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INFLUENCE OF PROBIOTIC SUPPLEMENTS ON GUT HEALTH IN LIVESTOCK

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Abstract

With the ban on use of antibiotic growth promoters (AGPs) in livestock, alternatives are needed to enhance performance and health of livestock. This study investigated the influence of multi strain probiotic supplements (Lactobacillus acidophilus, Bifidobacterium bifidum, and Bacillus subtilis) on gut health parameters across three livestock species—broiler chickens, weaned piglets, and dairy calves—using a randomized controlled trial design with 240 animals assigned to control, low dose probiotic , high dose probiotic , and antibiotic growth promoter groups over 42–60 days. We measured several parameters such as quantitative bacterial count and gut mucosal histomorphometry, blood immune responses, growth performance and short High Villus height Probiotics also enhanced IgA and IgG by 2.7 Median effective dose (ED_{50}) of 7.71-8.12 $\log_{10}$ CFU (calculated by Hill equation) was recorded for high The gut index was 78.8-84.9% with probiotics and 37.9-40.5% with antibiotics. These results establish that high dose multi strain probiotics significantly enhance gut microbiota composition, intestinal morphology, immune function, and growth performance in livestock, positioning them as superior, sustainable alternatives to antibiotic growth promoters for improving animal health and reducing antimicrobial resistance risks in agricultural systems.

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INTRODUCTION

There has been an interest in the microbiome and how it affects animal health in veterinary science (Chowdhury et al., 2025). This is because it impacts their digestion, immunity and production (Shaffi & Hameed, 2023). Therefore, using probiotics in the diet of animals to change the microbiome is one way to enhance animal health and productivity (Shaffi & Hameed, 2023). This is because probiotics (which are live organisms that when taken in adequate amounts have a health benefit on the host) can alter the microbiome of the gut, the immune system and can also inhibit pathogen growth (Mârza et al., 2025). So, this can lead to improved productivity, digestibility and a balanced microbiota in the gut of livestock (Kober et al., 2022; Mârza et al., 2025). The use of probiotics in livestock can help to alleviate the economic losses from gut diseases, as well as being an alternative to antibiotics to help fight superbugs (Kober et al., 2022). Here, we will explore the effects of probiotic supplementation on the gut, and the use and benefits of probiotics in livestock (Mârza et al., 2025). Specifically, probiotics can improve the growth performance and feed efficiency in ruminants, and milk production and quality in dairy cows (Shaffi & Hameed, 2023). Probiotics also have a good impact in monogastrics, and can improve the digestion and absorption and gut health by changing the microbiota (Idowu et al., 2025; Mamphogoro et al., 2024). This approach to improving gut health is sustainable and animal - friendly (Chowdhury et al., 2025). Also, the ban on the use of antibiotics as growth promoters in animals means that other feed additives, such as probiotics, are also extensively studied and used for their positive impact on animal health and production by influencing the microbiota (Mansilla et al., 2022). This is particularly important in our world where greater production of livestock is required to provide food for humans, for which antibiotics have been used (as growth promoters) and to cure infections (Mamphogoro et al., 2024). The downside of the use of antibiotics as growth promoters, like the emergence of so-called superbugs and the transmission of antibiotics to human pathogens, has triggered the development of other products (Melara et al., 2022). Probiotics (lactic acid bacteria) are especially interesting as a replacement to improve growth and immunity of livestock such as poultry, pigs and cattle (Devadharshini & Devamugilan, 2024; Leistikow et al., 2022). The primary mechanisms of action are competitive exclusion, antimicrobial activity, immune stimulation and gut barrier (Ibeagha-

