90 (2026) 100012

Contents lists available at ScienceDirect

European Poultry Science

journal homepage: www.sciencedirect.com/journal/european-poultry-science

Synbiotic containing fructooligosaccharides and multi-species probiotic strains and its effects on growth, intestinal morphology, tibia strength, and caecal microbiota in Cobb 500 and Ross 308 broilers

Sharmila Ahmad [a,*], Siti Nur Atasya Sanosi [a], Nur Iffah Izzati Rosidi [a], Nadwatul 'Asyiqin Jamlus [a], Zulkifli Idrus [a,b]

[a] Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, UPM Serdang, 43400, Selangor, Malaysia
[b] Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, UPM, Serdang, Selangor, 43400, Malaysia

ARTICLE INFO

Keywords:
Biotic
Poultry
Skeletal health
Welfare

ABSTRACT

The study assessed the effect of a synbiotic-supplemented diet on the performance, gut health, skeletal health, and fear response of two commercial broiler strains. Three-hundred-day-old Cobb 500 and Ross 308 chicks were randomly allotted to either only a basal diet or a diet supplemented with antibiotic or synbiotic (consisting of fructooligosaccharide, *Enterococcus* sp., *Bifidobacterium* sp., *Pediococcus* sp., and *Lactobacillus* spp. at 0.5 g/kg of diet). The birds' body weight (BW) and feed intake were measured weekly, and the tonic immobility and latency tests were conducted on days 32 and 35 of age, respectively. The birds were slaughtered on day 35, and the analysis included quantification of duodenum morphometrics and caecal bacteria and evaluation of leg and tibia strength. Diets had a negligible effect ($p > 0.05$) on bird BW, feed intake, relative internal organs (liver, gizzards, and heart weight), caecal bacterial population, and fear response. The synbiotic did not improve the birds' leg strength, although they had higher tibia weight ($p = 0.02$) and dry matter ($p = 0.008$) than antibiotic-supplemented birds. Additionally, synbiotic-supplemented birds had higher villus height and shorter villus width of duodenum compared to the other groups ($p < 0.01$), and broilers fed only the basal diet ($p < 0.05$), respectively. The Cobb 500 birds consumed more feed ($p = 0.0001$) and were heavier ($p < 0.0001$) than the Ross 308 birds, but no strain differences ($p > 0.05$) were observed for the tibia strength and caecal bacterial counts. The findings of this study indicate that Cobb 500 birds had a better performance than Ross 308. Although the synbiotic increased the duodenal villi's height and tibia's weight, it did not affect both bird strains' growth performance and leg strength. Therefore, the optimum amount of synbiotic required to enhance the overall performance of broiler chickens warrants further study.

1. Introduction

Broiler chickens often experience stress in commercial production due to fast growth and discomfort, particularly in the hot and humid tropics, which can negatively affect their welfare and productivity (Tainika et al., 2023). The skeletal health of the broiler is crucial for successful broiler production, as leg issues such as lameness can result in production loss due to poor performance, high culling, and mortality rates (as reviewed in Waldenstedt, 2006; Liu et al., 2023; Wilcox et al., 2024)). Lameness is a common issue in broiler production and is a serious welfare concern because it causes pain and discomfort and impairs the mobility of broiler chickens (Liu et al., 2023). The lameness in broiler chickens is associated with their rapid weight gain and growth rate, and as a result, the bone's mineral density and ash content decrease, which causes bone porosity (Biesek et al., 2022). Factors such as nutrition, genetics, sex, and growth performance can affect the skeletal health of the broiler (Rabia and Abbas, 2021).

The genetic differences in the commercial broiler strains can be reflected in the productive performance (Marcato et al., 2006; Awad et al., 2020; Badar et al., 2021), the prevalence of leg abnormalities (Guevara-Torres et al., 2021), the caecal microbiota composition (Richards et al., 2019), and immunity (Sakomura et al., 2006) between Ross 308 and Cobb 500. However, discrepancies exist on which commercial broiler strains are better. Guevara-Torres et al. (2021) noted that

* Corresponding author.
E-mail address: sharmila@upm.edu.my (S. Ahmad).

https://doi.org/10.1016/j.eups.2025.100012
Received 29 October 2024; Received in revised form 8 November 2025; Accepted 4 December 2025
Available online 10 December 2025
0003-9098/© 2025 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

the locomotion problems can be observed in Ross 308 and Cobb 500 chickens as early as the first week and gradually increase over time (Guevara-Torres et al., 2021). The authors concluded that Cobb 500 broilers and males were more susceptible to locomotion problems than Ross 308 and females (Guevara-Torres et al., 2021).

