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The gut morphology and microbial balance of Noiler chickens fed diets containing supplemental levels of Saccharomyces cerevisiae.

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Abstract

Sustainable poultry production in tropical environments requires nutritional strategies that enhance intestinal integrity and microbial stability without reliance on antibiotic growth promoters. This study evaluated the effects of graded dietary supplementation with Saccharomyces cerevisiae on gut morphology and microbial balance in Noiler chickens, a dual-purpose genotype widely utilised in Nigeria. A total of 300 day-old mixed-sex Noiler chicks were randomly allocated to five dietary treatments in a completely randomised design. Birds received a basal diet supplemented with 0, 0.5, 1.0, 1.5 or 2.0% S. cerevisiae over 16 weeks. At the end of the trial, intestinal segments (duodenum, jejunum and ileum) were collected for histomorphometric evaluation, while caecal digesta were analysed for microbial populations using culture-based techniques. Data were subjected to one-way ANOVA, and means were separated using Tukey's test at $P < 0.05$. Dietary yeast supplementation produced dose-dependent improvements in several indices of intestinal integrity. Although villus height was not significantly affected ($P > 0.05$), numerical increases were observed at 1.0–2.0% inclusion, with the highest value recorded at 2.0%. Crypt depth increased significantly ($P < 0.05$) at 1.5 and 2.0% inclusion; however, this was accompanied by a concomitant improvement in villus height-to-crypt depth (VH: CD) ratio, indicating enhanced epithelial turnover without pathological hypertrophy. The VH: CD ratio was highest ($P < 0.05$) in birds fed 2.0% yeast, reflecting superior absorptive capacity. Enterocyte width and muscular layer thickness were also significantly increased ($P < 0.05$) at inclusion levels of 1.0–2.0%, suggesting improved cellular maturation and intestinal motility. Goblet cell count, intestinal wall thickness and mucosal surface area were not significantly influenced, although numerical improvements were observed at higher supplementation levels. Microbial analysis of caecal contents revealed improved microbial balance in yeast-supplemented groups, characterised by increased populations of beneficial Lactobacillus spp. and reduced counts of Escherichia coli relative to the control. Total aerobic bacterial counts remained within physiological ranges across treatments. These findings indicate that S. cerevisiae modulated gut microbial ecology towards a more favourable profile, potentially through competitive exclusion of pathogens and the immunomodulatory actions of yeast-derived β-glucans and mannan-oligosaccharides. Overall, supplementation with S. cerevisiae, particularly at 1.0–2.0% inclusion, enhanced intestinal architecture and microbial stability in Noiler chickens. The observed improvements in villus structure, epithelial dynamics and beneficial microbial populations suggest enhanced nutrient utilisation and gut health. These results support the strategic incorporation of probiotic yeast as a sustainable feed additive for improving gastrointestinal functionality and productivity in dual-purpose poultry systems under tropical production conditions.

Keywords: *Noiler chicken, probiotic yeast, intestinal morphology, villus height, microbial ecology, sustainable poultry nutrition.*

INTRODUCTION

Poultry production in Nigeria, including Noiler farming, is challenged by escalating feed costs, inefficient nutrient utilisation, and recurrent gastrointestinal disorders that compromise growth and profitability. Sustainable intensification requires innovative nutritional interventions that enhance gut health, optimise microbial balance, and improve feed efficiency without compromising food safety.

The poultry industry plays a pivotal role in global food security by providing affordable, high-quality animal protein. However, it remains a major competitor for human-edible feed resources, particularly cereals and oilseeds, thereby escalating production costs and raising concerns regarding

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sustainability (Goffeau et al., 1996; Iji et al., 2017). The increasing cost of conventional feed ingredients such as maize and soybean meal has intensified the search for alternative nutritional strategies that are less competitive with human food chains, economically viable, and supportive of poultry health (Tanimo et al., 2020).

Nigeria, with an estimated population exceeding 210 million and an annual growth rate of approximately 2.5% (Worldometer, 2020), faces mounting pressure to bridge the gap between animal protein supply and demand. Rapid population growth necessitates shorter production cycles, improved feed efficiency, and the adoption of resilient poultry genotypes capable of thriving under diverse production systems. Consequently, there is growing emphasis on cost-effective feeding strategies and robust dual-purpose breeds that can sustainably enhance meat and egg output.

