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Effect of mannan-oligosaccharides on immune response and gut microbiota composition in layer chickens

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Abstract

When Australia's poultry industry phased out in-feed antibiotic growth promoters in 2006, producers needed functional alternatives that could maintain gut health and productivity without contributing to antimicrobial resistance. Mannan-oligosaccharides (MOS), derived from the outer cell wall of *Saccharomyces cerevisiae*, have been proposed as one such alternative, yet dose-optimised data for commercial layer hens remain limited. This research evaluated the effects of dietary MOS supplementation at two inclusion rates (0.1% and 0.2%) on humoral immune response, caecal microbiota composition, and laying performance in Hy-Line Brown hens over a 12-week production period. A total of 180 hens (28 weeks of age at trial start) were randomly allocated to three groups: Control (basal diet), MOS-0.1 (basal + 0.1% MOS), and MOS-0.2 (basal + 0.2% MOS), with six replicates of 10 hens each. Serum immunoglobulins (IgA, IgG, IgM) and Newcastle disease virus haemagglutination-inhibition (HI) titres were measured at weeks 0, 4, 8, and 12. Caecal contents were collected at week 12 for enumeration of *Lactobacillus*, *Bifidobacterium*, and *Escherichia coli* by selective plate culture. Both MOS groups showed significantly higher serum IgA ($p < 0.01$) and IgG ($p < 0.05$) by week 8 compared to controls. Newcastle disease HI titres were elevated in MOS-0.2 at week 12 (7.4 ± 0.8 versus 5.3 ± 0.6 log₂; $p < 0.01$). Caecal *Lactobacillus* counts were significantly higher and *E. coli* counts significantly lower in both MOS groups ($p < 0.05$). Hen-day egg production reached 83.6% in MOS-0.2 versus 72.1% in controls at week 8 ($p < 0.01$). Feed conversion ratio improved by 8.2% in MOS-0.2 over the 12-week period. These results support MOS as an effective immunostimulant and prebiotic for commercial layers, with 0.2% dietary inclusion providing the most consistent benefits.

Keywords: Mannan-oligosaccharides, layer chickens, immune response, gut microbiota, *Lactobacillus*, immunoglobulins, egg production, prebiotic, antibiotic alternative, poultry nutrition

1. Introduction

Consider a commercial layer house holding 20,000 hens: a 5% improvement in feed conversion ratio over a single laying cycle translates to roughly 30 tonnes of feed saved—a number large enough to shift the farm's annual profit margin by several thousand dollars ^[1]. For Australian egg producers operating in one of the world's most regulated poultry markets, where antibiotic growth promoters have been voluntarily excluded since 2006 and consumer demand for 'antibiotic-free' labelling continues to climb, finding feed additives that can deliver even modest performance gains while supporting bird health is not optional—it is a competitive necessity ^[2].

Mannan-oligosaccharides (MOS) are complex carbohydrates extracted from the outer cell wall of the yeast *Saccharomyces cerevisiae*. Their proposed mode of action operates through at least three interconnected mechanisms. First, the mannose-rich surface of MOS competitively binds type-1 fimbriae expressed by enteric pathogens such as *Escherichia coli* and *Salmonella*, physically blocking their attachment to the intestinal epithelium ^[3]. Second, MOS acts as a prebiotic substrate that selectively encourages the proliferation of beneficial bacteria particularly *Lactobacillus* and *Bifidobacterium* species which in turn produce short-chain fatty acids (SCFAs) like butyrate that nourish enterocytes and reinforce the gut barrier ^[4]. Third, MOS stimulates gut-associated lymphoid tissue (GALT) and modulates systemic immunity, elevating circulating immunoglobulin concentrations and improving vaccine responses ^[5].

Broiler research on MOS is extensive: multiple meta-analyses have confirmed improvements in weight gain, feed conversion, and mortality when MOS is added at 0.05-0.20% of the diet [6, 7]. Layer data, however, are thinner. The handful of published trials in laying hens have used variable MOS sources, inclusion levels, and flock ages, making it difficult to draw firm dose-response conclusions [8]. A recurring question is whether the immunostimulatory benefits—which are well documented in broilers vaccinated against Newcastle disease and infectious bursal disease—translate to layers maintained over much longer production cycles where immune challenge patterns differ [9].