Interest in studying the potential of biotics, such as probiotics (Mookiah et al., 2014; De Cesare et al., 2017; Popov et al., 2024), prebiotics (Houshmand et al., 2012; Mookiah et al., 2014; Popov et al., 2024), and synbiotics (Mohammed et al., 2018, 2019, 2022; Abdel--Wareth et al., 2019), to enhance broiler chickens' growth, health, and welfare is increasing. Synbiotic, the focus of this study, have been shown to effectively enhance broiler performance, improve leg and foot health (Hamasalim, 2016; Hu et al., 2022), and reduce fearfulness (Mohammed et al., 2021). These benefits are related to enhanced gut health (Tuohy et al., 2005).

A synbiotic comprises probiotics and prebiotics that benefit the host by promoting the growth and activity of beneficial gut bacteria (Tuohy et al., 2005). In synbiotic products, the prebiotics help nourish the probiotics and enhance the production of their beneficial byproducts (Gyawali et al., 2019). By acting in concert, the probiotics and prebiotics also improve the function of the gut-brain axis and the chickens' performance, health, and behaviour (Cao et al., 2021). The following studies have shown that providing synbiotic supplementation can improve the growth performance of Cobb 500 broilers (Fornazier et al., 2019) and Ross 308 broilers (Naghi Shokri et al., 2017) at different inclusion rates. It has been observed that Cobb 500 broilers generally outperform Ross 308 broilers in terms of body weight, weight gain, and feed conversion ratio (Do Nascimento et al., 2018; Hassan et al., 2021; Hammemi et al., 2024). Cobb 500 broilers also have a higher breast meat yield but a lower leg meat yield than Ross 308 birds (Olanrewaju et al., 2014).

Research has shown that Ross 308 chickens exhibit better growth performance and higher meat yield than Ross x Ross 708 (Olanrewaju et al., 2014). Additionally, a study highlighted that Ross 308 and Ross 708 can significantly differ in metabolic performance and respond differently to dietary tributyrin supplementation (Bedford et al., 2016). However, previous studies have not investigated the effects of synbiotics on growth performance, skeletal health, and overall well-being across commercial broiler strains. Consequently, this study is the first to assess how two broiler strains, Cobb 500 and Ross 308, respond to synbiotic supplementation.

This study aimed to investigate the impact of synbiotic supplementation on growth performance, tibia and leg health, caecal microbiota, and duodenum histomorphology in two modern commercial broiler strains, Ross 308 and Cobb 500. We hypothesised that synbiotic supplementation could enhance growth performance by promoting the colonisation of beneficial gut microbiota, improving gut integrity, reducing the incidence of leg abnormalities, and mitigating fear responses in Ross 308 and Cobb 500 broilers. This is based on previous evidence that synbiotic supplementation can serve as a biotherapeutic method to address these issues in fast-growth broilers, considering the genetic differences between commercial broiler strains.

2. Material and methods

2.1. Ethics statement

The experimental and animal handling was conducted following the Institutional Animal Care and Use Committee (IACUC) guidelines at Universiti Putra Malaysia (UPM/IACUC/AUP-T003/2023).

2.2. Experimental design, diets, and animals

The experiment was conducted at the Institute of Tropical Agriculture and Food Security research facilities, Universiti Putra Malaysia. Six-hundred-day-old male Cobb 500 and Ross 308 chicks, consisting of 300 Cobb 500 and Ross 308 chicks, were used in a complete randomised design experiment. The day-old chicks of both strains were purchased from a similar local hatchery. The Cobb 500 and Ross 308 chicks were randomly assigned to one of three diets: a basal diet supplemented with antibiotic (STAFAC 500-Virginiamycin, Phibro Animal Health, Canada) or a synbiotic (PoultryStar® me, BIOMIN, Singapore). The ingredients and nutrient composition of the basal diet are detailed in Table 1. The antibiotic and synbiotic were added at 44 g and 0.5 kg per ton of feed, respectively. Each strain-diet subgroup had five replicates, with 20 birds per replicate. The synbiotic contains fructooligosaccharides and four strains of beneficial bacteria: *Enterococcus* sp., *Bifidobacterium* sp., *Pediococcus* sp., and *Lactobacillus* sp. The diets were divided into starter (days 1–21) and finisher (days 22–35). The chemical composition of the experimental diet is listed in Table 1. The feed was provided *ad libitum*, and the chickens had free access to drinking water. The birds were raised on a pen floor with wood shaving in a mechanically ventilated house. Each pen measured 120 in length and 120 cm in width. The chickens were vaccinated against Newcastle Disease and Infectious Bronchitis on days 7 and 21. The experimental feeding trial lasted 35 days. The house's temperature was set at 32 ± 1 °C on the first day and gradually reduced to 24 ± 1 °C by day 21 and maintained at this level until 35 d of age.

