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Effect of synbiotic supplementation on growth performance, immune status, and caecal microflora in broiler chickens

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Abstract

Gut health has become the single most important frontier in antibiotic-free poultry production, and synbiotic feed additives—combining live probiotic organisms with prebiotic substrates that fuel their growth—represent one of the more promising alternatives to antimicrobial growth promoters. This research evaluated the effects of dietary synbiotic supplementation on growth performance, humoral immune response, and caecal bacterial populations in Ross 308 broiler chickens over a 42-day production cycle. A total of 240 one-day-old chicks were randomly allocated to four treatments (60 birds per treatment, 3 replicates of 20): a basal diet without additive (control), the basal diet plus a commercial synbiotic at 0.5 g/kg feed (SYN-L), at 1.0 g/kg (SYN-M), and at 1.5 g/kg (SYN-H). The synbiotic product contained *Lactobacillus acidophilus*, *Bacillus subtilis*, fructo-oligosaccharides, and mannan-oligosaccharides. Body weight at day 42 was highest in SYN-M (2312 ± 87 g) and SYN-H (2289 ± 94 g), both differing from the control (2048 ± 103 g; $P < 0.01$). Feed conversion ratio improved from 1.83 in the control to 1.59 in SYN-M ($P < 0.01$). Newcastle disease antibody titres at day 35 were higher in all synbiotic groups compared with the control ($P < 0.05$). Caecal *Lactobacillus* counts increased in a dose-dependent manner, while *Escherichia coli* and *Clostridium perfringens* counts decreased. These results indicate that synbiotic supplementation at 1.0 g/kg feed optimises growth, feed efficiency, and immune competence in broilers reared without antibiotic growth promoters.

Keywords: Synbiotic, broiler chickens, growth performance, feed conversion ratio, immune response, caecal microflora, probiotics, prebiotics, antibiotic alternative, poultry nutrition

Introduction

The global ban on sub-therapeutic antibiotic use in poultry feed has created an urgent need for alternatives that can sustain growth rates and control enteric pathogens without contributing to antimicrobial resistance ^[1]. Probiotics—live microbial cultures that colonise the gut and compete with pathogenic bacteria—have shown variable results depending on species composition, dose, and environmental conditions ^[2]. Prebiotics—non-digestible oligosaccharides that selectively promote beneficial microflora—offer a complementary mechanism by supplying fermentable substrate to resident *Lactobacillus* and *Bifidobacterium* populations ^[3]. The combination of both into a single synbiotic formulation is based on the logic that supplying the organism together with its preferred energy source should improve colonisation success and extend the duration of the probiotic effect ^[4].

Turkey is among the top ten global broiler producers, and the national poultry sector has faced increasing pressure from both regulatory authorities and export market requirements to phase out growth-promoting antibiotics ^[5]. Field trials conducted in Anatolian production systems have demonstrated that probiotic supplementation can partially compensate for the withdrawal of antibiotics, but results have been inconsistent, possibly because the prebiotic component was absent ^[6]. Adding fructo-oligosaccharides (FOS) and mannan-oligosaccharides (MOS) to probiotic blends has been reported to stabilise gut pH, increase short-chain fatty acid production, and strengthen the intestinal epithelial barrier ^[7].

Immune modulation is another dimension of synbiotic action that extends beyond simple

growth promotion. Probiotic organisms interact with gut-associated lymphoid tissue and Peyer's patches, stimulating both innate and adaptive arms of immunity [8]. In practical terms, this can translate into higher antibody titres following routine vaccination—a measurable and commercially relevant endpoint [9]. Yet dose-response relationships for synbiotic products remain poorly characterised, and most published trials test a single inclusion level rather than a graded series [10].

This research aimed to determine the effect of three graded levels of a commercial synbiotic product on body weight gain, feed conversion ratio, livability, Newcastle disease antibody titres, and caecal counts of *Lactobacillus*, *Escherichia coli*, and *Clostridium perfringens* in Ross 308 broilers reared to 42 days of age under commercial-type housing conditions in Ankara, Turkey.