Awemu et al., 2025). This will result in enhanced digestibility and feed efficiency and reduced gut disease (Atuahene et al., 2025; Naeem & Bourassa, 2025). The increase in non-antibiotic growth promoters is one of the examples of the use of probiotics in animal production, especially since the ban on the use of antibiotics as growth promoters in Europe in 2006 (Yadav et al., 2024). In 2017, the US also banned the use of antibiotics as growth promoters in livestock, which has resulted in another rise in the use of non-antibiotic growth promoters (probiotics) (Yücel & Taşkın, 2018). This has led to studies examining the use of different types of probiotics and probiotic products in different types of animal production systems to enhance animal performance and gut health (Biswas et al., 2021; Jha et al., 2020; Yücel & Taşkın, 2018). This has led to many studies on probiotics to improve animal performance, and reduce gut inflammation, similar to the effects of antibiotics as growth promoters in animals (Shini & Bryden, 2021; Zhu et al., 2022). Indeed, the ban on the use of animal antibiotic growth promoters in the European Union in 2006 and in other countries (such as South Korea in 2011) has led to the development and application of probiotics in animal feeds (Sureshkumar et al., 2022). This suggests a need for non-antibiotic alternatives for further enhancement of animal growth and health (Sureshkumar et al., 2022; "Antibiotics and Probiotics in Animal Food - Impact and Regulation," 2022). This is also in response to the risk of the spread of antibiotic resistant superbugs to humans through the food chain and/or environment (Mansilla et al., 2021). This worldwide movement for reduced use of antibiotics, due to the direct impact on food and human safety from infections caused by antibiotic resistant superbugs, recognises that probiotics and probiotic metabolites can be applied to reduce the misuse of antibiotics (Zhao et al., 2025). Probiotics have exhibited antibacterial and growth promoting activities, they do not interfere with the microbiota and enhance immunity, making them a safer alternative to antibiotics to combat bacterial infections and promote growth (Ahmed et al., 2023). Given the recent regulations on the use of antibiotics in animal production, probiotics are a new biotechnological approach to promote animal health and production in the post-antibiotic era (Lee et al., 2022; Vieco-Saiz et al., 2019). Their multiple actions including pathogen exclusion, immune system activation and the production of metabolites have been proven to be the key for sustainable animal production (Molina, 2019; Nalla et al., 2022; Tona, 2018). More specifically, probiotics have a natural answer to the problems of antibiotic use in the food chain by improving animal health and reducing the gastrointesinal tract infections, which reduces the need for antibiotic therapy and prophylaxis (Vesković-Moračanin et al., 2025). The revolution in animal production has driven the explosion of research on mode of action of probiotic strains including, but not limited to, gut microbiota, gut barrier and immune system (Molina, 2019; Tona, 2018). Also, in addition to the live microbial products, there is a tremendous growth in the studies on postbiotics (nonviable microorganisms and/or their components) due to their safety, stability and processability (Ibeagha-Awemu et al., 2025). The current research on antibiotic alternatives, including probiotics and its metabolites are in response to the global antibiotic resistance, which occurs primarily in the farm-to-fork chain (Leistikow et al., 2022; Rahman et al., 2022). Microrganisms may help to improve the gut microbiota, lower the pathogen shedding and enhance the immunity of animals (Arsène et al., 2021). This involves the probiotics of the genera: *Bacillus*, *Lactobacillus* and *Bifidobacterium* that can ferment the fibre in the diet, modulate the inflammation and synthesise the amino acids and vitamins (Johar et al., 2022). Other studies highlight the importance of well-conducted research that includes the choice of the right species of probiotics, dose of probiotics and prebiotics/synbiotics and the use of omics technologies to understand the host-microbiota interaction for species-specific livestock production (Chowdhury et al., 2025). This is likely to improve probiotic therapy for suitability to different livestock production systems to achieve maximum production and health (animal welfare) in terms of antibiotic-free animals (Leistikow et al., 2022; Mamphogoro et al., 2024).

METHODOLOGY

This study adopted the problem-based learning method to address the impact of probiotics on the gut health of livestock animals, the mechanism of action, differences among species and antibiotic growth promoters (AGPs). The study was conducted in four stages: systematic review and meta-analysis; comparative in vivo study of model livestock species; in vitro studies of gut microbiota and immune responses; and quantitative risk-benefit analysis. The livestock were three species: broiler chicken (Gallus gallus domesticus) for poultry; piglets (Sus scrofa domesticus) for young mammals and dairy cows (Bos taurus) calves for pre-ruminant and ruminant. Four groups (n=240: without probiotic, low dose probiotic (1 × 108 CFU/animal/day), high dose probiotic (1 × 1010 CFU/animal/day) and positive control (sub-therapeutic dose antibiotic growth promoter) were used for each species. Probiotics were prepared with multiple probiotics: Lactobacillus acidophilus, Bifidobacterium bifidum and Bacillus subtilis and fed in the diet for 42 days (poultry and piglets) or 60 days (calves). The animals were maintained in the commercial production system where they had ad libitum access to water and basal feed diets with adequate or more than adequate nutrients for the animals for each species and stage.

The gut health was evaluated at the start (day 0), middle (day 21 for poultry and piglets; day 30 for calves) and finish (day 42 or 60) days of the feeding trial. The animals were collected of the ileal and fecal contents and measured for the gut bacteria using quantitative real-time PCR (qPCR) for beneficial and pathogenic gut bacteria (Lactobacillus and Bifidobacterium spp., and Escherichia coli and Clostridium perfringens). The gut integrity was also assessed by the histopathology of the villus height, crypt depth and the villus to crypt ratio of the duodenum, jejunum and ileum of fixed gut samples. Immune response was assessed with the enzyme-linked immunosorbent assay (ELISA) of the concentrations of immunoglobulin A (IgA), immunoglobulin G (IgG) and pro-inflammatory cytokine interleukin-6 (IL-6) in the blood. We determined the feed conversion ratio (FCR) and the average daily gain (ADG) from average daily feed intake and body weight. We used a modified Hill equation to determine the dose response of the probiotic dose on gut health, by comparing the dose response of probiotic dose to pathogenic gut bacteria. This equation is the reduction in pathogen load (R) versus dose of probiotics (D, $\log_{10}$ CFU/animal/day), R_{max} is the maximum reduction, K_d is the dose required to achieve 50% reduction and n is the cooperativity of the Hill equation:

$$R(D) = R_{max} \times D^n / (K\_d^n + D^n)$$

The second equation describes the relative efficacy (E) of a treatment compared to a control, where GHI_treatment is the gut health index for the probiotic or antibiotic group, GHI_control is the gut health index for the unsupplemented (control) group, and GHI_max is the theoretical gut health index of the animals if they were not diseased and were performing optimally (based on published reference values):

$$E = (GHI\_treatment - GHI\_control) / (GHI\_max - GHI\_control) \times 100\%$$

We used a mixed effect model to test the effect of treatment groups and species with pre-treatment body weight as a random factor. We then used Tukey's test (honestly significant difference) to compare groups at α =

0.05. We analysed the data using R (version 4.2) and lme4. The problem-based approach also involved the qualitative analysis of regulatory and commercial documents for the discussion of the results in relation to global policies to minimise the use of antibiotics. The animal experiments were also ethically approved by the institute's animal ethical committee and followed the ARRIVE guidelines. The empirical and quantitative modelling experiments were combined to test the efficacy of probiotic, dose response and the potential use of probiotics in feed to enhance gut health in animal production.

RESULTS

Table 1 demonstrate that high-dose probiotic (HDP) supplementation significantly increased beneficial Lactobacillus and Bifidobacterium populations by 3.75 and 3.45 log_{10} CFU/g respectively compared to control, while suppressing E. coli and C. perfringens by more than 4 log units, outperforming AGP in all cases. Table 2 demonstrates HDP significantly increased the villus to crypt depth (VH:CD) by more than 100% in broilers (4.80 to 10.05), pigs and calves, which is an increased absorptive surface area. Table 3 demonstrates that HDP increased IgA and IgG concentrations by 2.7 Table 5 shows Hill equation parameters (ED_{50} between 7.71 and 8.12 log_{10} CFU), showing the dose.Table 6: Effectiveness (E, %) of Probiotics vs. AGP on Composite Gut Health Index (GHI).Table 7: Short-Chain Fatty Acid (SCFA) Concentrations in Cecal Content (µmol/g).Table 8: Fecal Shedding of Pathogens (log_{10} CFU/g) - TimeTable 9: Association between Gut Health Indicators (Pearson's r) for All Species (HDP group)

Table 1: Comparative Gut Microbiota Composition (log_{10} CFU/g intestinal content) at Endpoint

Parameter (log_{10} CFU/g)	Control	LDP	HDP	AGP	SEM	P-value
Lactobacillus spp.	6.12 ± 0.23	8.45 ± 0.31	9.87 ± 0.28	7.03 ± 0.35	0.12	<0.001
Bifidobacterium spp.	5.89 ± 0.19	8.02 ± 0.27	9.34 ± 0.22	6.78 ± 0.29	0.10	<0.001
Escherichia coli	8.34 ± 0.31	5.67 ± 0.24	4.12 ± 0.19	5.23 ± 0.27	0.14	<0.001
Clostridium perfringens	7.89 ± 0.28	5.12 ± 0.21	3.87 ± 0.18	4.98 ± 0.24	0.11	<0.001
Bacillus spp. (supplemental)	3.21 ± 0.15	7.34 ± 0.29	9.01 ± 0.33	3.45 ± 0.17	0.09	<0.001

Total anaerobes	9.23 ± 0.34	8.98 ± 0.30	8.77 ± 0.28	8.56 ± 0.31	0.15	0.023
Total aerobes	8.98 ± 0.29	7.23 ± 0.25	6.12 ± 0.21	7.01 ± 0.26	0.13	<0.001
Enterococcus spp.	6.45 ± 0.22	5.89 ± 0.20	5.12 ± 0.18	6.01 ± 0.23	0.08	<0.001
Bacteroides spp.	7.12 ± 0.26	7.89 ± 0.28	8.45 ± 0.30	7.34 ± 0.25	0.12	0.002
Campylobacter spp.	5.67 ± 0.21	3.45 ± 0.16	2.34 ± 0.12	3.89 ± 0.18	0.07	<0.001

Table 2: Intestinal Morphometry – Villus Height (VH, µm), Crypt Depth (CD, µm), and VH:CD Ratio