2.3. Tonic immobility test

On day 32, 15 birds per dietary group (3 birds x 5 replicates) were randomly chosen for the tonic immobility (TI) test following the previously described method (Zulkifli and Nor, 2004). An experimenter quietly entered the pens and caught one bird with both hands, immediately carrying it to a separate room to measure tonic immobility. It is important to catch and handle each chicken separately to avoid

Table 1
Experimental diet composition (as-fed basis).

Diets	Starter (1–21 d)	Finisher (22–35 d)
Ingredients (%)		
Corn	55.0	61.3
Soybean meal, 44 %	37.0	29.9
Palm Oil	3.84	5.30
Limestone	1.55	1.00
Monocalcium Phosphate	1.50	1.35
L-Threonine	0.100	0.100
Common Salt	0.250	0.250
Vitamin Premix	0.050	0.050
Mineral Premix	0.100	0.100
DL-Methionine	0.180	0.190
L-Lysine HCl	0.20	0.210
Choline Cl −70 %	0.050	0.050
Sodium Bicarbonate	0.180	0.200
Calculated		
Energy (Kcal/g)	3050	3200
Crude protein (%)	22.2	19.5
Crude fat (%)	7.71	7.51
Calcium (%)	0.960	0.850
Total phosphorus (%)	0.630	0.610
Available phosphorus (%)	0.450	0.350
Lysine (%)	1.24	1.02
Methionine (%)	0.530	0.480
Methionine + Cysteine (%)	0.900	0.820
Threonine	0.980	0.860
Tryptophan (%)	0.670	0.700

Note: Antibiotic (Virginiamycin) was supplemented at 44 g per kilogram of basal diets, and synbiotic (PoultryStar®, DSM) was supplemented at 0.5 kg per ton of basal diets. The provided Vitamin Premix contains the following nutrients per kg of diet: vitamin A, 24,000 IU; vitamin D3, 6000 IU; vitamin E, 80 mg; vitamin K3, 4 mg; vitamin B1, 4 mg; vitamin B2, 10 mg; vitamin B6, 6 mg; vitamin B12, 0.04 mg; niacin, 80 mg; pantothenic acid, 20 mg; folic acid, 2 mg; biotin, 0.3 mg. Mineral Premix provided the following nutrients per kg diet: Fe, 176 mg; Cu, 145.2 mg; Zn, 120 mg; Mn, 132 mg; I, 1.98 mg; Co, 0.66 mg; Se, 0.44 mg.

impacting other flock members. The birds were induced into TI by gently restraining them on their right side and wings for 15 s. A stopwatch was started to record latencies until the bird righted itself. If the bird righted itself in less than 10 s, it was recaptured, and the restraining procedure was repeated. If TI was not induced after three attempts, the duration of TI would have been considered 0 s. The maximum duration of tonic immobility was 10 min. After taking the measurements, the birds were spray-painted on their backs and returned to their home pens. The maximum duration of tonic immobility was 10 min.

2.4. Latency to-lie-test

The leg strength of fifteen birds in each dietary group (3 birds x 5 replicates) at 35 days of age. The latency test (LTL) was conducted using a modified method (Berg and Sanotra, 2003; Santos et al., 2022). A container containing 3 cm of warm water (28 °C) was prepared to test the birds. The test involved placing one bird per pen into the water and recording the time for the bird to lie down. If a bird did not lie in the water, the maximum recorded time was 600 s. A bird was considered lying down when its breast touched the water for at least 5 s, with the last second of these 5 s considered as the latency-to-lie. The water was replaced as needed, especially if it became soiled due to bird droppings, before placing additional birds into the container.

2.5. Broiler performance parameters and weight of internal organs

In the five-week experimental period, the body weight (BW) and feed intake were recorded weekly for each pen. The feed conversion ratio (FCR) was calculated as the weight of feed consumed divided by BW per pen (Abdel-Wareth et al., 2019). The feed intake was adjusted for bird mortalities (Abdel-Wareth et al., 2019).