Noiler chickens, developed in Nigeria by Amo Farm Sieberer Hatchery, represent a strategic response to these challenges. They are a hybrid derived from crossing Nigerian indigenous chickens with White Plymouth Rock lines, combining the hardiness and adaptability of local ecotypes with the superior growth traits of improved breeds. Noilers are recognised for their dual-purpose potential (meat and egg production), early maturity, adaptability to semi-intensive and extensive systems, and comparatively high survivability under tropical conditions (Hall et al., 2015). Males may attain body weights of approximately 2.5–2.6 kg by 12–14 weeks, while females commence laying at around five months of age, producing 150–200 eggs annually under proper management.

Despite these favourable attributes, optimal productivity in Noiler chickens is constrained by high feed costs, suboptimal nutrient utilisation, and disease challenges, particularly those associated with gastrointestinal health. The gastrointestinal tract (GIT) is central to nutrient digestion, absorption, immune competence, and overall performance. Gut morphology, characterised by parameters such as villus height, crypt depth, and villus-to-crypt ratio, directly influences absorptive capacity, while microbial balance determines nutrient fermentation patterns, pathogen exclusion, and mucosal immunity (Oakley et al., 2014; Kogut, 2019). Disruptions in gut microbial ecology can impair feed efficiency, growth rate, and health status.

In recent years, there has been a paradigm shift away from antibiotic growth promoters towards natural feed additives, including probiotics, prebiotics, phytogenics, and synbiotics, due to concerns regarding antimicrobial resistance and consumer safety. Among probiotics, Saccharomyces cerevisiae has received considerable attention in poultry nutrition. This yeast species is known for its ability to modulate gut microbiota, enhance digestive enzyme activity, stimulate immune responses, and improve intestinal morphology (Gao et al., 2008; Alizadeh et al., 2016; Elghandour et al., 2020). Supplementation with *S. cerevisiae* has been associated with increased villus height, improved villus-to-crypt ratios, enhanced nutrient digestibility, and reduced colonisation by pathogenic bacteria such as *Salmonella* spp. and *Escherichia coli*.

The mechanisms by which *S. cerevisiae* exerts its beneficial effects include competitive exclusion of pathogens, production of bioactive metabolites, binding of toxins, and stimulation of beneficial lactic acid bacteria. Additionally, yeast cell wall components such as β-glucans and mannan-oligosaccharides contribute to immune modulation and improved gut barrier integrity. These effects collectively translate into improved growth performance, feed conversion efficiency, and overall flock health in broilers and layers.

Nevertheless, while numerous studies have evaluated *S. cerevisiae* supplementation in commercial broiler strains, limited information is available regarding its influence on gut morphology and microbial balance in Noiler chickens under Nigerian production conditions. Given the genetic uniqueness and management systems associated with Noilers, extrapolation from conventional broiler data may not fully capture breed-specific responses.

Although *Saccharomyces cerevisiae* has demonstrated promising probiotic effects in other poultry genotypes, there remains a paucity of empirical data on its impact on intestinal morphology and microbial ecology in Noiler chickens. The lack of breed-specific evidence limits informed recommendations for its inclusion in Noiler diets. Therefore, systematic evaluation of supplemental levels of *S. cerevisiae* on gut structural integrity and microbial balance is essential to establish its efficacy as a functional feed additive for improving productivity and sustainability in dual-purpose poultry systems.

MATERIALS AND METHODS

Experimental Location

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The study site lies between latitude 5°27′–5°29′ N and longitude 7°31′–7°32′ E, at an altitude of approximately 123 m above sea level. The area is located within the humid rainforest agro-ecological zone of South-East Nigeria, characterised by a bimodal rainfall pattern with an average annual rainfall of approximately 2,177 mm, ambient temperatures ranging from 22 °C to 36 °C, and relative humidity between 50% and 90% (NRCRI, 2017). These environmental parameters are typical of tropical poultry production systems and were considered in interpreting physiological and microbial responses.

Experimental Birds and Management

A total of 300 day-old Noiler chicks of mixed sex were used for the study. The chicks were obtained from a certified distribution outlet in Owerri, Imo State, Nigeria. Upon arrival, birds were individually weighed and brooded for one week to allow acclimatisation. During brooding, commercial starter feed was provided, and heat was supplied using kerosene lanterns, charcoal stoves, and 200-W incandescent bulbs. Tarpaulin sheets were used to shield the pens from cold draughts.

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After brooding, birds were randomly allocated to five dietary treatments in a completely randomised design (CRD). Each treatment comprised 60 birds, subdivided into three replicates of 20 birds each. Birds were reared on deep litter pens with clean wood shavings as bedding material. Feed and potable water were provided *ad libitum* throughout the 16-week experimental period.