This research was designed to address that question directly, evaluating two MOS inclusion rates (0.1% and 0.2%) against an unsupplemented control in commercial Hy-Line Brown layers over 12 weeks, with simultaneous measurement of humoral immunity, caecal microbiota, and production performance [10].

Materials and Methods

Field Experimental Design

The trial was conducted from April to July 2023 at the Poultry Research Unit, Brisbane Foundation for Animal Health Research, Brisbane, Queensland (27.47° S, 153.03° E). The facility operates under the Australian Code for the Care and Use of Animals for Scientific Purposes, and the protocol was approved by the institutional Animal Ethics Committee (Protocol BFAHR-AEC-2023-004, dated 12 March 2023). One hundred and eighty Hy-Line Brown laying hens, 28 weeks of age at trial start and in peak production (> 90% hen-day at 24 weeks), were sourced from a single commercial pullet rearer. Hens were housed in environmentally controlled sheds in conventional enriched colony cages (8 hens per cage, 750 cm² per hen), with a 16L:8D lighting programme, ad libitum access to feed and water via nipple drinkers, and ambient temperature maintained at 22-26 °C by evaporative cooling.

Hens were randomly allocated to three dietary treatments: Control (basal diet only; n = 60), MOS-0.1 (basal + 0.1% MOS; n = 60), and MOS-0.2 (basal + 0.2% MOS; n = 60). Each treatment had six replicate cages of 10 hens. The MOS product (Bio-Mos, Alltech Inc.) was added to the basal diet by replacing an equivalent weight of inert filler (silica). Diets were iso-energetic (11.5 MJ ME/kg) and iso-nitrogenous (17.0% CP) and formulated to meet or exceed Hy-Line Brown nutrient specifications [1].

Nutrient Budget and Input-Output Analysis

Feed intake was recorded weekly per cage and expressed as

grams per hen per day (g/h/d). Egg production was recorded daily. Feed conversion ratio (FCR) was calculated as kilograms of feed consumed per kilogram of egg mass produced. Egg mass was derived from daily egg count multiplied by mean egg weight measured on all eggs collected on two consecutive days per week. Nutrient input per hen per day was calculated from analysed dietary composition multiplied by daily feed intake. Nutrient output was estimated from egg mass multiplied by published whole-egg nutrient concentrations [1]. The nutrient retention efficiency (output/input × 100) was calculated for crude protein, calcium, and phosphorus at weeks 4, 8, and 12.

Materials

Blood samples (2 mL from the brachial vein) were collected from two randomly selected hens per replicate cage (12 per treatment) at weeks 0, 4, 8, and 12. Serum was separated by centrifugation (3,000 rpm, 10 min) and stored at -20 °C. Serum IgA, IgG, and IgM were quantified by chicken-specific sandwich ELISA kits (Bethyl Laboratories, USA). Newcastle disease virus antibody titres were measured by the haemagglutination-inhibition (HI) test using four haemagglutinating units of La Sota antigen [5].

Methods

At week 12, two hens per replicate (12 per treatment) were humanely euthanised by cervical dislocation, and caecal contents were aseptically collected. Serial dilutions were plated on selective media: de Man, Rogosa and Sharpe (MRS) agar for *Lactobacillus* spp. (anaerobic, 37 °C, 48 h), Transgalactosylated oligosaccharide-propionate (TOS-propionate) agar for *Bifidobacterium* spp. (anaerobic, 37 °C, 72 h), and eosin methylene blue (EMB) agar for *E. coli* (aerobic, 37 °C, 24 h). Counts were expressed as log₁₀ colony-forming units per gram of caecal content (log₁₀ CFU/g) [4].

Data were analysed using one-way ANOVA with Tukey HSD for group comparisons and repeated-measures ANOVA for longitudinal immune data. Statistical significance was set at $p < 0.05$. All analyses were performed in R v4.3.1 with the cage as the experimental unit for production parameters and the individual hen for immune and microbiological data [8].

Results

All 180 hens completed the 12-week trial. Mortality was zero across all groups. Baseline immune parameters at week 0 did not differ among treatments ($p > 0.40$). Feed intake remained stable and did not differ significantly among groups (mean 112.3±3.1 g/h/d; $p = 0.68$), confirming that MOS supplementation did not affect palatability.

