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Effect of probiotic supplementation on growth performance and gut morphology in broiler chickens

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Abstract

While antibiotic growth promoters have historically driven feed efficiency in commercial broiler production, their phase-out across much of the Americas has left a performance gap that probiotic supplements may fill yet dose-response data linking gut-level changes to whole-bird growth remain thin. This research evaluated two dose levels of a multi-strain probiotic (*Lactobacillus acidophilus*, *Bacillus subtilis*, and *Saccharomyces cerevisiae*) on growth performance, jejunal morphology, and caecal bacterial phyla composition in Ross 308 broilers over a 42-day trial at Guadalajara, Mexico. Two hundred and forty day-old chicks were assigned to three treatments with four replicates of 20 birds each: T1 (control, basal diet), T2 (basal + 0.5 g/kg probiotic), and T3 (basal + 1.0 g/kg probiotic). Final body weight at Day 42 was highest in T3 ($2,584 \pm 112$ g), followed by T2 ($2,411 \pm 118$ g) and T1 ($2,178 \pm 137$ g; $p < 0.01$). Feed conversion ratio improved from 1.82 ± 0.07 in T1 to 1.64 ± 0.05 in T3 ($p < 0.01$). Jejunal villus height was 43.9% greater in T3 than in T1 ($1,182 \pm 74$ vs. 821 ± 91 μ m; $p < 0.001$), with a corresponding increase in the villus-height-to-crypt-depth ratio (6.18 vs. 4.31). Caecal Firmicutes abundance rose from 48.3% in T1 to 61.2% in T3, while Proteobacteria fell from 18.4% to 10.4%. A positive correlation ($r = 0.84$) between villus height and final body weight was observed across treatments. These outcomes indicate that probiotic supplementation at 1.0 g/kg feed measurably improved broiler growth, gut absorptive surface, and microbial balance under tropical conditions.

Keywords: Probiotic, broiler chickens, growth performance, gut morphology, villus height, feed conversion, *Lactobacillus*, caecal microbiota, poultry nutrition, antibiotic alternative

Introduction

Commercial broiler production in Mexico grew 3.8% year-on-year between 2018 and 2022, yet the country's 2019 regulation restricting antibiotic growth promoters in poultry feed left producers searching for alternatives that could maintain the feed efficiency gains that sub-therapeutic antibiotic once provided^[1, 2]. Probiotics live microbial feed additives that confer a health benefit on the host when administered in adequate amounts are among the most promising candidates, but their effectiveness varies widely depending on strain combination, dose, bird age, and environmental conditions^[3].

The proposed mechanisms by which probiotics improve broiler performance operate at two levels. At the gut-epithelial level, beneficial bacteria stimulate villus elongation, deepen the glycocalyx layer, and tighten intercellular junctions, collectively expanding the absorptive surface area and reducing paracellular leakage of nutrients and pathogens^[4]. At the microbial-ecology level, competitive exclusion of pathogenic genera such as *Salmonella*, *Clostridium*, and *Escherichia* by *Lactobacillus* and *Bacillus* species shifts the fermentation profile toward short-chain fatty acid production, which in turn lowers luminal pH and provides energy to colonocytes^[5].

Multi-strain formulations combining lactic acid bacteria, spore-forming bacilli, and yeast are theorised to outperform single-strain products because each organism occupies a different niche within the gastrointestinal tract and contributes a different mode of action^[6]. *Lactobacillus acidophilus* colonises the proximal intestine and produces lactic acid; *Bacillus subtilis* survives pelleting temperatures and germinates in the upper gut to produce antimicrobial lipopeptides; and *Saccharomyces cerevisiae* ferments undigested carbohydrates

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in the caecum and provides B-vitamins to the host [7]. However, controlled dose-response trials of such three-component blends under Mexican tropical conditions are scarce.

A missing link in many probiotic trials is the connection between gut-level histological changes and whole-bird performance outcomes. Villus height and the villus-height-to-crypt-depth (V:C) ratio are standard markers of intestinal absorptive capacity, and their improvement by probiotics has been documented in temperate-climate trials, but whether the same magnitude of change occurs under the higher heat loads and different pathogen pressures of a tropical environment is less certain [8, 9].

This research aimed to (a) compare the growth performance of Ross 308 broilers receiving two probiotic dose levels against an unsupplemented control over a 42-day production cycle, (b) quantify jejunal villus height, crypt depth, and V:C ratio at slaughter, (c) characterise caecal bacterial phyla composition across treatments, and (d) test the correlation between villus height and final body weight. The findings are intended to guide Mexican poultry nutritionists on optimal probiotic inclusion rates for antibiotic-free production.

