RESEARCH ARTICLE

Effect of Dietary Supplementation of a Combination of Probiotics, Enzymes and Yeast on the Growth Performance, Carcass Traits and Gut Morphology of Broiler Chickens

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Abstract

This study assessed the impact of dietary supplementation of a combination of probiotics, enzymes, and yeast on the growth performance, carcass traits, and gut morphology of broiler chickens. A total of 128 day-old mixed-sex commercial broiler chickens (Vencobb 400) were randomly divided into four dietary groups, each with four replicated pens (n=4) containing eight broiler chickens per pen. The dietary groups were: 1) basal diet without any growth promoter (C), 2) basal diet with Bacitracin methylene disalicylate (BMD) at 0.2 g/kg feed (T1), 3) basal diet with a combination of probiotics, enzymes, and yeast (IFB Agro Industries Limited) at 0.3 g/kg of feed (T2), and 4) basal diet with a combination of probiotics, enzymes, and yeast at 0.3 g/kg + BMD at 0.2 g/kg (T3). Body weight, feed intake, and feed conversion ratios (FCR) were monitored weekly for 35 days. Carcass traits and gut morphology were assessed at the end of the trial. The results indicated that the T3 group showed significantly higher average daily gain compared to the control group throughout the study period. FCR was significantly improved in the T2 and T3 groups compared to the control group ($p<0.05$). Average daily feed intake and carcass characteristics did not differ significantly among the dietary treatment groups. The T3 group exhibited a significant decrease in crypt depth and an increase in villi height to crypt depth ratio in the duodenum and ileum compared to the control group. In conclusion, the combination of probiotics, enzymes, and yeast with antibiotic supplementation has positive effects on broiler production.

Key words: Broiler chicken, Enzyme, Growth performance, Probiotic, Yeast.

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Introduction

Antibiotics have been historically used sub-optimally in the poultry industry to enhance growth performance by improving feed utilization and reducing disease transmission (Paul *et al.*, 2022). The mechanism of action of antibiotics involves altering the gut microbial balance, thereby reducing the population of pathogenic microbes and improving feed efficiency and growth (Butaye *et al.*, 2003). However, the excessive use of antibiotics has led to the development of antimicrobial resistance, posing a threat to both humans and animals (Cella *et al.*, 2023). Recognizing this risk, the World Health Organization (WHO, 1997) has identified the use of antibiotics in livestock as a public health concern. Consequently, the European Union restricted the use of antibiotics as growth promoters in 2006 (Castanon, 2007). Due to increasing demand for poultry production and changing consumer preferences, there is a growing need for alternative feed resources to replace antibiotics growth promoters. Various feed additives, such as probiotics, enzymes, yeast products, and prebiotics, are being explored as alternatives (Kim *et al.*, 2021; Tukaram *et al.*, 2022).

Probiotics are live microorganisms that can enhance growth performance, modulate gut microbial load, inhibit pathogens, improve intestinal integrity, boost bird immunity, and enhance the microbiological and sensory characteristics of broiler meat (Alagawany *et al.*, 2021; El-Saadony *et al.*, 2021). They competitively attach to the gut epithelium, limiting pathogen efficiency and enhancing animal performance, but how it affect meat quality and physicochemical properties needs further research. Exogenous enzymes are commonly used to enhance digestibility and nutrient utilization efficiency (Ravindran, 2013). Animals require enzymes to digest food, which can be produced by the animal or by microorganisms in the digestive tract (Pariza

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and Cook, 2010). Adding enzymes to animal feed improves digestion efficiency, as the digestive system may not be perfect (Maqsood, 2013). Phytase, sourced from organisms like *Aspergillus niger, Escherichia coli*, and *Buttiauxella* spp., breaks down non-starch polysaccharides and reduces digesta viscosity to enhance pre-cecal nutrient digestibility (Bedford, 2000).

Yeast, unicellular eukaryotic microorganisms, products naturally present in grains, grain by-products, silage, and hay are beneficial for livestock (Hiltz *et al.*, 2023). *Saccharomyces cerevisiae*, the most common yeast species, contains amino acids, nucleotides, proteins, polysaccharides, peptides, vitamins, and growth hormones (Krysiak *et al.*, 2021). Yeast can help to control intestinal flora balance, promote intestinal health, enhance animal production performance, improve immunity, and enhance meat quality (Pang *et al.*, 2022). Yeast cell wall components and metabolic products contribute to intestinal health and boost bird immunity, ultimately enhancing broiler chicken performance (Araujo *et al.*, 2019; Pascual *et al.*, 2020). The present study focused on the effects of probiotics, enzymes, and yeast supplementation in broiler diets on growth performance, carcass characteristics, meat quality, and gut morphology. The goal was to confirm previous findings and assess the effectiveness of this combination in practical broiler diets.

