY supplementation suggests that phase-specific HY provision did not disrupt the core microbial community. Generally, the limited microbiome shifts observed in this study suggest that phased HY supplementation yields microbial profiles statistically similar to those obtained with continuous supplementation under high stocking density. These findings suggest that targeted nutritional strategies may help maintain microbial stability under the conditions of this study, although overall microbiome changes were limited. These findings align with established knowledge that dietary yeast-based products, such as HY, can modulate gut microbial activity and support intestinal function without inducing large-scale alterations in the microbial community structure (Ovi et al., 2025).

5. Conclusions

Phase-specific HY supplementation produced growth performance comparable to continuous supplementation in broilers reared under high stocking density, with the greatest benefits observed in birds supplemented in the grower and finisher phases. Although AGP supplementation resulted in superior growth performance, HY supplementation improved selected haematological and physiological responses, while exerting limited effects on caecal microbial composition and diversity. The elevated starter-phase FCR could not be fully explained by the recorded data and should be interpreted with caution. Additionally, the absence of gut barrier function measurements, economic evaluations, and functional microbiome analyses limits the interpretation of the findings. Another limitation is that physiological and microbial measurements were obtained only at the end of the experimental period. Consequently, although birds received different supplementation schedules during the starter, grower, and finisher phases, the study cannot determine the temporal progression of physiological or microbial responses within individual production phases. Future studies incorporating longitudinal sampling would provide greater insight into the dynamics of these responses.

Ethical statement

All experimental procedures involving animals were conducted in accordance with internationally accepted standards for the care and use of animals in research and were approved by the University of Mpumalanga's Research Ethics Committee – Animal Sciences (UMP REC-AS; approval no. UMP/Mlambo/1/2024) and the North-West University Animal Production Research Ethics Committee (NWU–ANIMPROD REC; approval no. NWU-00818-24-A5).

Birds were handled and managed to minimize stress and discomfort throughout the experimental period. All sampling and slaughter procedures were performed humanely in accordance with institutional and national animal welfare guidelines.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors utilized Grammarly to enhance readability and language use in certain sections of the paper. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

CRediT authorship contribution statement

Nicole Moreane: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Caven M. Mnisi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Victory O. Sumanu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Funding acquisition, Formal analysis, Data curation. **Omolola E. Fayemi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition. **Ghaneshree Moonsamy:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology,

Investigation, Funding acquisition, Conceptualization. **Elijah G. Kiarie:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis. **Rajesh Jha:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Funding acquisition, Conceptualization. **Victor Mlambo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All relevant data are included in the manuscript. Phylogenetic datasets generated and/or analysed during the current study have been deposited in the National Center for Biotechnology Information (NCBI), hosted by the National Institutes of Health, under BioProject accession number PRJNA1451950 [https://www.ncbi.nlm.nih.gov/sra/PRJNA1451950].

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