2.6. Sampling of internal organs, duodenum, the legs and caecal digesta

Two birds per pen (60 birds in total) were randomly selected and slaughtered on day 35. The birds were individually weighed before slaughtering. The birds were halal slaughtered, according to Farouk et al. (2014). The liver, empty gizzard, and heart were sampled and weighed. The weight of the liver, heart, and empty gizzard was measured and expressed as a percentage of live BW. The duodenum was cut, flushed with normal saline, and kept in a pill box containing 10 % formaldehyde solution for the histological analysis. The cecum digesta of the birds were immediately removed after being slaughtered and collected into sterile tubes for bacterial quantification and stored at −20 °C pending the DNA extraction analysis. The left tibia and femur will be dissected, removed from the birds, placed in individual plastic bags, and kept at 20 °C) until the analysis (Yan et al., 2019).

2.7. Tissue preparation and histological analysis

Intestinal morphology was analysed using the method outlined by Awad (2016) with slight modifications. The duodenum tissue was fixed in a 10 % formaldehyde solution pending analysis. Before embedding, the tissues were trimmed and immersed for 2 h in an 80 % ethanol solution and then in a 95 % ethanol solution for another 2 h. This was followed by immersion in a 100 % ethanol solution for 3 h, then 3 h in a chloroform solution, and finally 5 and a half hours in paraffin solution. Sections were then cut at 4 μm and subsequently stained. The tissue sections were stained with Harris's haematoxylin and eosin.

2.8. Histomorphometric analysis

The slides were viewed under a microscope (Novex B series, Holland) at 40× magnification. The camera used to capture the images of the slides was CMEX DC. 1300x, euromax, Holland, which was attached to the microscope. The villi height, width, and crypt depth of the duodenum on the slides were measured using Image Focus software (ImageFocus v3). The villus height was measured from the top of the villus to the base, the width of the villus was measured at the basal and apical parts, and the crypt depth was measured from the base of the crypt to the level of the crypt opening as per the reference. Ten measurements were taken for each parameter on each slide.

2.9. Caecal DNA extraction

The DNA isolated from the caecal samples was extracted following the manufacturer's protocol using the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). In short, approximately 180–220 mg of stool was weighed and transferred to a 2 mL microcentrifuge tube. The stool was homogenised with 1 ml InhibitEX buffer, heated at 70 °C for 5 min, and then centrifuged to pellet the stool particles. After adding 15 mL of proteinase K and 200 mL of buffer AL, the samples were incubated at 70 °C for 10 min, and 96 % ethanol was added. The lysate was then applied to a QIAamp spin column, and the DNA was eluted in 200 μL Buffer ATE. The concentration and integrity of isolated DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.10. Quantitative real-time PCR analysis

The caecal microbiota was quantified following the previously established method (Navidshad et al., 2012). The cDNA synthesis was performed by reverse transcription (RT) by applying QuantiNova™ SYBR SYBR Green PCR kit (Qiagen, Hilden, Germany). A total of 20 μL reaction mixture was prepared by adding 8.5 μL of 2X SYBR Green PCR Master Mix, 1 μL of each of forward and reverse primers, 1 μL of DNA samples, and 8.5 μL of RNase-free water. Following the manufacturer's protocols, the qPCR was performed using a BioRad CFXConnect (Biorad, USA). The PCR conditions used were as follows: denaturation at 94 °C for 5 min, annealing at 50 °C for *Salmonella* spp., 55 °C for total bacteria, 58 °C for *Lactobacillus* spp., and 60 °C for other bacteria (*Clostridium* spp. and *E. coli* spp.) for 30 s (refer to Table 2), and elongation at 72 °C for 20 s. These steps were repeated 40 times in each qPCR reaction (Rezaei et al., 2015).

2.11. Tibia bone preparation and tibia strength analysis

The bone strength analysis was performed according to the procedure outlined in a previously published article (Mohammed et al., 2021). Briefly, thirty-six samples of the right leg were boiled at 80 °C for 20 min to remove the muscle and other soft tissues. Subsequently, the weight of the tibia was measured, and its breaking strength at the mid-shaft was tested using compression testing (Instron, Model 3366 (10 kN)).

2.12. Statistical analyses

All data were analysed using a completely randomised factorial

Table 2
Bacteria, primer sequences, and annealing temperatures.