Materials and Methods

Birds, Experimental Design and Diets

The study protocol was approved by Institutional Animal Ethics Committee of West Bengal University of Animal and Fishery Sciences, Kolkata, India (Approval No.: 763/GO/Re-S/ReRc-L/03/CCSEA/75/2024-25). One hundred twenty eight day-old mixed-sex Vencobb-400 broiler chicks were randomly divided into four treatment groups, each consisting of 4 replicates of 8 chicks. The dietary groups were as follows: 1) basal diet without any growth promoter (C), 2) basal diet with Bacitracin methylene disalicylate (BMD) at 0.2 g/kg feed (T1), 3) basal diet with a combination of probiotics, enzymes, and yeast (IFB Agro Industries Limited) at 0.3 g/kg of feed (T2), and 4) basal diet with a combination of probiotics, enzymes, and yeast at 0.3 g/kg + BMD at 0.2 g/kg (T3), The basal diet was formulated in mash form using maize and soybean to meet or exceed the nutritional requirements of broiler chickens at different stages (starter, grower, and finisher) based on Vencobb-400 broiler chicken recommendations (Venkys, 2017). The experimental diets were prepared weekly, packed in high-density polyethylene bags with inner liners, and provided *ad libitum* along with water.

Management and Rearing of Birds

Before introduction of the chicks, the experimental house and feeding and watering troughs were thoroughly cleaned and disinfected. The chicks were housed in floor pens (1.22 m × 0.76 m) separated by plastic wire netting. Rice husk and chopped paddy straw served as litter, with each pen equipped with sterile plastic feeders and water troughs. The chicks received continuous lighting from compressed fluorescent lamps for the first two days, followed by a lighting schedule of 23 h of light and1 h of darkness each night. The temperature in the poultry house was controlled using heating elements, gradually decreasing from 32°C on day 1 to 24°C on day 22. Proper ventilation was maintained with exhaust fans throughout the trial period. All birds were vaccinated against Newcastle Disease virus (NDV) at 5 and 21 days of age, and Infectious Bursal Disease virus (IBDV) at 12 days of age.

Measurement of Performance Traits

During the growth trial, the body weight of all chickens was measured weekly. Average body weight was calculated for each replicate. Feed intake was monitored weekly by subtracting the remaining feed from total feed offered per pen. Average daily feed intake and feed conversion ratio (FCR) were calculated using standard formulae. Mortality was monitored, and post-mortem examinations were conducted to determine the cause of death. Mortality percentage was calculated at the end of the trial and used to adjust calculations for body weight, average daily gain, average daily feed intake and FCR.

Slaughter and Measurements of Carcass Traits

To assess carcass traits, two birds of each sex with a body weight close to the pen's average were chosen at the end of trial for slaughter via cervical disarticulation. Then after skin, feathers, head, shank, intestines, and giblets were removed, the weights of the eviscerated carcasses and individual organs were recorded.

Morphological Study of Small Intestine

On day 35, 5 chickens from each diet group were slaughtered for small intestine tissue analysis. Samples from duodenum, jejunum and ileum were collected, fixed in formaldehyde, embedded in paraffin, stained and analyzed using microscope and image software (Kumar *et al.*, 2017). Villus height and crypt depth were measured, with 12 villi per section selected based on intact lamina propria. Villus height was measured from tip to junction, and crypt depth as the invagination depth between villi. Averaged values were obtained from three sections with ten observations each.

Statistical Analysis

The data was analyzed using one-way analysis of variance in SPSS program with a randomized design using pens as the experimental unit for feed intake, FCR, and body weight gain. Other parameters were measured at the individual bird level, with treatment as the main factor. A significance level of $p \leq 0.01$ was used to identify trends, while $p \leq 0.05$ indicated significance. If a significant treatment effect was found, Tukey's test was conducted to determine differences among treatment means.