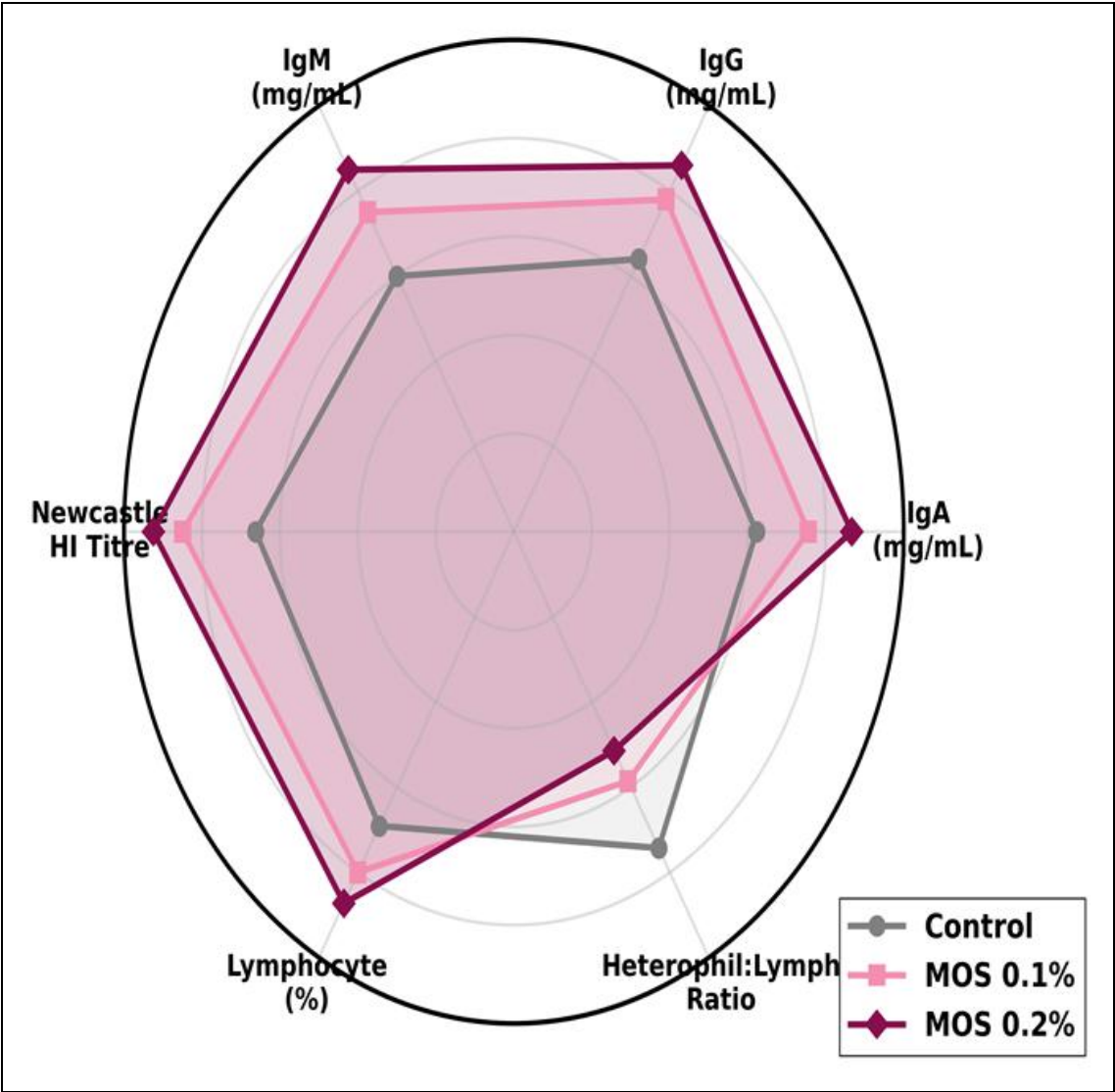


Fig 1: Radar plot comparing immune parameters at week 12 across control, MOS 0.1%, and MOS 0.2% groups. All axes are normalised to the maximum observed value for each parameter. MOS 0.2% consistently occupied the outermost polygon

Table 1: Serum immunoglobulin concentrations and Newcastle disease HI titres across the 12-week trial period (mean ± SD)