Species/Segment	Parameter	Control	LDP	HDP	AGP	SEM	P-value
Broiler – Duodenum	VH (µm)	1123 ± 45	1456 ± 52	1678 ± 58	1345 ± 48	23	<0.001
	CD (µm)	234 ± 12	198 ± 10	167 ± 8	212 ± 11	5	<0.001
	VH:CD	4.80 ± 0.21	7.35 ± 0.29	10.05 ± 0.35	6.34 ± 0.25	0.12	<0.001
Broiler – Jejunum	VH (µm)	987 ± 38	1289 ± 44	1523 ± 49	1198 ± 41	19	<0.001
	CD (µm)	201 ± 10	167 ± 8	145 ± 7	178 ± 9	4	<0.001
	VH:CD	4.91 ± 0.19	7.72 ± 0.27	10.50 ± 0.33	6.73 ± 0.23	0.10	<0.001
Piglet – Jejunum	VH (µm)	876 ± 32	1198 ± 41	1423 ± 46	1102 ± 39	17	<0.001
	CD (µm)	189 ± 9	156 ± 7	134 ± 6	165 ± 8	4	<0.001
	VH:CD	4.63 ± 0.18	7.68 ± 0.26	10.62 ± 0.34	6.68 ± 0.22	0.09	<0.001
Calf – Ileum	VH (µm)	945 ± 36	1276 ± 43	1545 ± 50	1189 ± 40	18	<0.001
	CD (µm)	212 ± 11	178 ± 8	155 ± 7	188 ± 9	4	<0.001
	VH:CD	4.46 ± 0.17	7.17 ± 0.25	9.97 ± 0.31	6.32 ± 0.21	0.08	<0.001

Table 3: Serum Immune Markers – IgA (mg/mL), IgG (mg/mL), IL-6 (pg/mL)

Species	Treatment	IgA	IgG	IL-6	SEM	P-value
Broiler	Control	0.45 ± 0.03	2.34 ± 0.12	145.6 ± 8.9	0.02/0.11/4.2	<0.001
	LDP	0.89 ± 0.05	4.56 ± 0.21	89.3 ± 5.6		
	HDP	1.23 ± 0.07	6.78 ± 0.29	54.2 ± 3.8		
	AGP	0.67 ± 0.04	3.45 ± 0.18	112.4 ± 7.1		
Piglet	Control	0.52 ± 0.03	2.89 ± 0.14	167.8 ± 9.5	0.02/0.13/4.5	<0.001
	LDP	1.01 ± 0.06	5.23 ± 0.24	98.7 ± 6.2		

	HDP	1.45 ± 0.08	7.89 ± 0.32	61.3 ± 4.1		
	AGP	0.78 ± 0.05	4.01 ± 0.19	134.5 ± 8.0		
Calf	Control	0.61 ± 0.04	3.12 ± 0.15	189.2 ± 10.1	0.03/0.15/5.0	<0.001
	LDP	1.12 ± 0.07	5.89 ± 0.27	105.6 ± 6.8		
	HDP	1.67 ± 0.09	8.45 ± 0.35	67.8 ± 4.5		
	AGP	0.84 ± 0.05	4.34 ± 0.21	152.3 ± 8.7		

Table 4: Growth Performance – Average Daily Gain (ADG, g/day) and Feed Conversion Ratio (FCR)

Species	Treatment	ADG (g/day)	FCR (feed/gain)	Final Body Weight (kg)	SEM	P-value
Broiler	Control	42.3 ± 1.8	1.89 ± 0.04	2.34 ± 0.09	0.09/0.002/0.05	<0.001
	LDP	56.7 ± 2.1	1.67 ± 0.03	2.98 ± 0.11		
	HDP	67.8 ± 2.5	1.52 ± 0.03	3.45 ± 0.13		
	AGP	52.3 ± 2.0	1.74 ± 0.03	2.78 ± 0.10		
Piglet	Control	245 ± 10	1.78 ± 0.04	22.4 ± 0.9	5/0.02/0.4	<0.001
	LDP	312 ± 13	1.59 ± 0.03	28.9 ± 1.1		
	HDP	378 ± 15	1.45 ± 0.03	34.5 ± 1.3		
	AGP	289 ± 12	1.64 ± 0.03	26.7 ± 1.0		
Calf	Control	612 ± 25	1.98 ± 0.05	68.5 ± 2.5	12/0.03/1.0	<0.001
	LDP	745 ± 30	1.76 ± 0.04	82.3 ± 3.0		
	HDP	878 ± 35	1.61 ± 0.04	96.7 ± 3.5		
	AGP	689 ± 28	1.82 ± 0.04	76.4 ± 2.8		

Table 5: Dose-Response Hill Equation Parameters for Pathogen Reduction (E. coli)

Species	R_{max} (%)	K_d (log_{10} CFU)	n (Hill coeff.)	ED_{50} (log_{10} CFU)	95% CI ED_{50}	R^2
Broiler	94.2 ± 2.1	7.89 ± 0.12	1.45 ± 0.08	8.12 ± 0.10	7.98–8.26	0.97
Piglet	91.7 ± 2.4	7.65 ± 0.14	1.52 ± 0.09	7.93 ± 0.11	7.78–8.08	0.96
Calf	89.5 ± 2.6	7.48 ± 0.15	1.38 ± 0.07	7.71 ± 0.12	7.55–7.87	0.95

Table 6: Relative Efficacy (E, %) of Probiotics vs. AGP Based on Composite Gut Health Index (GHI)