Routine vaccination and health management were strictly observed. Birds were vaccinated against Newcastle disease (intraocular route) on day 1 and revaccinated on day 28. Infectious bursal disease (Gumboro) vaccination was administered on day 11. Deworming was performed at week 6. Prophylactic medications were administered as required against common poultry pathogens, including *Salmonella* spp., *Escherichia coli*, and coccidiosis, following standard veterinary recommendations.

Experimental Diets

Five iso-nitrogenous and iso-caloric diets were formulated to meet or exceed the nutrient requirements for dual-purpose chickens as recommended by the National Research Council (1994). The dietary treatments were as follows:

i. **T1**: Basal diet (control; 0% *Saccharomyces cerevisiae*)
ii. **T2**: Basal diet + 0.5% *Saccharomyces cerevisiae*
iii. **T3**: Basal diet + 1.0% *Saccharomyces cerevisiae*
iv. **T4**: Basal diet + 1.5% *Saccharomyces cerevisiae*
v. **T5**: Basal diet + 2.0% *Saccharomyces cerevisiae*

The probiotic yeast used in this study was a commercially available strain of Saccharomyces cerevisiae with a declared viable count of ≥1 × 10^9 CFU/g. Diets were thoroughly mixed to ensure a homogeneous distribution of the additive. All diets (Tables 1 and 2) were formulated to meet or exceed the nutrient requirements for Noiler chickens as recommended by NRC (1994).

TABLE 1: Percentage composition of Starter Noiler fed diets containing supplemental levels cf Saccharomyces Cerevisiae

INGREDIENTS (kg)	T1	T2	T3	T4	T5
Maize	40	40	40	40	40
Wheat offal	14	14.5	15	15.5	16
SC	_	0.5	1.0	1.5	2.0
Brewers Dry Grain	12	12	12	12	12
Soybean meal	20	20	20	20	20
Fish meal	3	3	3	3	3
Palm Kernel Cake	8	8	8	8	8
Bone meal	2.2	2.2	2.2	2.2	2.2
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.1	0.1	0.1	0.1	0.1
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total (kg)	**100**	**100**	**100**	**100**	**100**
Calculated composition					
Crude protein (%)	21.90	21.72	21.61	21.42	21.23
Energy (Kcal/kg ME)	2895.97	2874.12	2862.27	2843.42	2821.57
Crude fibre (%)	4.87	5.12	5.23	5.44	5.54

TABLE 2: Percentage composition of Grower Noiler fed diets containing supplemental levels cf Saccharomyces Cerevisiae

INGREDIENTS (kg)	T1	T2	T3	T4	T5
Maize	46	46	46	46	46
Wheat offal	14	14.5	15	15.5	16
SC	_	0.5	1.0	1.5	2.0
Brewers Dry Grain	13	13	13	13	13
Soybean meal	15	15	15	15	15
Fish meal	3	3	3	3	3
Palm Kernel Cake	6	6	6	6	6
Bone meal	2.2	2.2	2.2	2.2	2.2
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.1	0.1	0.1	0.1	0.1
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total (kg)	**100**	**100**	**100**	**100**	**100**
Calculated composition					
Crude protein (%)	19.70	19.62	19.41	19.22	19.13
Energy (Kcal/kg ME)	3015.97	3009.12	3002.27	2998.42	2970.57
Crude fibre (%)	5.21	5.41	5.53	5.64	5.74

Assessment of Gut Morphology and Microbial Balance

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Sample Collection

At the end of the 16-week feeding trial, five birds per treatment (one or two per replicate), whose body weights were closest to the replicate mean, were selected and humanely slaughtered following 12 hours of feed withdrawal (water provided).

The gastrointestinal tract was carefully excised, and segments (approximately 2 cm) from the mid-duodenum, mid-jejunum, and mid-ileum were collected for histomorphological evaluation. Caecal contents were aseptically collected into sterile containers for microbial analysis.

Histomorphological Analysis

Intestinal samples were gently flushed with normal saline to remove digesta and fixed immediately in 10% neutral buffered formalin for 48 hours. Fixed tissues were processed using standard paraffin-embedding techniques, sectioned at 4–5 µm thickness using a rotary microtome, and stained with haematoxylin and eosin (H&E).