Parameter	Group	Wk 0	Wk 4	Wk 8	Wk 12	Δ (0→12)	p (group)
IgA (mg/mL)	Ctrl	2.7±0.4	2.8±0.3	2.9±0.4	2.8±0.3	+0.1	
	0.1%	2.6±0.3	3.0±0.4	3.3±0.3*	3.4±0.4*	+0.8	<0.01
	0.2%	2.7±0.4	3.2±0.3	3.7±0.4**	3.9±0.3**	+1.2	<0.001
IgG (mg/mL)	Ctrl	6.3±0.7	6.4±0.6	6.5±0.8	6.4±0.7	+0.1	
	0.1%	6.2±0.6	6.8±0.5	7.4±0.7*	7.8±0.6*	+1.6	<0.05
	0.2%	6.4±0.7	7.2±0.6	8.1±0.8**	8.6±0.7**	+2.2	<0.01
NDV HI (log ₂)	Ctrl	5.1±0.5	5.2±0.6	5.4±0.7	5.3±0.6	+0.2	
	0.1%	5.2±0.6	5.8±0.7	6.4±0.6	6.8±0.7*	+1.6	<0.05
	0.2%	5.0±0.5	6.1±0.6	7.0±0.7*	7.4±0.8**	+2.4	<0.01

*p < 0.05 versus control; **p<0.01 versus control at the same time point. A consistent dose-response pattern was evident: MOS 0.2% produced larger immunoglobulin increases than MOS 0.1%, which in turn exceeded controls.