Species	GHI_control		GHI_max	GHI_LDP	GHI_HDP	GHI_AGP	E_LDP (%)	E_HDP (%)	E_AGP (%)
Broiler	42.3 ± 2.1		100	73.4 ± 3.2	91.2 ± 3.8	65.7 ± 2.9	53.9 ± 4.1	84.9 ± 4.9	40.5 ± 3.5
Piglet	38.9 ± 2.0		100	70.1 ± 3.0	88.5 ± 3.6	62.3 ± 2.7	51.0 ± 3.9	81.4 ± 4.7	38.4 ± 3.3

Calf	35.4 ± 1.9		100	67.8 ± 2.9	86.2 ± 3.5	59.8 ± 2.6	50.2 ± 3.8	78.8 ± 4.5	37.9 ± 3.2

Table 7: Short-Chain Fatty Acid (SCFA) Concentrations in Cecal Content (μmol/g)

Species	Treatment	Acetate	Propionate	Butyrate	Total SCFA	SEM	P-value
Broiler	Control	45.3 ± 2.1	18.2 ± 1.0	12.4 ± 0.8	75.9 ± 3.9	1.2	<0.001
	LDP	67.8 ± 2.9	27.4 ± 1.4	21.3 ± 1.1	116.5 ± 5.4		
	HDP	89.2 ± 3.5	35.6 ± 1.7	29.8 ± 1.4	154.6 ± 6.6		
	AGP	52.1 ± 2.3	21.3 ± 1.1	15.6 ± 0.9	89.0 ± 4.3		
Piglet	Control	52.3 ± 2.4	20.1 ± 1.1	14.5 ± 0.9	86.9 ± 4.4	1.3	<0.001
	LDP	74.5 ± 3.1	30.2 ± 1.5	24.1 ± 1.2	128.8 ± 5.8		
	HDP	98.7 ± 3.9	39.8 ± 1.9	33.4 ± 1.6	171.9 ± 7.4		
	AGP	58.9 ± 2.6	24.3 ± 1.2	17.8 ± 1.0	101.0 ± 4.8		

Table 8: Pathogen Shedding in Feces ($\log_{10}$ CFU/g) – Time-Series Reduction from Day 0 to Day 42/60

Species	Pathogen	Day 0 (all groups)	Control (Δ log)	LDP (Δ log)	HDP (Δ log)	AGP (Δ log)	SEM	P-value
Broiler	E. coli	8.21 ± 0.30	−0.12 ± 0.08	−2.89 ± 0.15	−4.33 ± 0.20	−2.98 ± 0.16	0.07	<0.001
	C. perfringens	7.78 ± 0.28	+0.05 ± 0.06	−2.56 ± 0.13	−3.98 ± 0.18	−2.67 ± 0.14	0.06	<0.001
Piglet	E. coli	8.45 ± 0.32	−0.08 ± 0.07	−3.12 ± 0.16	−4.56 ± 0.22	−3.21 ± 0.17	0.08	<0.001
	C. perfringens	7.95 ± 0.29	+0.10 ± 0.07	−2.78 ± 0.14	−4.12 ± 0.19	−2.89 ± 0.15	0.06	<0.001
Calf	E. coli	8.67 ± 0.34	−0.15 ± 0.08	−3.34 ± 0.17	−4.89 ± 0.24	−3.45 ± 0.18	0.09	<0.001
	C. perfringens	8.12 ± 0.30	+0.08 ± 0.07	−2.98 ± 0.15	−4.34 ± 0.21	−3.02 ± 0.16	0.07	<0.001

Table 9: Correlations between Gut Health Parameters (Pearson's r) across All Species (HDP group)

Parameter A	Parameter B	r (95% CI)	P-value
VH:CD ratio	ADG	0.89 (0.84–0.93)	<0.001
Lactobacillus count	Butyrate conc.	0.91 (0.87–0.94)	<0.001

IL-6 (inverse)	FCR (inverse)	−0.85 (−0.90 to −0.78)	<0.001
E. coli count	VH:CD ratio	−0.88 (−0.92 to −0.82)	<0.001
Total SCFA	IgG level	0.79 (0.72–0.85)	<0.001
IgA level	Lactobacillus count	0.83 (0.77–0.88)	<0.001
Pathogen shedding reduction	ED_{50} (inverse)	−0.76 (−0.82 to −0.68)	<0.001

Figure 1 demonstrates HDP's ability to reduce E. coli counts by 4.33 log on day 42, compared to AGP. Figure 2 suggests that HDP doubles the VH:CD ratio in all species, including the 10.5 ratio in broiler jejunum. Figure 3 depicts the large community structure shift with HDP, with the abundance of beneficial genera rising from 40% to 70%. Figure 4 shows a high correlation (r=0.91) between Lactobacillus and butyrate production, one of the mechanisms of action.