Bacteria	Primer Sequence (5′-3′)	Annealing temperature (°C)
Total bacteria	F-CATCCACTGCAAACCTAAGAG R- GATCCGCTTGCCTTCCGA	55
Lactobacillus spp.	F: CAT CCA GTG CAA ACC TAA GAG R: GAT CCG CTT GCC TTC GCA	58
Clostridium spp.	F: GAG TTT GAT CMT GGC TCA G R: CCC TTT ACA CCC AGT AA	60
Escherichia coli	F: GTG TGA TAT CTA CCC GCT TCG C R: AGA ACG CTT TGT GGT TAA TCA GGA	60
Salmonella spp.	F: TCG TCA TTC CAT TAC CTA CC R: AAA CGT TGA AAA ACT GAG GA	50

design with fixed effects of diet, strain, and diet-by-strain interactions using ANOVA (Chambers et al., 2017) in the R studio statistical software (Team, 2024), except for mortality. Mortality data were analysed using a Chi-square test. Statistical differences between means were assessed using the Tukey multiple test. The normality of the model was checked using the Shapiro-Wilk test and norm plots of the residuals. If the model did not adhere to a normal distribution, log transformation was applied to normalise the model. Post hoc analysis was conducted using Tukey's multiple comparison tests when one of the main effects or their interactions was significant. The data are presented as mean ± SE, and the p-values are based on ANOVA. The level of significance was set at $p < 0.05$.

3. Results

3.1. Growth performance

Mortality rates did not differ among the experimental groups ($\chi^2 = 0.96$), as shown in Fig. 1. The mortality rates for Cobb broilers fed a basal diet, a basal diet supplemented with a synbiotic, and an antibiotic are 3 %, 5 %, and 5 %, respectively. In contrast, Ross, fed with the same diets, exhibits mortality rates of 1 %, 1.7 %, and 2 %. The interaction effect of diet and strain on broiler chickens' growth performance was listed in (Table 3). However, Cobb 500 broilers consumed more feed throughout the rearing period (days 1–21: $p < 0.0001$; days 22–23: $p = 0.01$ and days 1–35: $p = 0.0001$) and achieved a significantly higher weight gain at only days 1–21 ($p < 0.0001$) and days 1–35 ($p = 0.01$) but not days 22–35 ($p = 0.29$) compared to Ross 308 chickens.

3.2. Tonic immobility test

The results indicate that diet ($p = 0.33$), strain ($p = 0.44$), and their interactions ($p = 0.75$) did not significantly affect TI (Fig. 2).

3.3. Latency-to-lie test

The time spent by the broiler standing before sitting down following the LTL test is presented in (Table 2). Diet ($p = 0.08$), strain ($p = 0.45$), and their interactions ($p = 0.42$) had no significant effects on the time spent by the broiler to stand before sitting down in the LTL test.

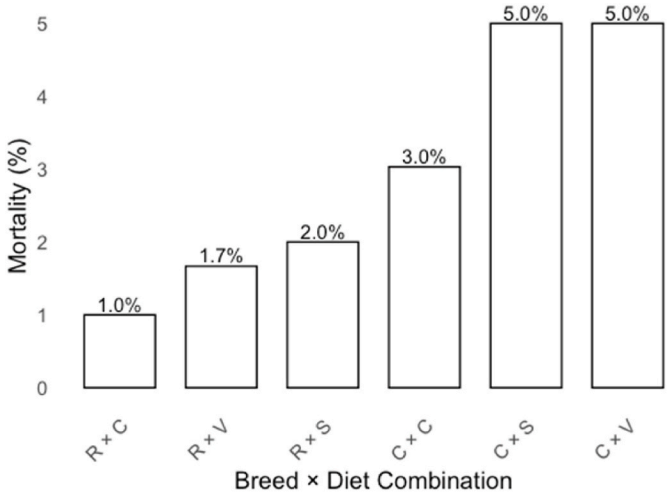

Fig. 1. The percentage of mortality in broiler chickens by combined breed x diet. Diet: C, Control (basal diet); V, Antibiotic; S, Synbiotic. Breed C, Cobb 500; R, Ross 308.

3.4. The tibia's characteristics and strength

The weight, dry matter (DM), ash, and bone-breaking force of the tibia bone are presented in (Table 4). Birds given synbiotic showed significantly higher weight and DM of the tibia bone than antibiotic-supplemented birds ($p = 0.02$ and $p = 0.008$, respectively). The ash content of the tibia was not influenced by diet ($p = 0.17$), strain ($p = 0.21$), or their interaction ($p = 0.85$).