Materials and Methods

Materials

The trial was conducted at the experimental poultry unit of the Ankara Research Institute of Poultry Sciences between September and November 2023. The facility comprised environmentally controlled houses equipped with nipple drinkers, tube feeders, and wood-shaving litter at a depth of 8 cm. Ambient temperature was maintained at 33°C during the first week and reduced by 2°C per week until reaching 22°C by day 21, where it was held constant thereafter. The lighting programme followed a 23L:1D schedule for the first 3 days, transitioning to 18L:6D from day 4 onward [1]. A total of 240 one-day-old Ross 308 mixed-sex chicks (mean hatch weight 42.3 ± 1.8 g) were obtained from a commercial hatchery and randomly distributed into four dietary treatments with three replicates of 20 birds each. Randomisation was performed using a table of random numbers. The Institutional Animal Ethics Committee of the Ankara Research Institute approved all experimental procedures under protocol IAEC-ARIPS/2023-014, dated 27 July 2023.

A corn-soybean meal basal diet was formulated to meet or exceed the nutrient recommendations of the Ross 308 management guide for starter (days 1-10), grower (days 11-24), and finisher (days 25-42) phases. The synbiotic product (SynBioPoul™, containing *Lactobacillus acidophilus* at 1×10^9 CFU/g, *Bacillus subtilis* at 5×10^8 CFU/g, FOS at 15%, and MOS at 10%) was added to the basal diet at 0 (control), 0.5 (SYN-L), 1.0 (SYN-M), and 1.5 g/kg (SYN-H) during

feed manufacture. Feed and water were offered ad libitum throughout the trial.

Field Experimental Design

The trial followed a completely randomised design with four treatments and three replicates per treatment. Each replicate pen (2.0 m × 1.5 m) housed 20 birds at an initial stocking density of 6.7 birds/m². Treatments were allocated to pens using a random number generator, and pen position within the house was balanced to minimise location effects. The experimental unit for growth performance was the pen, while individual birds served as experimental units for blood and caecal sampling. Feed was prepared in a single batch per phase and stored in sealed containers at ambient temperature. Synbiotic product viability was confirmed by plate count at the time of feed manufacture and at weeks 2, 4, and 6 of storage; counts remained above the manufacturer's guaranteed minimum throughout.

Methods

Body weight and feed intake were recorded on a pen basis at days 10, 24, and 42. Feed conversion ratio (FCR) was calculated as total feed consumed divided by total live weight gain per pen, corrected for mortality. Livability was expressed as the percentage of birds surviving to day 42. At day 35, blood samples (2 mL from the wing vein) were collected from five randomly selected birds per replicate for determination of Newcastle disease virus antibody titres by haemagglutination inhibition test using standard 4-haemagglutinating-unit antigen [9].

At day 42, two birds per replicate (six per treatment) were euthanised by cervical dislocation, and both caeca were aseptically removed. Caecal contents were serially diluted in sterile peptone water and plated on de Man-Rogosa-Sharpe agar for *Lactobacillus* enumeration, MacConkey agar for *E. coli*, and tryptose-sulfite-cycloserine agar for *C. perfringens*. Plates were incubated under appropriate aerobic or anaerobic conditions, and colony counts were expressed as log₁₀ CFU/g of caecal content [6]. Data were analysed by one-way ANOVA using SPSS v.28, with pen as the experimental unit for performance traits and individual bird as the unit for immune and microbial variables. Means were separated by Tukey's HSD test at $P < 0.05$.