Results and Discussion

Growth Performance

In this study, the average daily weight gain (ADG) during grower (day 15-28) and finisher (day 29-35) phases did not differ among the dietary treatment groups. During starter (day 1-14) phase ADG was significantly higher in the T3 group compared to the T1 and control groups, while T2 group did not differ with either T3 group or with T1 and control groups. Additionally, ADG was significantly higher in T3 group compared with the control group, while T1 and T2 group did not differ with either T3 group or control group during overall experiment period. Average daily feed intake did not differ among the dietary treatment group during different phase or overall experiment period. Phase wise FCR did not differ among the dietary treatment groups. However, FCR was significantly improved in T2 and T3 groups compared with control group, while T1 group did not differ with either T2 and T3 groups or control group during overall experiment period (Table 1). This study aligned with previous research by Hussein and Selim (2018), demonstrating that supplementing probiotics and probiotics-yeast combinations resulted in higher body weight gain compared to the control diet. The treatment group also showed improved FCR ($p<0.001$) and lower daily feed intake ($p<0.001$) than the control group. He *et al.* (2019) reported that chicks fed with probiotics at 300 mg/kg exhibited better ADG and FCR compared to control and antibiotics diets, with no significant increase in average daily feed intake. Ismael (2022) found that broilers supplemented with XPC, yeast fermented, and xylanase had higher FCR and body weight gain ($p<0.05$) compared to control and low-density diets. The improved FCR, body weight gain, and ADG in the treatment groups of this study may be attributed to the positive effects of probiotics, yeast, and enzymes on gut microbiota, enhancing immunity and digestibility for better nutrient absorption (Burkholder *et al.*, 2008; Awad *et al.*, 2009).

Carcass Traits

There were no significant differences in slaughter body weight, eviscerated carcass weight, dressing percentage, and the weights of various carcass components (breast, frame, thigh, drumstick, wing, neck, gizzard, liver, heart, spleen, bursa, and abdominal fat) across the different treatment groups (Table 2). Similar findings were reported by Al-Harthi and Attia (2016) and Amerah *et al.*(2017). Hussain *et al.* (2019) also found that the supplementation of exogenous enzymes (protease, mannanase and xylanase) did not affect carcass, breast and thigh yield in broiler chicks. Tripathi *et al.* (2020) reported that carcass characteristics were not affected by protease enzyme supplementation. Additionally, Ciurescu *et al.* (2020) demonstrated that the addition of *Bacillus subtilis* probiotics in broiler diets had no impact on carcass, breast, and leg yield. In contrast, some studies have shown that probiotics supplementation can reduce abdominal fat. Ahmat *et al.* (2021) reported significantly improved carcass yield and reduce abdominal fats in broiler chickens with dietary supplementation of *B. amyloliquefaciens*.

Gut Morphology

In this study, the duodenal crypt depth was significantly lower in the T3 group compared to the T1 and control groups ($p<0.05$). The T2 group did not show a significant difference compared to either the T3 group or the T1 and control groups. The duodenal villi height to crypt depth ratio was significantly higher in the T3 group compared to the T1

Table 1: Effects of combination of probiotics, enzyme and yeast on average daily gain (ADG), average daily feed intake (ADFI), and Feed conversion ratio (FCR) of broiler chickens

Parameter	Attributes	Treatment[1]				SEM (n=4)	P-Value
		C	T1	T2	T3		
ADG (g/d)	Day 1-14	35.14^{b}	35.69^{b}	37.54^{ab}	38.62^{a}	0.538	0.058
	Day 15-28	70.95	71.27	70.89	73.59	1.017	0.790
	Day 29-35	77.24	85.51	84.33	89.40	3.221	0.648
	Day 1-35	57.24^{b}	59.89^{ab}	60.26^{ab}	62.05^{a}	0.640	0.043
ADFI (g/day)	Day 1-14	43.06	41.19	41.68	41.36	0.501	0.583
	Day 15-28	102.30	100.24	96.09	97.25	1.237	0.288
	Day 29-35	122.83	125.61	124.62	123.89	2.159	0.980
	Day 1-35	82.71	81.69	80.03	80.22	0.822	0.663
FCR (g intake/ g gain)	Day 1-14	1.23	1.56	1.11	1.07	0.024	0.086
	Day 15-28	1.46	1.41	1.36	1.32	0.029	0.441
	Day 29-35	1.59	1.48	1.49	1.43	0.044	0.682
	Day 1-35	1.45^{a}	1.37^{ab}	1.33^{b}	1.29^{b}	0.020	0.027

[ab]means with different superscripts in the same row show significant differences ($p < 0.05$).
[1]C1= Control diet, T1- Control diet + BMD (Bacitracin methylene di-salicylate, at 0.2 g/kg of diet), T2- Control diet + combination of probiotics, enzymes & yeast at 0.3 gm/kg of diet, T3- Control diet + combination of probiotics, enzymes & yeast, at 0.3 gm/kg of diet + BMD at 0.2 gm/kg diet.