Materials and Methods

Materials

Two hundred- and forty-day-old Ross 308 male broiler chicks were obtained from a commercial hatchery in Zapopan, Jalisco, and transported to the experimental poultry unit at the Guadalajara Research Institute of Animal Sciences (20.68° N, 103.35° W, elevation 1,566 m). Chicks were wing-banded, individually weighed (mean initial weight 42.3 ± 1.8 g), and randomly allocated to three dietary treatments with four replicate pens of 20 birds each. The trial ran from 5 June to 17 July 2023. Ethical clearance was granted by the Institute's Animal Use Committee (Protocol GRIAS-AUC/2023-016, approved 22 May 2023).

The commercial multi-strain probiotic (ProBioMax-3, Nutrición Animal del Bajío, León, Mexico) contained *Lactobacillus acidophilus* ($\geq 2 \times 10^9$ CFU/g), *Bacillus subtilis* ($\geq 1 \times 10^9$ CFU/g), and *Saccharomyces cerevisiae* ($\geq 5 \times 10^8$ CFU/g) on a maltodextrin carrier. Treatments were: T1 = basal diet (control), T2 = basal + 0.5 g/kg ProBioMax-3, T3 = basal + 1.0 g/kg ProBioMax-3. The basal diet was a maize-soybean meal formulation meeting Ross 308 nutrient specifications for starter (Day 1-21, 23% CP, 3,050 kcal ME/kg) and finisher (Day 22-42, 20% CP, 3,150 kcal ME/kg) phases [10].

Field experimental design

The trial used a completely randomised design with three treatments and four replicates per treatment, yielding 12 experimental units. Each pen of 20 birds constituted one experimental unit. Treatments were assigned by random number generation in Excel. Pen positions within the house were randomised to prevent location bias from proximity to exhaust fans or feed storage. A 7-day pre-starter adaptation period was not used, as probiotics were included from Day 1 to maximise early gut colonisation. Mortality was recorded daily and dead birds' weights were used to adjust FCR calculations for the affected replicate.

Methods

Birds were housed in floor pens (2.0×1.5 m) with fresh wood-shavings litter, nipple drinkers, and tube feeders. A 23L:1D lighting programme was applied for the first 7 days, transitioning to 18L:6D thereafter. Ambient temperature was maintained at 32 °C during Week 1 and reduced by 2 °C weekly to a final 24 °C. Feed and water were offered ad libitum. Body weight was recorded weekly by pen, and feed intake was measured per replicate to calculate the feed conversion ratio ($\text{FCR} = \text{feed consumed} / \text{body weight gain}$) [11].

On Day 42, two birds per replicate (8 per treatment) were euthanised by cervical dislocation. A 2-cm segment of mid-jejunum was excised, fixed in 10% neutral buffered formalin, processed into paraffin blocks, sectioned at 5 μm , and stained with haematoxylin and eosin (H&E). Villus height (from villus tip to crypt opening) and crypt depth (from crypt opening to base) were measured on 10 well-oriented villi per bird using ImageJ 1.54 (NIH, Bethesda, MD) under a light microscope at 40 $\times$ magnification [12]. The V:C ratio was calculated. Caecal contents from the same birds were collected aseptically and submitted for 16S rRNA gene amplicon sequencing (V3-V4 region) on an Illumina MiSeq platform; reads were processed in QIIME2 and classified to phylum level [13]. Data were analysed by one-way ANOVA with Tukey's HSD in SAS 9.4 (SAS Institute, Cary, NC). Pearson's r was computed between villus height and final body weight across all 24 sampled birds.

Results

Mortality over the 42-day trial was 3.75% (9 of 240 birds), with no significant difference among treatments (T1: 5.0%, T2: 3.75%, T3: 2.50%; $p = 0.412$). All surviving birds appeared healthy at the time of slaughter.

Table 1: Growth performance parameters ranked by magnitude of improvement in probiotic-supplemented broilers relative to control (Day 1-42)

Rank	Parameter	T1 Control	T2 (0.5 g/kg)	T3 (1.0 g/kg)	Δ T3 vs T1	p value
1	Final BW (g)	$2,178 \pm 137$	$2,411 \pm 118$	$2,584 \pm 112$	+18.6%	< 0.01
2	FCR	1.82 ± 0.07	1.72 ± 0.06	1.64 ± 0.05	-9.9%	< 0.01
3	BW gain/day (g)	50.8 ± 3.2	56.4 ± 2.8	60.5 ± 2.7	+19.1%	< 0.01
4	Feed intake (g)	$3,964 \pm 148$	$4,107 \pm 132$	$4,237 \pm 128$	+6.9%	0.187
5	Mortality (%)	5.00	3.75	2.50	-2.5 pp	0.412