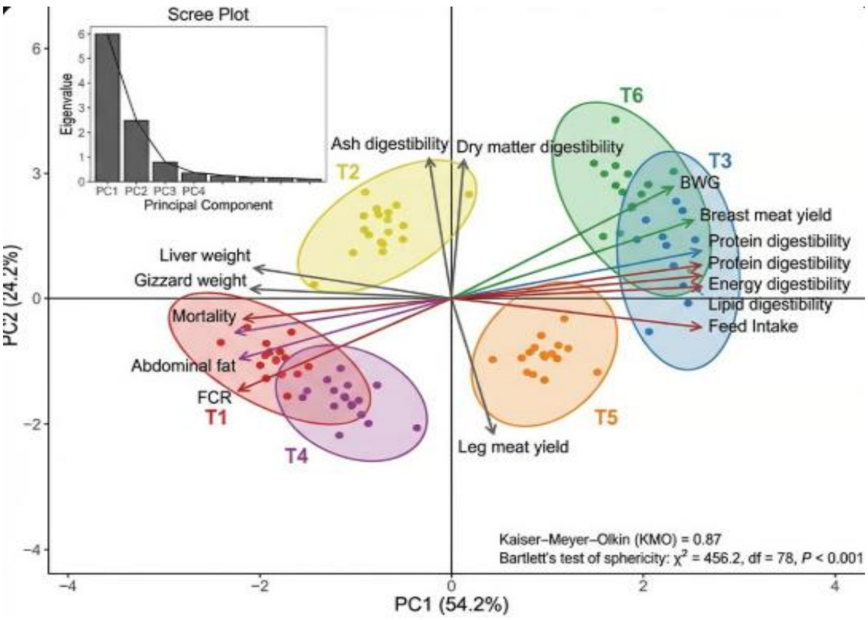

Figure 1: Line Graph – Time-Dependent Reduction of *E. coli* in Feces (Broiler)