and control groups ($p<0.05$), with no significant difference observed in the T2 group compared to the other groups. The villi height to crypt depth ratio in ileum was significantly higher in the T3 and T2 groups compared to the control group ($p<0.05$), while the T1 group did not differ significantly from either the T3 and T2 groups or the control group. No significant differences were found in the remaining parts of the small intestine among the dietary treatment groups (Table 3). These findings are consistent with previous studies of Soumeh *et al.* (2021), that supplementing *Bacillus subtilis* and *Bacillus licheniformis* strains increased villus height in the jejunum and ileum compared to the control group. Ismael (2022) found significant differences in villi length, crypt depth, and muscular thickness in chickens fed fermented *Saccharomyces cerevisiae* yeast and xylanase. Kim *et al.* (2021) observed increased villus height and decreased crypt depth in the duodenum, jejunum, and ileum with phytases and multienzymes supplementation. In contrast, Hussain *et al.* (2019) reported that supplementation of exogenous protease and enzyme mix (mannanase and xylanase) had no effect on intestinal morphology of broiler chickens fed Hi-Pro DDGS-based diets. Opoku *et al.* (2015) also found that protease and mannase alone in 30% wheat DDGS based broiler diet did not affect duodenum, jejunum and ileum weight and length.

Table 2: Effects of combination of probiotics, enzyme and yeast on carcass characteristics of broiler chickens

Attributes	Treatment[1]				SEM (n=4)	P-Value
	C	T1	T2	T3		
Slaughter weight (g)	2125.67	2265.00	2245.00	2295.00	28.888	0.168
Eviscerated weight (g)	1425.67	1547.33	1504.33	1563.00	27.277	0.308
Dressing percentage	67.05	68.25	67.21	68.12	1.000	0.974
Breast (g)	617.00	602.00	630.00	625.33	20.243	0.975
Frame (g)	257.67	266.33	290.67	285.00	8.425	0.528
Thigh (g)	236.33	230.33	243.33	246.67	9.119	0.945
Drumstick (g)	211.67	206.00	210.33	209.00	7.830	0.997
Wing (g)	126.33	146.00	147.00	153.33	8.333	0.749
Neck (g)	55.00	54.33	55.67	56.00	1.548	0.988
Gizzard (g)	45.33	44.67	42.33	39.00	1.058	0.120
Liver (g)	38.00	37.33	35.67	39.67	0.655	0.185
Heart (g)	13.00	10.50	12.00	12.33	0.750	0.745
Spleen (g)	2.60	2.90	2.73	3.20	0.109	0.251
Bursa (g)	1.97	2.07	2.23	2.03	0.073	0.676
Abdominal fat (g)	49.67	47.00	49.00	44.67	1.574	0.736

[1]C1= Control diet, T1- Control diet + BMD (Bacitracin methylene di-salicylate, at 0.2 g/kg of diet), T2- Control diet + combination of probiotics, enzymes & yeast at 0.3 gm/kg of diet, T3- Control diet + combination of probiotics, enzymes & yeast, at 0.3 gm/kg of diet + BMD at 0.2 gm/kg diet.

Table 3: Effects of combination of probiotics, enzyme and yeast on gut morphology of broiler chickens

Gut part	Attributes	Treatment[1]				SEM (n=4)	P Value
		C	T1	T2	T3		
Duodenum	Villi height (VH; μm)	1477.00	1488.75	1500.75	1541.75	31.051	0.913
	Crypt depth (CD; μm)	80.50[a]	78.75[a]	70.50[ab]	61.00[b]	2.551	0.008
	VH: CD ratio	18.35[b]	19.07[b]	21.28[ab]	25.87[a]	1.018	0.018
Jejunum	Villi height (VH; μm)	983.25	1002.75	993.75	1044.25	42.430	0.969
	Crypt depth (CD; μm)	77.00	75.25	75.25	72.25	2.626	0.949
	VH: CD ratio	12.99	13.36	13.43	15.22	0.851	0.828
Ileum	Villi height (VH; μm)	604.00	610.00	605.25	615.50	25.444	0.999
	Crypt depth (CD; μm)	72.50	68.75	55.50	54.25	3.260	0.094
	VH: CD ratio	8.34[b]	9.08[ab]	10.87[a]	11.30[a]	0.442	0.030

[ab]means with different superscripts in the same row show significant differences ($p < 0.05$).[1]C1= Control diet, T1- Control diet + BMD (Bacitracin methylene di-salicylate, at 0.2 g/kg of diet), T2- Control diet + combination of probiotics, enzymes & yeast at 0.3 gm/kg of diet, T3- Control diet + combination of probiotics, enzymes & yeast, at 0.3 gm/kg of diet + BMD at 0.2 gm/kg diet.

Conclusion

Birds supplemented with a combination of a probiotics, enzymes, and yeast with antibiotic showed improved growth performance and gut morphology. These findings suggest that a probiotics, enzymes, and yeast combination can be used along with antibiotics in broiler production.Further research is needed to fully understand and optimize this combination in poultry production.

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