3.5. Duodenum histomorphometric

There were significant ($p < 0.05$) interactions between diet and strain for the crypt depth of the duodenum (Table 6). The broilers that received synbiotic supplementation had longer villus height (VH) compared to the other groups ($p < 0.01$) (Table 5). Additionally, the synbiotic supplementation significantly reduced the duodenum's villus width compared to broilers fed the basal diet ($p < 0.05$).

3.6. Caecal bacterial quantification

Neither diet nor strain had a significant effect on the caecal bacteria of *E. coli* ($p = 0.730$ and $p = 0.64$ for diet and strain, respectively), *Clostridium* spp. ($p = 0.18$ and $p = 0.18$ for diet and strain, respectively), *Lactobacillus* spp. ($p = 0.18$ and $p = 0.18$ for diet and strain, respectively), and total bacteria counts ($p = 0.52$ and $p = 0.45$ for diet and strain, respectively) (Table 7). Conversely, *Salmonella* spp. was not detected.

4. Discussion

The present study evaluated the effect of synbiotic and broiler strains on performance, duodenum histomorphology, caecal bacteria count, tibia and leg strength, and the fear response of Cobb 500 and Ross 308 commercial broiler strains. The study found that synbiotic did not impact the broilers' growth performance, the caecal bacteria population, or leg strength. However, synbiotic supplementation increased the tibia's weight and dry matter. Additionally, differences in strains were observed, with Cobb 500 consuming more feed, having a higher final body weight, and exhibiting shorter duodenum crypt depth compared to Ross 308.

The genetic differences in the commercial broiler strains can be reflected in the productive performance (Marcato et al., 2006; Awad et al., 2020; Badar et al., 2021), the prevalence of leg abnormalities (Guevara-Torres et al., 2021), the caecal microbiota composition (Richards et al., 2019), and immunity (Sakomura et al., 2006) between Ross 308 and Cobb 500. However, discrepancies exist on which strains are better. Several studies have indicated that Cobb 500 broilers outperform Ross 308 broilers regarding body weight, weight gain (WG), and feed conversion ratio (Do Nascimento et al., 2018; Hassan et al., 2021; Hammemi et al., 2024). However, other studies have reported conflicting results, suggesting higher WG and final body weight for Ross 308 broilers (Awad et al., 2020). Besides that, studies also showed that Cobb 500 had a greater breast meat yield (Do Nascimento et al., 2018; Hammemi et al., 2024) but a lower leg meat yield than Ross 308 birds (Hammemi et al., 2024). Their rapid growth and increased body weight (Sterling et al., 2006; Khalid et al., 2021) made Cobb 500 chickens more vulnerable to high temperatures and have a higher risk of locomotion problems (Guevara-Torres et al., 2021). In this study, Cobb 500 chickens were found to have higher final body weight, consumed more feed, and gained more weight than Ross 308 chickens, consistent with previous research (Sterling et al., 2006; Hassan et al., 2021; Hammemi et al., 2024). No difference in FCR was also observed, which aligns with the previous findings (Petričević et al., 2011). Despite this, contrary to earlier findings (Guevara-Torres et al., 2021), there were no observed differences in leg and tibia strength between the Cobb 500 and Ross 308 chicken strains. Previous studies indicate that the Cobb 500 strain has a

Table 3
Effect of diet[a] and strain on broiler chickens' feed intake, weight gain and feed conversion ratio from days 1–35 (Means ± SE).