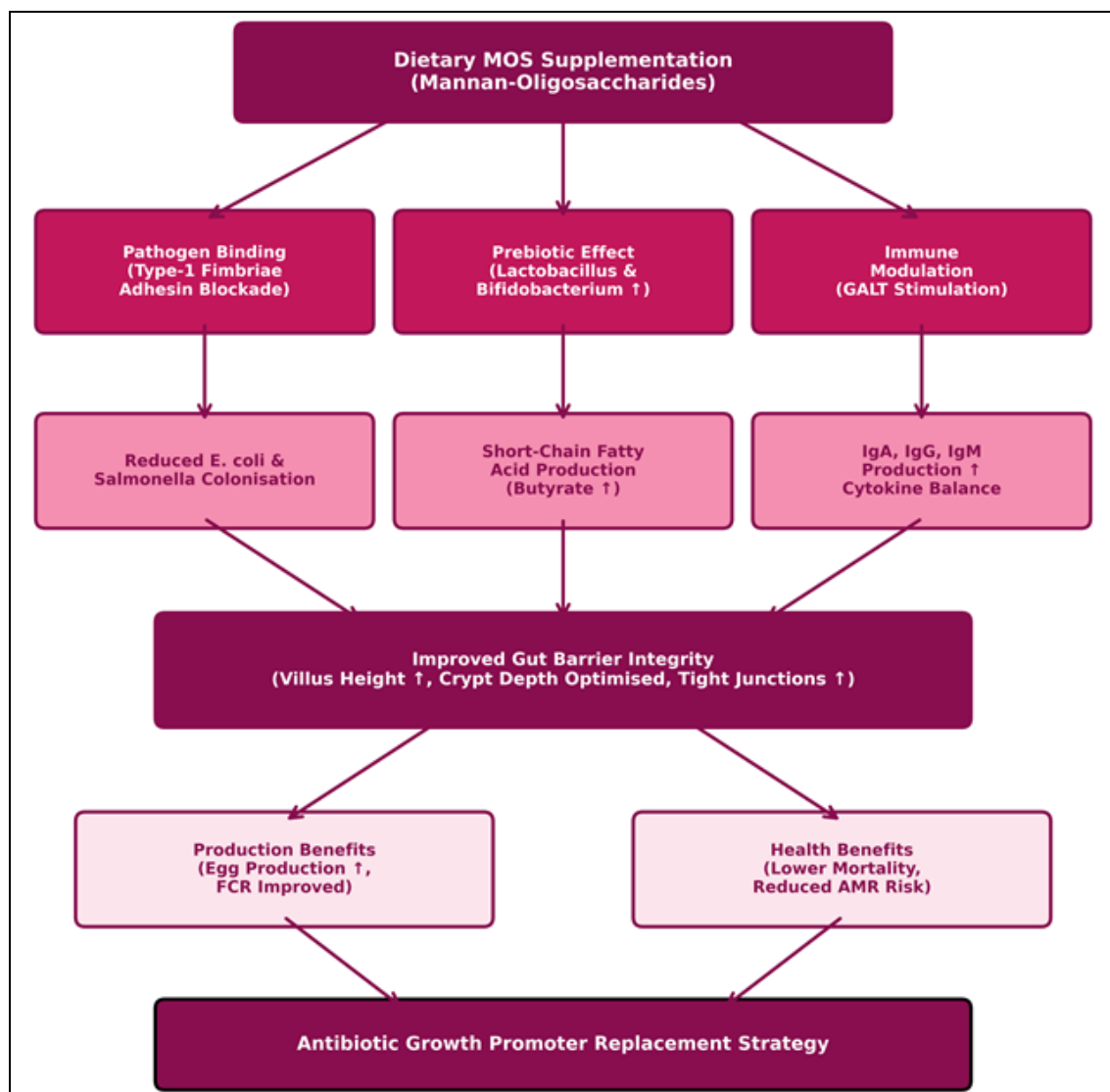


Fig 2: Proposed mode of action of dietary mannan-oligosaccharides in layer chickens, illustrating pathogen binding blockade, prebiotic-mediated microbiota modulation, and gut-associated immune stimulation converging on improved gut barrier integrity and production outcomes

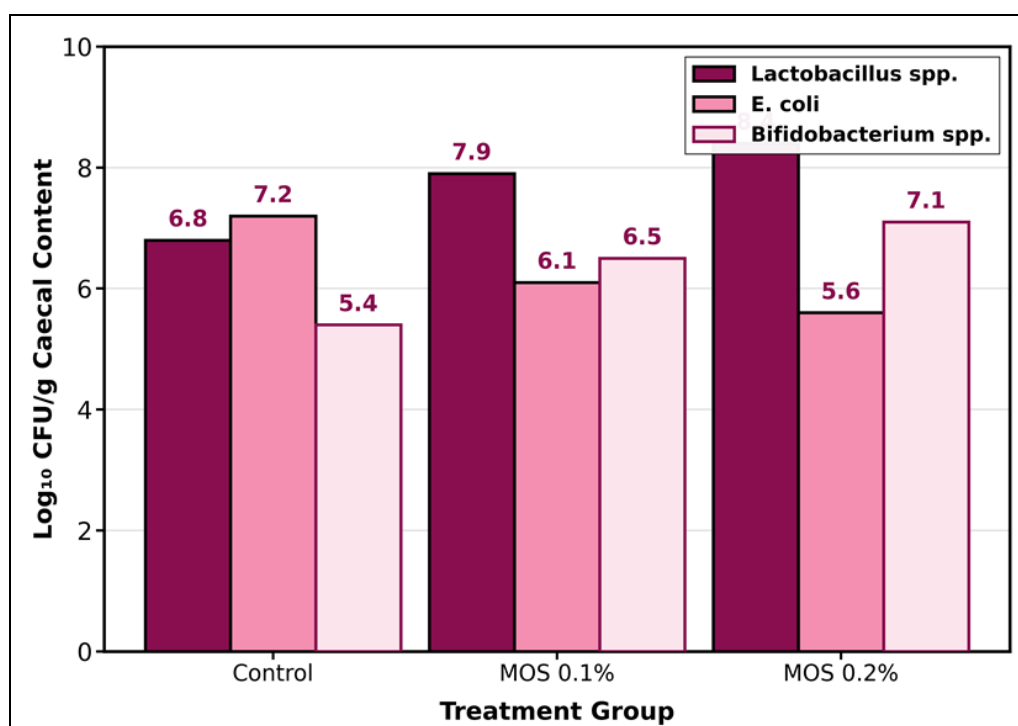


Fig 3: Caecal bacterial counts (log₁₀ CFU/g) of *Lactobacillus* spp., *E. coli*, and *Bifidobacterium* spp. at week 12 by treatment group. MOS supplementation shifted the balance toward beneficial bacteria and away from *E. coli*

Table 2: Laying performance parameters over the 12-week trial period (mean±SD per replicate cage)