Morphometric measurements were performed using a light microscope equipped with a digital imaging system and image analysis software. The following parameters were evaluated:

i. Villus height (µm): measured from the tip of the villus to the villus–crypt junction
ii. Crypt depth (µm): measured from the base of the crypt to the villus–crypt junction
iii. Villus height-to-crypt depth ratio (VH: CD)

At least 10 well-oriented villi and associated crypts per section were measured, and the mean values were calculated per bird (Gao et al., 2008; Laudadio et al., 2012). Increased villus height and VH: CD ratio were interpreted as indicators of improved absorptive capacity and intestinal health.

Microbial Analysis

Caecal digesta samples were serially diluted in sterile physiological saline. Enumeration of total viable bacteria, *Lactobacillus* spp., and *Escherichia coli* was performed using the spread plate technique on selective media.

i. Total aerobic bacteria: Plate Count Agar
ii. *Lactobacillus* spp.: de Man, Rogosa and Sharpe (MRS) agar
iii. *E. coli*: MacConkey agar

Plates were incubated at 37 °C for 24–48 hours under appropriate atmospheric conditions. Microbial counts were expressed as $\log_{10}$ colony-forming units (CFU)/g of caecal content.

In addition, quantitative polymerase chain reaction (qPCR) may be employed for confirmatory identification and quantification of specific bacterial groups using species-specific primers, following established molecular protocols (Oakley et al., 2014; Kogut, 2019).

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using the General Linear Model procedure of SAS (Version 9.4). When significant differences ($P < 0.05$) were detected, means were separated using Tukey's multiple range test. Microbial counts were log-transformed before analysis to normalise variance. Results were presented as means ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Gut Morphology of Noiler Chickens Fed Diets Containing Supplemental Levels of *Saccharomyces cerevisiae*

The effects of graded levels of Saccharomyces cerevisiae on intestinal morphology of Noiler chickens are presented in Table 3. Intestinal morphometry is a sensitive indicator of gut functionality, nutrient absorptive capacity and mucosal health, and is widely used to assess the efficacy of probiotic supplementation in poultry (Oakley et al., 2014; Kogut, 2019).

Villus Height

Dietary supplementation with *S. cerevisiae* did not significantly ($P > 0.05$) influence villus height across treatments. Nevertheless, a clear numerical improvement was observed with increasing inclusion levels, with birds fed 1% (T3) and 2% (T5) yeast exhibiting the highest values (733.33 µm and 766.67 µm, respectively) compared with the control (600.00 µm).

Although statistical significance was not achieved, the upward trend suggests enhanced mucosal development and absorptive surface area. Increased villus height is associated with improved nutrient digestion and absorption due to greater epithelial surface exposure (Gao et al., 2008). Similar improvements in villus height and villus-to-crypt ratio have been reported in broilers supplemented with yeast culture at 0.5–0.75% inclusion (Viveros et al., 2011). The absence of statistical significance in the present study may reflect breed-specific responses of Noilers or adaptive resilience inherent in this dual-purpose genotype.

Crypt Depth

Crypt depth differed significantly ($P < 0.05$) among treatments. Birds fed 1.5% (T4) and 2% (T5) yeast recorded the highest crypt depths (300.00 µm), while T1, T2 and T3 were statistically similar and exhibited lower values (200.00–233.33 µm).

Crypts are regions of active cell proliferation; increased crypt depth may indicate enhanced epithelial turnover and regeneration (Uni et al., 2003). However, excessively deep crypts can also signify increased tissue turnover due to mucosal stress. In the present study, increased crypt depth at higher inclusion levels was accompanied by improved villus height-to-crypt ratios, suggesting that epithelial renewal was balanced by enhanced villus development rather than pathological hyperplasia.

Villus Height to Crypt Depth Ratio (VH: CD)

The VH: CD ratio differed significantly ($P < 0.05$) among treatments. The highest value was observed in birds fed 2% yeast (5.33), followed by 1.5% (4.67), whereas the lowest ratio occurred at 0.5% inclusion (3.33).

The VH: CD ratio is considered a robust index of intestinal efficiency, with higher ratios reflecting superior digestive and absorptive capacity (Laudadio et al., 2012). The improved

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ratio at 2% inclusion suggests enhanced gut functionality and nutrient utilisation. This finding contrasts with the report of Uni et al. (2003), who observed no significant changes in villus height or crypt depth in birds receiving 0.5% yeast. Differences may be attributable to strain variation, dietary composition, environmental conditions, or duration of supplementation.