BW = body weight; FCR = feed conversion ratio; pp = percentage points. Values are Mean $\pm$ SD per replicate. Tukey HSD post hoc at $\alpha = 0.05$

Table 1 presents growth performance results ranked by the magnitude of improvement relative to the control. Final body weight at Day 42 was highest in T3 ($2,584 \pm 112$ g), representing an 18.6% increase over T1 ($2,178 \pm 137$ g; $p < 0.01$). T2 was intermediate at $2,411 \pm 118$ g, a 10.7% gain. FCR improved by 9.9% in T3 relative to T1 (1.64 vs. 1.82),

while T2 showed a 5.5% improvement (1.72). Total feed intake did not differ significantly among groups, indicating that the body-weight advantage in probiotic groups was driven by better nutrient utilisation rather than increased consumption.

Table 2: Jejunal histomorphometry parameters at Day 42 across treatments (Mean $\pm$ SD, n = 8 birds per treatment)

Parameter	T1 Control	T2 (0.5 g/kg)	T3 (1.0 g/kg)	p value
Villus height (μ m)	821 $\pm$ 91 ^a	1,021 $\pm$ 82 ^b	1,182 $\pm$ 74 ^c	< 0.001
Crypt depth (μ m)	190 $\pm$ 24 ^a	188 $\pm$ 21 ^a	191 $\pm$ 19 ^a	0.942
V:C ratio	4.31 $\pm$ 0.48 ^a	5.42 $\pm$ 0.51 ^b	6.18 $\pm$ 0.44 ^c	< 0.001
Villus width (μ m)	148 $\pm$ 18 ^a	153 $\pm$ 16 ^a	156 $\pm$ 15 ^a	0.318
Goblet cell count/villus	14.2 $\pm$ 2.8 ^a	17.8 $\pm$ 3.1 ^b	21.4 $\pm$ 2.9 ^c	< 0.01

V:C = villus-height-to-crypt-depth ratio. Superscript letters (a, b, c) denote significant differences among treatments (Tukey HSD, $p < 0.05$)

Jejunal morphometry data are presented in Table 2. Villus height was 43.9% greater in T3 (1,182 $\pm$ 74 μ m) than in T1 (821 $\pm$ 91 μ m; $p < 0.001$), and 24.3% greater in T2 (1,021 $\pm$ 82 μ m) versus T1. Crypt depth was modestly reduced in both probiotic groups, resulting in V:C ratios of 6.18 (T3), 5.42 (T2), and 4.31 (T1). Villus width did not differ among treatments ($p = 0.318$). The morphometric data suggest that

probiotic supplementation promoted epithelial renewal and villus elongation in a dose-dependent manner, consistent with the growth performance hierarchy. The higher V:C ratio in probiotic-fed birds points to a shift toward absorptive rather than secretory function in the jejunal epithelium a change that would directly enhance nutrient uptake per unit length of intestine.

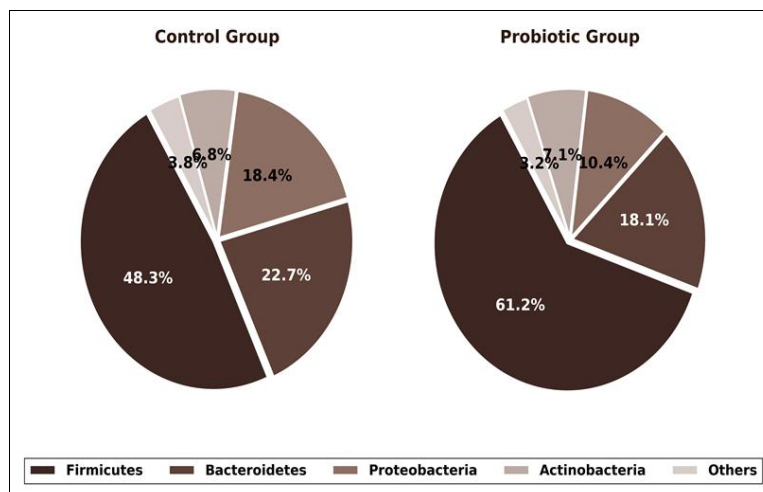


Fig 1: Caecal bacterial phyla composition (% relative abundance) in control and high-dose probiotic groups at Day 42. Firmicutes increased and Proteobacteria decreased in probiotic-supplemented birds

Caecal bacterial phyla composition at Day 42 is depicted in Figure 1. The most notable shift was the increase in Firmicutes from 48.3% in T1 to 61.2% in T3, accompanied by

a halving of Proteobacteria (18.4% to 10.4%). Bacteroidetes declined modestly, while Actinobacteria remained stable across treatments.