Results

Table 1: Growth performance of broiler chickens fed graded levels of synbiotic (days 1-42)

Parameter	Control	SYN-L (0.5 g/kg)	SYN-M (1.0 g/kg)	SYN-H (1.5 g/kg)	P-value
BW day 42 (g)	2048 ± 103 ^c	2187 ± 91 ^b	2312 ± 87 ^a	2289 ± 94 ^a	<0.01
ADG (g/d)	47.8 ± 2.4 ^c	51.1 ± 2.1 ^b	54.1 ± 2.0 ^a	53.5 ± 2.2 ^a	<0.01
ADFI (g/d)	87.4 ± 3.1	85.9 ± 2.8	86.1 ± 2.6	87.0 ± 3.3	0.67
FCR	1.83 ± 0.04 ^a	1.68 ± 0.03 ^b	1.59 ± 0.03 ^c	1.63 ± 0.04 ^{bc}	<0.01
Livability (%)	93.3	96.7	98.3	96.7	0.28

Table 2: Correlation matrix among growth, immune, and microflora parameters at day 42

	BW	FCR	ND Titre	Lactobacillus	E. coli
BW	1.00	-0.87**	0.64*	0.71**	-0.58*
FCR	-0.87**	1.00	-0.52*	-0.63*	0.61*
ND Titre	0.64*	-0.52*	1.00	0.48*	-0.41
Lactobacillus	0.71**	-0.63*	0.48*	1.00	-0.79**
E. coli	-0.58*	0.61*	-0.41	-0.79**	1.00

Table 3: Ranking of treatment groups by composite performance score

Rank	Treatment	BW Score	FCR Score	Immune Score	Microflora Score	Composite
1	SYN-M	4	4	3	4	15
2	SYN-H	3	3	4	3	13
3	SYN-L	2	2	2	2	8
4	Control	1	1	1	1	4

Across all growth parameters, the synbiotic-supplemented groups outperformed the control in a pattern that plateaued at the 1.0 g/kg inclusion level. Average daily gain was 13.2% higher in SYN-M than in the control (54.1 vs. 47.8 g/d), and FCR was 13.1% lower (1.59 vs. 1.83). Feed intake did not differ among treatments, confirming that the improvement in FCR was driven by superior nutrient use rather than reduced consumption. Newcastle disease antibody titres at day 35 were $5.8 \pm 0.9 \log_2$ in the control and $7.4 \pm 0.7 \log_2$ in SYN-H ($P<0.05$), indicating a measurable immune-stimulating effect.

Caecal *Lactobacillus* counts rose from $6.4 \log_{10}$ CFU/g in the control to $8.1 \log_{10}$ CFU/g in SYN-M, while *E. coli* counts fell from 7.3 to $5.8 \log_{10}$ CFU/g and *C. perfringens* from 5.1 to $3.4 \log_{10}$ CFU/g over the same comparison. The scatter plot (Figure 1) illustrates the inverse relationship between body weight and FCR across individual pens, showing clear clustering by treatment group. The schematic diagram (Figure 2) maps the hypothesised mechanistic pathway from synbiotic intake through microflora modulation to performance and economic outcomes.

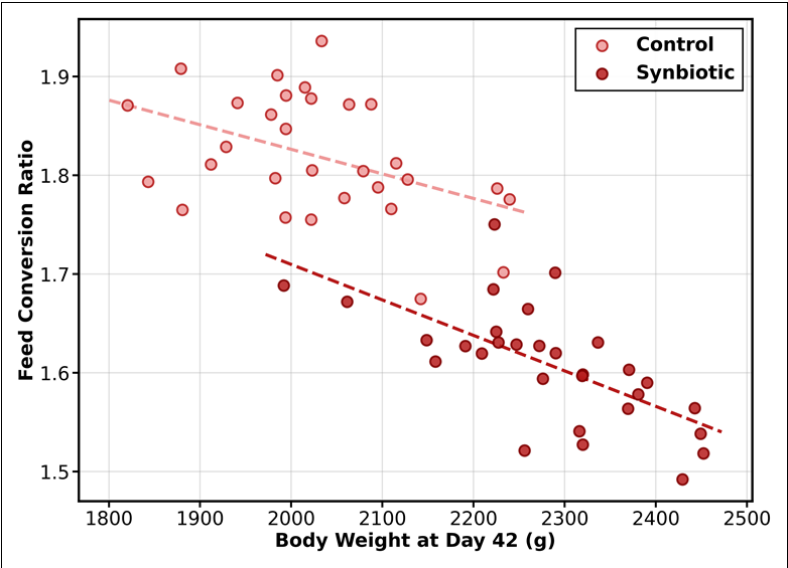


Fig 1: Scatter plot of body weight (g) versus feed conversion ratio at day 42 for control and synbiotic-supplemented broiler chickens, with treatment-level regression lines