	Diet[a]			Strain		p-value		
Parameters	C	V	PS	Cobb 500	Ross 308	Diet	Strain	Diet x Strain
Feed intake, g/bird								
Days								
1–21	1364 ± 29.8	1327 ± 29.9	1314 ± 32.9	1418 ± 9.8[a]	1265 ± 16.8[b]	0.270	<0.000	0.990
22–35	2386 ± 23.2	2359 ± 35.1	2401 ± 50.0	2439 ± 21.8[a]	2332 ± 30.6[b]	0.680	0.010	0.350
1–35	3746 ± 49.4	3676 ± 54.2	3716 ± 81.7	3859 ± 21.2[a]	3598 ± 42.7[b]	0.860	0.000	0.490
Weight gain, g/bird								
Days								
1–21	1037 ± 24.27	1039 ± 17.94	1033 ± 26.18	1093 ± 11.69[a]	980 ± 9.55[b]	0.950	<0.000	0.300
22–35	1549 ± 26.68	1483 ± 31.37	1536 ± 22.54	1539 ± 11.64	1506 ± 29.7	0.200	0.290	0.290
1–35	2587 ± 29.92	2522 ± 35.06	2570 ± 39.48	2633 ± 12.47[a]	2486 ± 27.5[b]	0.210	<0.000	0.410
FCR, feed/gain								
Days								
1–21	1.26 ± 0.010	1.225 ± 0.011	1.24 ± 0.010	1.24 ± 0.010	1.24 ± 0.010	0.140	0.990	0.670
22–35	1.56 ± 0.020	1.61 ± 0.030	1.57 ± 0.030	1.59 ± 0.010	1.57 ± 0.030	0.390	0.470	0.230
1–35	1.44 ± 0.010	1.45 ± 0.020	1.43 ± 0.010	1.45 ± 0.010	1.43 ± 0.010	0.570	0.430	0.510

[a,b]Means within a row-subgroup with no common letters differ at ($p \leq 0.05$).
FCR: Feed conversion ratio.
[a] C: control; V: Antibiotic; PS: Synbiotic.

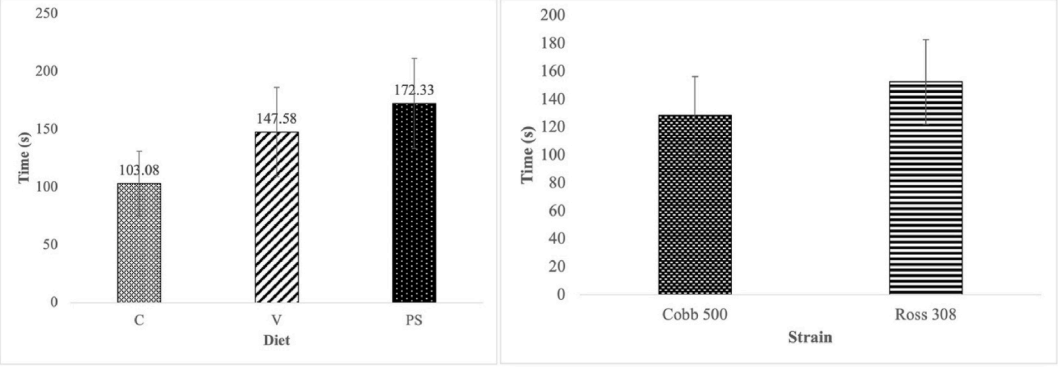

Fig. 2. The effect of diet[1] and strain on the tonic immobility (TI) responses of 32-day-old broiler chickens (Means ± SE). The number of broilers in each dietary group was 15, with 3 birds in each group and 5 replicates.
[1]C: control; V: Antibiotic; PS: Synbiotic.

Table 4
Effect of diet[1] and strain on the leg and tibia strength, as well as broiler chickens' tibia dry matter and ash (Means ± SE).

Item	Diet			Strain		p-value		
	C	V	PS	Cobb 500	Ross 308	Diet	Strain	Diet x Strain
LTL	75.4 ± 8.29	98.8 ± 11.49	62.6 ± 7.02	73.8 ± 7.65	83.8 ± 7.84	0.080	0.450	0.420
Tibia								
Strength (MPa)	57.1 ± 4.40	57.9 ± 4.41	49.5 ± 4.41	52.3 ± 3.60	57.4 ± 3.60	0.350	0.330	0.90
Weight (g)	8.58 ± 0.290[ab]	8.30 ± 0.320[b]	9.51 ± 0.300[a]	8.94 ± 0.240	8.66 ± 0.250	0.020	0.620	0.06
DM (%)	5.70 ± 0.170[ab]	5.28 ± 0.170[b]	6.13 ± 0.170[a]	5.87 ± 0.140	5.53 ± 0.140	0.008	0.100	0.52
ASH (%)	2.81 ± 0.130	2.72 ± 0.130	3.08 ± 0.140	2.97 ± 0.110	2.77 ± 0.100	0.170	0.210	0.85

[a,b]Means within a row-subgroup with no common letters differ at ($p \leq 0.05$).

Table 5
The effect of diet and strain on the villus height (VH), villus width (VW), villus crypt depth (CD), and the villus height and crypt depth ratio of the duodenum of broiler chickens (Means ± SE).