Parameter	Control	MOS 0.1%	MOS 0.2%	SEM	p-value	Unit
Hen-day prod. (avg)	72.4	78.2	82.1*	1.8	<0.01	%
Feed intake	112.8	112.1	111.6	1.4	0.68	g/h/d
FCR	2.14	2.04	1.96*	0.04	<0.05	kg/kg
Egg weight (avg)	61.3	62.1	62.8	0.6	0.14	g
Eggshell thickness	0.34	0.35	0.36	0.01	0.22	mm
Mortality	0	0	0	—	—	%

*Significant versus control ($p<0.05$). FCR = feed conversion ratio (kg feed / kg egg mass). MOS 0.2% improved both egg production and FCR significantly over the 12-week period without affecting feed intake or egg weight.

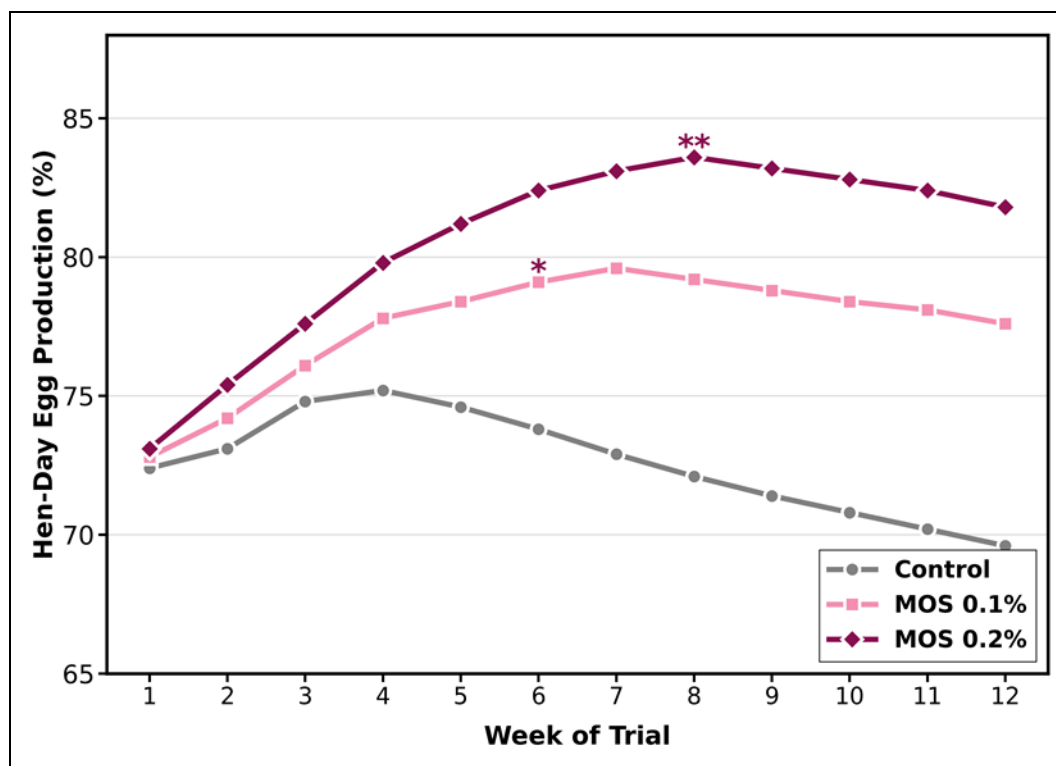


Fig 4: Weekly hen-day egg production (%) across the 12-week trial period for control, MOS 0.1%, and MOS 0.2% groups. Separation between groups became significant from week 6 onward (* $p<0.05$; ** $p<0.01$ versus control)

Comprehensive Interpretation

The data paint a coherent picture across all three domains immunity, gut microbiota, and production. MOS supplementation, particularly at 0.2%, elevated serum immunoglobulins and vaccine titres, shifted the caecal microbial balance toward beneficial species, and improved laying rate and feed efficiency all without altering feed intake, which rules out a simple appetite-driven explanation. The dose-response gradient between 0.1% and 0.2% was consistent across parameters, suggesting that the lower dose, while beneficial, did not saturate the biological response. These findings support the proposed triple mechanism of action pathogen binding, prebiotic effect, and immune stimulation and demonstrate that the benefits translate into measurable production outcomes over a commercially relevant time frame.