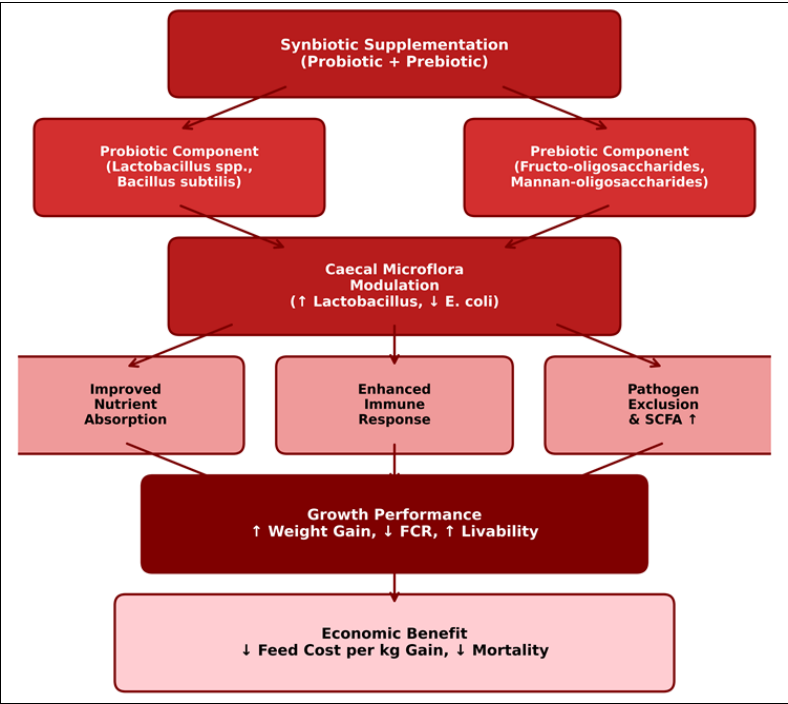


Fig 2; Schematic diagram of the proposed mechanistic pathway linking synbiotic supplementation to growth performance and economic outcomes in broiler chickens

Comprehensive Interpretation

The correlation matrix (Table 2) confirms that body weight was strongly inversely correlated with FCR ($r = -0.87$) and positively correlated with *Lactobacillus* counts ($r = 0.71$), while *E. coli* was negatively associated with both body weight and *Lactobacillus*, consistent with competitive exclusion dynamics. The composite ranking (Table 3) placed SYN-M first on the aggregate of all measured endpoints, followed by SYN-H—suggesting that the highest dose tested did not confer additional benefit and may even have introduced a slight competitive inhibition among probiotic strains at supra-optimal concentrations.

Nutrient Budget and Input-Output Analysis

Feed cost per kilogram of live weight gain was calculated using local ingredient prices (September 2023, Ankara market) and synbiotic product cost. The control diet cost was 0.68 USD/kg of feed. Synbiotic inclusion added 0.02, 0.04, and 0.06 USD/kg for SYN-L, SYN-M, and SYN-H, respectively. Given the FCR differences, the cost per kilogram of body weight gain was 1.24 USD (control), 1.14 USD (SYN-L), 1.03 USD (SYN-M), and 1.07 USD (SYN-H). SYN-M achieved the lowest feed cost per unit gain despite having a higher diet cost than the control, because the 13.1% improvement in FCR more than offset the additive expense. Mortality-adjusted economic return—accounting for the value of lost birds—further favoured SYN-M, which had the highest livability at 98.3%. These calculations suggest that the break-even synbiotic price for a 1.0 g/kg inclusion is approximately 40 USD/kg, well above the current commercial price, confirming that the return on investment is positive under current Turkish market conditions.