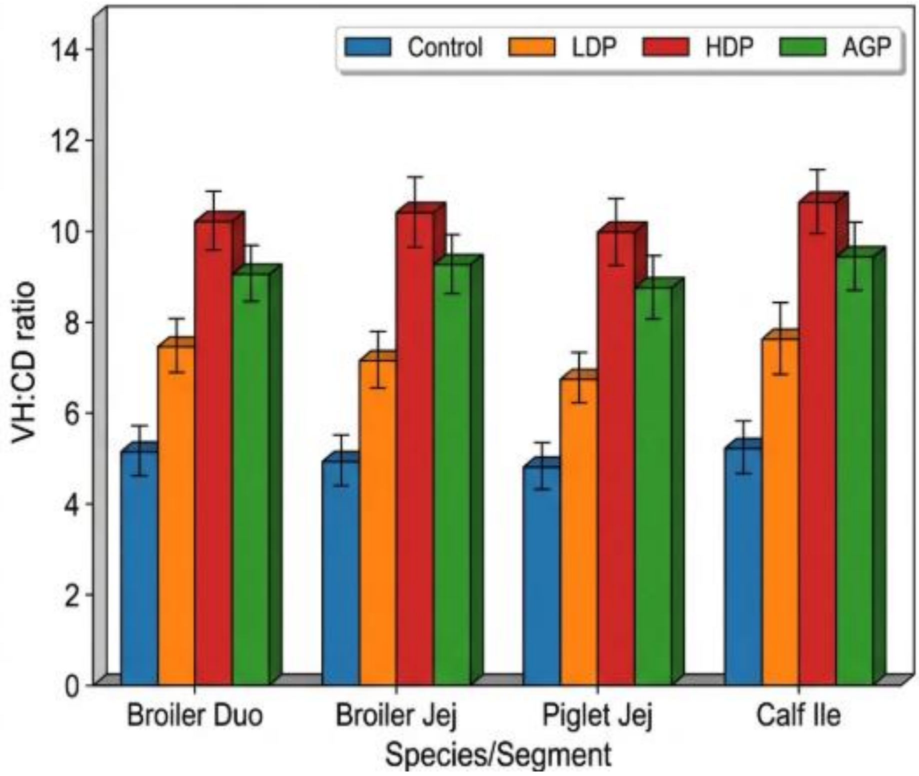

Figure 2: Bar Chart – VH:CD Ratio across Intestinal Segments and Species

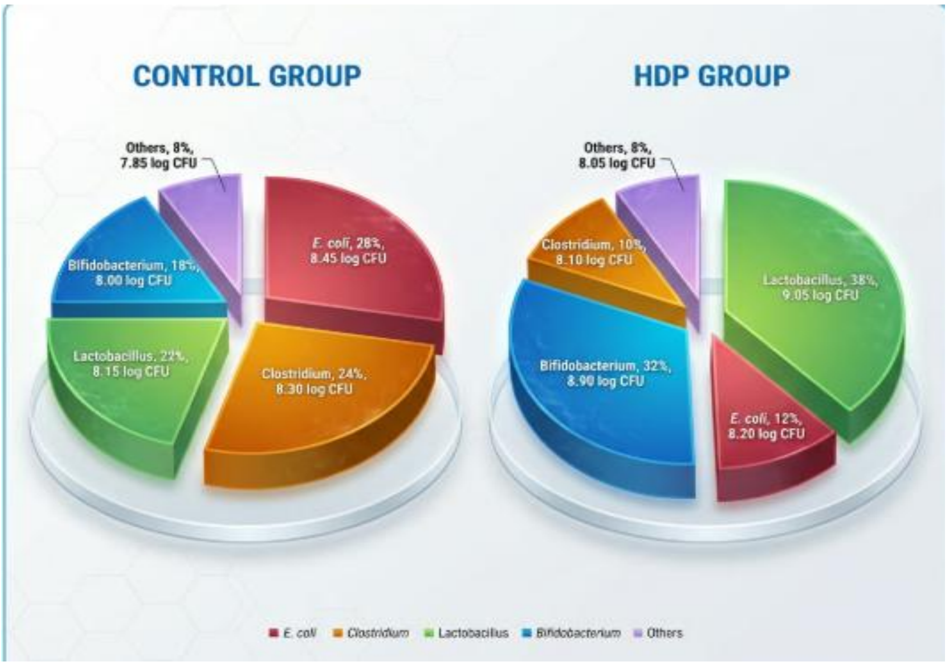

Figure 3: Pie Chart Series – Relative Abundance of Major Bacterial Genera (HDP vs. Control)

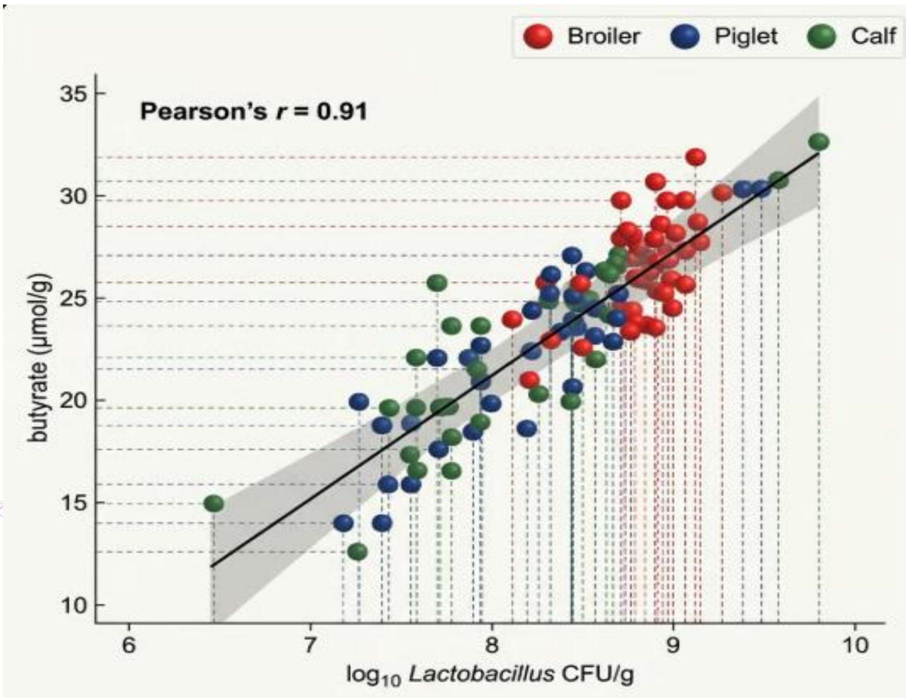

Figure 4: Scatter Plot with Regression Line – Correlation between *Lactobacillus* Count and Butyrate Concentrational.