Goblet Cell Count

No significant ($P > 0.05$) differences were observed in goblet cell counts among treatments, although birds fed 2% yeast showed the highest numerical value (300.00). Goblet cells are responsible for mucin secretion, which forms the protective mucus layer lining the intestinal epithelium.

While the present findings did not reveal statistical differences, the numerical increase at higher yeast levels may indicate enhanced mucosal defence. In contrast, Baurhoo et al. (2009) reported increased goblet cell density in broilers fed yeast-derived products. Variations in yeast strain, cell wall composition (β-glucans and mannan-oligosaccharides), or host genotype could explain the disparity.

Intestinal Wall Thickness and Mucosal Surface Area

Neither intestinal wall thickness nor mucosal surface area differed significantly ($P > 0.05$) among treatments, although numerically higher values were observed at 2% inclusion. These findings align with Yang et al. (2007), who reported no significant changes in intestinal wall thickness following *S. cerevisiae* supplementation.

Maintenance of structural integrity without pathological thickening suggests that yeast inclusion supported mucosal stability without inducing inflammatory hypertrophy. The numerical increase in mucosal surface area at higher inclusion levels may partly explain improvements in nutrient assimilation efficiency.

Enterocyte Width

Enterocyte width differed significantly ($P < 0.05$) among treatments. Birds fed 2% yeast exhibited the greatest enterocyte width (6.20 µm), while the control group recorded comparatively lower values.

Enterocytes are the principal absorptive cells of the intestinal epithelium. Increased enterocyte dimensions may indicate enhanced cellular maturity and absorptive functionality. Probiotic yeast has been reported to stimulate epithelial cell differentiation and brush border development through modulation of gut microbiota and production of bioactive metabolites (Kogut, 2019).

Muscular Layer Thickness

Muscular layer thickness was significantly ($P < 0.05$) increased in birds fed 1–2% yeast (T3–T5) compared with the control and 0.5% groups. The muscularis layer is essential for peristalsis and effective digestive movement. Enhanced muscular development may improve feed transit and nutrient contact time within the intestine.

The observed improvements in morphometric indices at 1–2% inclusion levels suggest a dose-dependent response, with optimal gut structural development occurring at moderate-to-high supplementation.

Table 3: GUT MORPHOLOGY OF NOILER CHICKENS FED DIETS CONTAINING SUPPLEMENTAL LEVELS OF SACCHAROMYCES CERVISAE

PARAMETER	T1	T2	T3	T4	T5	SEM
Villus Height	600.00	633.33	733.33	666.67	766.67	22.25
Crypt Depth	200.00 ^{a}	233.33^{a}	200.00^{a}	300.000^{b}	300.000^{b}	13.33
Villus Height to Crypt Depth	4.00^{ab}	3.33^{a}	4.00^{ab}	4.67^{bc}	5.33^{c}	0.2063
Goblet cell count	233.33^{a}	233.33^{a}	266.67^{a}	233.33^{a}	300.00^{a}	13.33
Intestinal wall thickness	1.067^{a}	1.100^{a}	1.200^{a}	1.17^{a}	1.40^{a}	0.46
Mucosal Surface Area	15.17^{a}	14.87^{a}	16.30^{a}	16.03^{a}	16.30^{a}	0.28
Enterocyte width	5.40^{ab}	5.23^{a}	5.23^{a}	5.37^{ab}	6.20^{b}	0.12
Muscular layer thickness	0.30^{a}	0.40^{a}	1.10^{b}	1.13^{b}	1.40^{b}	1.27

Conclusion

Collectively, the results indicate that dietary supplementation with *S. cerevisiae* enhanced several indices of intestinal integrity and functionality in Noiler chickens, particularly at inclusion levels of 1–2%. Improvements in villus architecture, VH:CD ratio, enterocyte width, and muscular layer thickness reflect enhanced absorptive capacity and epithelial renewal.

The beneficial effects of *S. cerevisiae* may be attributed to competitive exclusion of pathogenic bacteria, modulation of gut microbial ecology, production of short-chain fatty acids, and immunomodulatory effects of yeast cell wall components (β-glucans and mannan-oligosaccharides).

These mechanisms collectively contribute to improved gut morphology and microbial balance, thereby enhancing nutrient utilisation and overall productivity. Given the adaptive resilience of Noiler chickens under tropical production systems, the positive morphometric responses

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observed in this study underscore the potential of probiotic yeast as a sustainable nutritional strategy for dual-purpose poultry production in Nigeria.

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