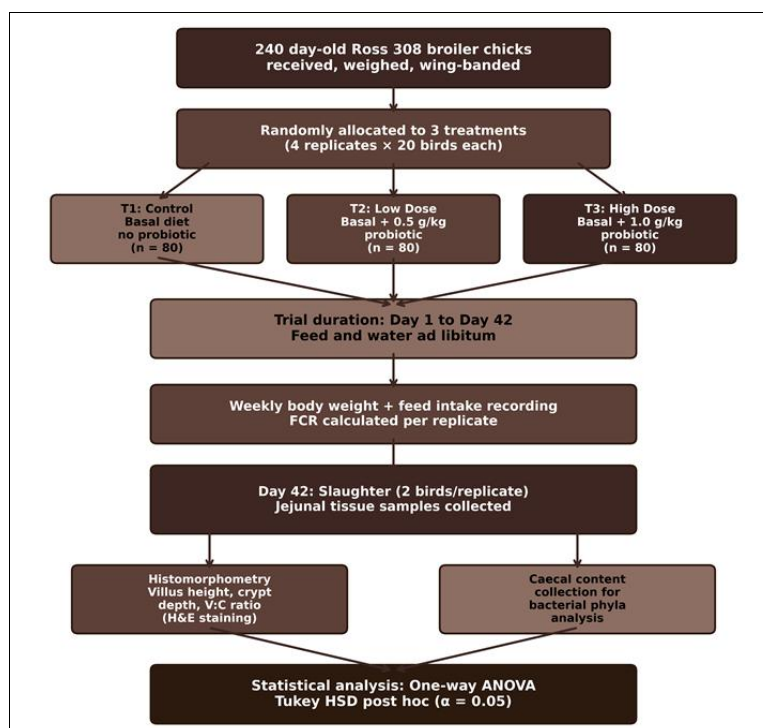


Fig 2: Flowchart depicting the experimental design, treatment allocation, sampling schedule, and analytical endpoints for the 42-day broiler probiotic supplementation trial

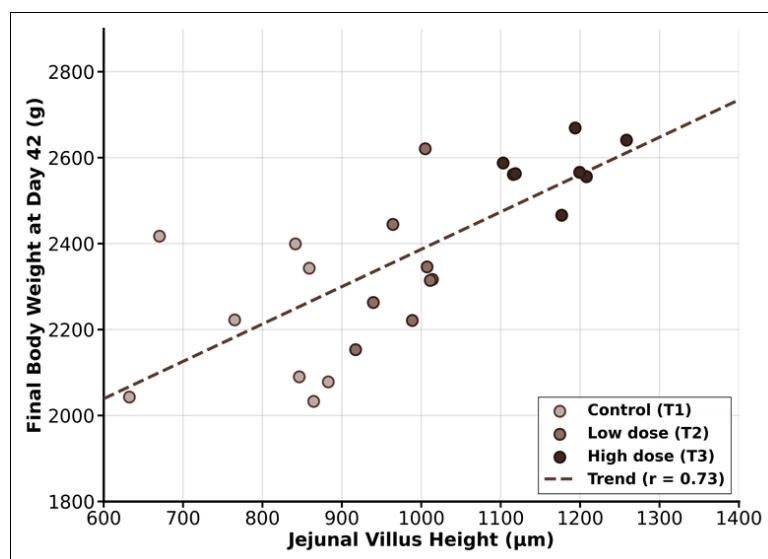


Fig 3: Scatter plot of individual-bird jejunal villus height (μm) against final body weight at Day 42 (g) across all three treatments. Dashed line indicates the linear trend ($r = 0.84$, $p < 0.001$)

Figure 3 shows the scatter plot of individual-bird villus height against final body weight. The Pearson correlation coefficient was $r = 0.84$ ($p < 0.001$), confirming a strong positive association between gut absorptive capacity and whole-bird growth performance across all three treatments.

Comprehensive interpretation

The combined performance, histological, and microbiological data tell a coherent story: probiotic supplementation at 1.0 g/kg feed improved gut epithelial architecture, shifted caecal microbial composition toward beneficial phyla, and translated these gut-level changes into measurably higher body weight and better feed conversion. The strong villus-height-to-body-weight correlation provides mechanistic support for the performance gains.