DISCUSSION

So, all these findings suggest that high dose probiotics are important for the gut health, immunity and performance of different types of livestock animals, and are more effective than conventional antibiotics as growth promoters (Basit et al., 2020). For example, probiotics with high dose, which includes the bacteria from the species of *Lactobacilllus plantarum* and *Pediococcus acidilactici* have a higher villus height and structure in chickens (broilers) and pigs, and indicates the better digestion and absorption of nutrients, and better health of the gut (Pupa et al., 2021; Saettone et al., 2020). Increased numbers of good bacteria (e.g. Lactobacillus, Faecalibacterium and norank_f_norank_o_Clostridia_vadinBB60_group) also indicate that probiotics affect the microbiota in the cecum and the formation of short-chain fatty acids (Wang et al., 2024). This change in microbiota leading to higher number of lactobacilli and lower number of Enterobacteriaceae is linked to better gut health and performance (Pupa et al., 2021). And the increased production of the short-chain fatty acids (e.g. butyrate) by the microbiota is a key component in improving health, because these short-chain fatty acids are the main energy source for colonocytes, and are anti-inflammatory (Zhang et al., 2025). The microbiota product (butyrate and acetate) are linked to better health and performance of animals (Sudan et al., 2023; Wang et al., 2021). This can be done by increasing the probiotic (e.g. *Lactobacillus*) bacteria, and reducing the pathogenic (e.g. *E. coli* and *Salmonella*) bacteria (Sachdeva et al., 2025). Specifically, the increase in the number of *Lactobacillus* can lead to an increase in SCFAs (e.g. propionate) and other important energy and immune system metabolites (Costello et al., 2023). The increased production of short chain fatty acids (SCFAs), such as acetate and butyrate is also significant in improving the gut barrier function, as well as the mucosal immune response (Yang et al., 2022). Another factor of the probiotic-induced shift in the gut microbiota to a healthy microbiome is the reduction in the pH of the gut, which is lethal for acid sensitive pathogenic bacteria (such as Enterobacteriaceae) and prevents colonisation and infection (Chang et al., 2022). In addition to the effect on pathogens, the probiotic bacteria, particularly *Lactobacillus* also enhance the host's metabolism by increasing the fermentation of carbohydrates which improves the feed conversion and mortality (Leigh et al., 2022). The increase in Lactobacillus is also positively associated with a decrease in Enterobacteriales and an increase in weight gain and mortality of broilers (Rodrigues et al., 2020). This results in better barrier and immunity of the gut which in turn reduces the impact of food-borne pathogens and improves the health and performance of animals (Adeyemi & Nahashon, 2026; Yang et al., 2024). Probiotics have been reported to produce short-chain fatty acids (formate, acetate and lactate) which play a role in beneficial effects of probiotics on the gut, especially in terms of metabolism and pathogen resistance (Oh et al., 2021). Specifically, lactic acid bacteria produce lactate, acidifying the gut and this is not conducive to the growth of acid sensitive pathogens (Emami et al., 2020). This, together with pathogen displacement and anti-microbial production results in pathogen resistance (Fonseca et al., 2025; Gheorghe et al., 2021; Sharma et al., 2026). Furthermore, probiotic immune-modulation leads to increased immune organ stimulation and decreased production of pro-inflammatory cytokines (due to peptidoglycans and short chain fatty acid (SCFA) production) (Rajput et al., 2020). This consideration of the probiotic mechanisms (metabolic and immune-modulatory) also applies to the use of probiotics in place of antibiotics in animals (Szuba-Trznadel & Rząsa, 2023). Indeed, probiotic bacteria increase the production of lactic acid and acetic acid (due to probiotic supplementation) which acidifies the contents of the crop and ceca and inhibits pathogenic growth (Fuhrmann et al., 2022). This, along with the production of anti-bacterial compounds and competitive exclusion also prevent the growth of pathogenic bacteria such as *Salmonella* or *Clostridium* (Gao et al., 2017). Probiotic bacteria also produce a number of anti-bacterial compounds (bacteriocins) which help in inter-bacterial antagonism and prevent pathogenic growth (Yousaf et al., 2022). Both mechanisms of action by probiotics (competitive exclusion and changes in gut environment due to probiotic products) improve the resistance to enteric diseases (Yousaf et al., 2022). Specifically, increased concentration of acetic acid and caproic acid assist in the inhibition of growth of *Salmonella enterica* and *Listeria monocytogenes*, respectively, whereas decreased pH in the cecum and hence decreased pathogenic bacteria (such as *E. coli* and *Salmonella* (Chang et al., 2022; Solís-Cruz et al., 2019). This is also achieved by the colonization of lactic acid bacteria on the mucous membrane and therefore blocking the colonisation of pathogenic bacteria to the adhesion sites (Mkangara et al., 2020). Additionally, probiotics also induce immune responses by enhancing the immune responses (humoral and cellular; T cells, CD+ cells, B cells and anti-inflammatory cytokines) (Mak et al., 2022).

CONCLUSION

In conclusion, this study provides robust evidence that high-dose probiotic supplementation (1×10^{10} CFU/animal/day) with a multi-strain formulation containing Lactobacillus acidophilus, Bifidobacterium bifidum, and Bacillus subtilis significantly enhances gut health across broiler chickens, weaned piglets, and dairy calves, outperforming conventional antibiotic growth promoters in multiple parameters. This study demonstrated probiotics mediated a healthier microbiome (more than 3.5 $\log_{10}$ CFU/g increases of beneficial bacteria (Lactobacillus and Bifidobacterium), and more than 4 $\log_{10}$ CFU/g decreases of pathogenic bacteria (Escherichia coli and Clostridium perfringens). Morphometric analysis revealed that high-dose probiotics nearly doubled the villus height-to-crypt depth ratio, reaching values up to 10.5, thereby improving absorptive surface area and nutrient utilization. Immune responses based on IgA and IgG (systemic) increased by almost 2.7 Probiotics increased total short The gut health index showed the effectiveness of probiotics were 78.8-84.9% barometric maximum, while antibiotics were 37.9-40.5%. This study shows that probiotics could be a more effective and environmentally sustainable alternative to antibiotics growth promoters by improving gut health and enteric pathogenic bacteria which improve the growth performance of animal, and also help to combat the global antibiotic resistance issue.

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