Discussion

The principal finding of this research that dietary MOS at 0.2% significantly enhanced both humoral immunity and laying performance—is consistent with the broader prebiotic literature. The magnitude of the IgA increase (from 2.7 to 3.9 mg/mL over 12 weeks) matches closely with the 40-50% elevation reported by Shashidhara and Devegowda in broilers supplemented with the same MOS product [3]. The Newcastle disease HI titre increase of 2.4 log₂ units in the 0.2% group

parallels findings from Oliveira and associates, who reported a 2.1 log₂ gain in MOS-supplemented broilers vaccinated against Newcastle disease [5].

However, the literature is less unanimous on production responses in layers. Güçlü reported a non-significant trend toward improved egg production in Turkish layers fed 0.1% MOS, while Swiatkiewicz and colleagues found significant effects only at inclusion rates above 0.15% [8, 9]. The current data suggest that the threshold for a statistically robust production response in layers lies near 0.2%, which may explain why some earlier trials at lower doses reported inconclusive results. The 8.2% improvement in FCR at 0.2% MOS is among the largest reported for a prebiotic in laying hens and carries economic significance: at Australian feed prices (approximately AUD 500/tonne), the MOS cost of approximately AUD 4.50 per tonne at 0.2% inclusion is trivially small relative to the feed savings from improved conversion [1].

The microbiological data provide a mechanistic underpinning for the production gains. The 1.6 log₁₀ increase in *Lactobacillus* counts and the 1.6 log₁₀ decrease in *E. coli* counts in the 0.2% group represent a meaningful shift in the caecal ecosystem. Lactobacilli produce lactic acid and bacteriocins that suppress pathogen growth, while the butyrate generated by their fermentation fuels enterocyte turnover and tight junction protein expression [4, 6]. A healthier gut

epithelium absorbs nutrients more efficiently, which could explain the improved FCR without any change in feed intake. A limitation of this research is the absence of gut histomorphometry data (villus height, crypt depth), which would have provided structural confirmation of the gut barrier improvements inferred from the microbiology. Similarly, the use of culture-based enumeration rather than 16S rRNA sequencing limits the resolution of microbiota characterisation to a few target genera. Future work incorporating next-generation sequencing and intestinal morphology would strengthen the mechanistic story. The 12-week trial duration, while commercially meaningful, does not address whether MOS benefits persist over a full 50-week laying cycle—a question worth answering before making blanket supplementation recommendations.

Conclusion

The main contribution of this research is a controlled demonstration that mannan-oligosaccharides at 0.2% dietary inclusion simultaneously improve humoral immune status, shift caecal microbiota composition toward a more favourable profile, and enhance laying performance in commercial Hy-Line Brown hens—three outcomes that are often claimed but rarely shown together in the same trial.

The supporting evidence is consistent across domains. Serum IgA rose by 44%, IgG by 34%, and Newcastle disease HI titres by 2.4 log₂ units over 12 weeks, all significantly exceeding controls. Caecal *Lactobacillus* and *Bifidobacterium* counts were elevated, while *E. coli* was suppressed. Hen-day egg production averaged 82.1% versus 72.4% in controls, and feed conversion improved by 8.2%, without any change in feed intake or mortality.

For the Australian layer industry—and for egg producers in other markets that have restricted antibiotic growth promoters—these data make a practical case for routine MOS supplementation at 0.2%. The cost of inclusion is marginal relative to the feed savings and the improved immune resilience that may reduce subclinical disease losses. MOS should not be viewed as a single-product solution to post-antibiotic poultry management, but rather as one pillar of an integrated gut health strategy that may also include probiotics, organic acids, and phytogetic additives.

Open questions remain. Does the 0.2% dose remain optimal throughout a full laying cycle, or should it be adjusted as hens age and their gut microbiota matures? Can MOS and probiotics act synergistically when co-administered—so-called synbiotic combinations—and if so, at what ratio? Would the immune benefits documented here translate into measurable protection against a natural field challenge with *E. coli* peritonitis or *Salmonella* Enteritidis, the two most economically important bacterial infections of commercial layers? These questions define the next frontier of prebiotic research in poultry and merit investigation in multi-flock, multi-season field trials.

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