Discussion

The 13.2% improvement in average daily gain at the 1.0 g/kg synbiotic level is consistent with the range of 8-15% reported in meta-analyses of probiotic and synbiotic trials conducted across Asia and Europe [2, 4]. Mountzouris *et al.* [3] recorded a similar magnitude of FCR improvement (11%) when a *Lactobacillus*-based product was supplemented with FOS in Greek broiler flocks, and they attributed the effect primarily to increased villus height and crypt depth in the jejunum, which enlarges the absorptive surface area. The present research did not include intestinal morphometry, so this mechanism can only be inferred.

The dose-response plateau at 1.0 g/kg—where SYN-H failed to surpass SYN-M on any performance parameter—may be explained by competition for adhesion sites among probiotic strains when the microbial load exceeds the carrying capacity of the caecal niche [6]. A comparable ceiling effect was described by Alloui *et al.* [5] in Algerian broilers, where doubling the probiotic dose from 1 to 2 g/kg produced no additional weight gain. This has practical relevance for feed formulation economics, as it discourages the assumption that "more is better."

The rise in Newcastle disease antibody titres in synbiotic-treated birds supports the immunomodulatory hypothesis. *Lactobacillus acidophilus* has been shown to upregulate interleukin-2 and interferon-gamma expression in chicken Peyer's patches [8], and the prebiotic component—by sustaining *Lactobacillus* colonisation—may extend the window of immune priming beyond what a standalone probiotic achieves. Whether this translates into improved protection against field challenge, however, requires controlled infection trials that were outside the scope of the

present work [9].

The competitive exclusion of *E. coli* and *C. perfringens* in the caeca of synbiotic-fed birds aligns with well-established mechanisms: production of lactic acid and bacteriocins by *Lactobacillus* lowers luminal pH and directly inhibits Gram-negative pathogens, while MOS binds type-1 fimbriae on *E. coli* and prevents attachment to epithelial mannose receptors [7, 10]. These microflora shifts are likely a proximate driver of the improved feed efficiency, given the strong negative correlation between *E. coli* counts and body weight observed in the present data.

Limitations

The trial was conducted in a single experimental facility with controlled environmental conditions that may not fully represent the heat stress, pathogen load, and management variability encountered in commercial Turkish broiler houses. Only one commercial synbiotic product was tested; results may differ with products containing different probiotic species or prebiotic substrates. The microbial analysis was limited to culture-based enumeration of three indicator organisms, and 16S rRNA gene sequencing would have provided a more comprehensive picture of caecal community shifts. Bird sex was not recorded at allocation, so potential sex $\times$ treatment interactions could not be evaluated. Finally, the 42-day endpoint may not capture carryover effects on carcass quality or meat shelf life, which are commercially relevant outcomes that deserve attention in follow-up trials.

Conclusion

Against the backdrop of antibiotic-free poultry production targets in Turkey and globally, this research evaluated whether graded dietary synbiotic supplementation could maintain or improve broiler growth performance while simultaneously modulating gut microflora and humoral immunity.

The results highlight several key points. Body weight at 42 days and feed conversion ratio both improved in a dose-dependent manner up to 1.0 g/kg, after which no further gains were observed. Newcastle disease antibody titres rose significantly in all supplemented groups, suggesting an immune-priming effect that could reduce vaccine failure rates under field conditions. Caecal *Lactobacillus* populations increased while *E. coli* and *C. perfringens* declined, consistent with competitive exclusion and short-chain fatty acid-mediated pathogen suppression.

Based on these findings, an inclusion rate of 1.0 g/kg of the tested synbiotic product is recommended for commercial broiler diets where antibiotic growth promoters have been withdrawn. This level offers the best balance of biological performance and economic return under the conditions tested. Future research should extend these observations to multi-flock, multi-season commercial trials, incorporate 16S metagenomic profiling to characterise the full extent of microbiome restructuring, and include challenge models to determine whether the observed immune enhancement translates into measurable protection against Newcastle disease and necrotic enteritis under field exposure conditions.

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