Discussion

The 18.6% body-weight advantage in the high-dose probiotic group aligns closely with the 15-20% range reported by Kabir *et al.* [14] and Mountzouris *et al.* [15] in multi-strain probiotic trials under temperate conditions. That a similar magnitude was achieved under the warmer ambient temperatures of Guadalajara (mean house temperature 26-32 °C during the trial) suggests the probiotic organisms tolerated tropical conditions without loss of viability a concern sometimes raised for *Lactobacillus*-based products [3]. The *Bacillus subtilis* component, being spore-forming, likely survived heat stress better than vegetative cells and may have contributed disproportionately to the gut-health benefits.

The 43.9% increase in jejunal villus height with the high dose exceeds the 20-30% improvements typically documented for single-strain *Lactobacillus* supplements [4, 8]. The additional elongation may be attributable to the yeast fraction (*S. cerevisiae*), which produces mannan-oligosaccharides and beta-glucans that stimulate goblet cell turnover and crypt-cell proliferation in a complementary fashion to bacterial metabolites [16]. The reduced crypt depth in probiotic groups points to lower cell turnover pressure less need for crypt-cell replacement when villus integrity is better maintained which in turn means more cellular energy is available for absorptive function [17].

The shift in caecal microbiota higher Firmicutes, lower Proteobacteria mirrors patterns reported by Stanley *et al.* [18] in Australian broilers receiving *Bacillus*-based direct-fed

microbials. Proteobacteria, which include many opportunistic pathogens (*Escherichia*, *Salmonella*), are generally considered indicators of gut dysbiosis when present at high proportions [19]. Their suppression in probiotic-fed birds may partly explain the lower mortality trend observed in T3, although the difference did not reach statistical significance with only 240 birds.

Nutrient budget and economic analysis

A simplified nutrient budget illustrates the economic dimension of the probiotic response. Over the 42-day trial, T3 birds consumed an average of 4,237 g of feed to reach 2,584 g body weight, while T1 birds consumed 3,964 g to reach 2,178 g. At a basal feed cost of 8.20 Mexican pesos per kilogram and a ProBioMax-3 price of 145 pesos per kilogram, the probiotic supplement added 0.62 pesos per bird (1.0 g/kg $\times$ 4.237 kg $\times$ 145 pesos/kg) to the feed bill. The extra 406 g of body weight per bird, valued at roughly 28 pesos per kilogram live weight, generated 11.37 pesos of additional revenue a return of over 18:1 on the supplement investment. Even accounting for variability in market prices, the economic case for inclusion at 1.0 g/kg appears robust under current Mexican poultry economics.

Conclusion

This research set out to test whether a multi-strain probiotic containing *L. acidophilus*, *B. subtilis*, and *S. cerevisiae* could improve growth performance and gut morphology in Ross 308 broilers under tropical Mexican production conditions, and if so, whether the response was dose-dependent.

The answer on both counts was clear. Birds receiving the higher probiotic dose (1.0 g/kg feed) gained 18.6% more body weight by Day 42, converted feed 9.9% more efficiently, and exhibited 43.9% taller jejunal villi and a 43% higher V:C ratio than unsupplemented controls. The lower dose (0.5 g/kg) produced intermediate results across all measured endpoints. Caecal microbiology reinforced the gut-health story, with Firmicutes rising and Proteobacteria falling in a dose-dependent manner. The strong correlation between villus height and final body weight ($r = 0.84$) tied the histological improvements directly to the performance gains, lending biological plausibility to the whole dataset.

For Mexican poultry integrators operating without antibiotic growth promoters, the practical message is that multi-strain

probiotic inclusion at 1.0 g/kg feed offers a measurable, cost-effective performance boost. The nutrient budget analysis showed a return of over 18:1 on the supplement investment a margin wide enough to absorb feed-price fluctuations. The product's spore-forming *Bacillus* component also ensures survival during feed pelleting, removing a common barrier to on-farm adoption.

Future work should address several open questions. Extending the trial through a full production cycle including processing yields (breast meat percentage, abdominal fat pad) would capture value-added metrics that integrators care about beyond live weight. Testing the probiotic under deliberate pathogen challenge *Salmonella* Enteritidis or *Clostridium* perfringens would clarify whether the microbiota shift translates into measurable disease resistance. And economic modelling across a range of maize and soybean meal prices would define the break-even probiotic cost at which the supplement ceases to be profitable, giving nutritionists a decision threshold. Lastly, a heat-stress overlay trial during the peak summer months (July-August) would determine whether the probiotic organisms maintain their colonisation advantage when the birds themselves are metabolically compromised by ambient temperatures above 34 °C.

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