Item	Diet[a]			Strain		p-value		
	C	V	PS	Cobb 500	Ross 308	Diet	Strain	Diet x Strain
VH (μm)	684 ± 65.3[a]	697 ± 12.7[a]	749 ± 52.6[b]	706 ± 8.74	714 ± 8.74	< 0.010	0.554	0.140
VW (μm)	88.50 ± 4.48[a]	86.40 ± 4.43[ab]	83.80 ± 4.46[b]	85.30 ± 4.45	87.10 ± 4.47	< 0.050	0.193	0.510
CD (μm)	123 ± 4.80	123 ± 4.81	121.00 ± 4.81	118.00 ± 4.77[b]	127 ± 4.84[a]	0.830	< 0.018	< 0.001
VH:CD (μm)	4.81 ± 0.02	4.81 ± 0.02	4.80 ± 0.02	4.77 ± 0.02[b]	4.84 ± 0.12[a]	0.17	0.007	0.05

[a,b]Means within a row-subgroup with no common letters differ at ($p \leq 0.05$).
[a] C: Control; V: Antibiotic; PS: Synbiotic.

Table 6
The duodenum crypt depth of broiler chickens where diet[a] x strain interactions were significant (Means ± SE).

Item	Diet					
	C		V		PS	
	Cobb 500	Ross 308	Cobb 500	Ross 308	Cobb 500	Ross 308
Crypt dept (μm)	113 ± 3.45^{c}	134 ± 4.30^{a}	115 ± 3.53^{c}	130 ± 3.80^{ab}	126 ± 3.86^{abc}	116 ± 3.56^{bc}

^{a,b,c}Means within a row with no common letters differ at ($p \leq 0.05$).
[a] C: Control; V: Antibiotic; PS: Synbiotic.

lower leg meat yield than the Ross 308 strain (Hammemi et al., 2024). However, we did not measure muscle weight in this study and speculate that it may be similar for both strains, leading to no significant difference in leg strength.

In chickens, the influence of the host's genetic background on microbial colonisation and microbiota composition is limited (Schokker et al., 2015). However, it has been reported that the host's genotype and gender are among the factors that affect gut microbiota composition (Zhao et al., 2013). According to another study, breed variations (Cobb 500 vs. Ross 308) were evident only 3 and 7 days after hatching but vanished 14 days after (Richards et al., 2019). However, environmental factors, diet, and management practices influence the caecal microbiota more than genetics (Richards et al., 2019). Nevertheless, a study by Awad et al. demonstrated no difference in the population of caecal bacteria between Cobb 500 and Ross 308, which is in line with the present findings (Awad et al., 2020).

The avian gastrointestinal tract is specialised to maximise absorption of dietary components through its highly convoluted intestinal mucosa, which is folded into villi and microvilli, increasing the surface area for absorption by about 600-fold (De Verdal et al., 2010; Wijtten et al., 2012; Alshamy et al., 2018). In a study comparing Lohman Dual and Ross birds, researchers found strain differences in the anatomy of the digestive system (Alshamy et al., 2018). Lohman Dual birds were observed to have heavier gizzards, shorter intestines, higher jejunal villi, thicker ileal tunica muscularis, and smaller intestinal mucosal surface area, resulting in a slower growth rate than found in Ross 308 birds (Alshamy et al., 2018). This study suggested breed differences in the anatomy of the chickens' digestive system. Our study observed variations in duodenal crypt depth between Cobb 500 and Ross 308 birds. The Ross 308 birds had a deeper crypt depth than the Cobb 500 birds. While the crypt depth was significantly more profound in the Ross 308 birds, no differences were observed in villi height or height-to-crypt depth ratio. Deeper intestinal villi crypts suggest fast tissue metabolism for villi renewal (Baurhoo et al., 2007, 2009), while reducing crypt depth may lower nutrient absorption (Kuzmuk et al., 2005). Awad et al. (2020) reported that the Cobb 500 and Ross 308 exhibit different growth trajectories in embryo chicks, leading to distinct growth patterns due to physiological differences. In line with this, we observed that the Cobb 500 exhibited a distinct growth pattern compared to the Ross 308 broilers.

Broilers fed a synbiotic diet showed enhanced immunity (Abdulhasan, 2018; Mohammed et al., 2019; Cai et al., 2023), reduced fear responses (Abdulhasan, 2018; Mohammed et al., 2019; Cai et al., 2023), and improved growth performance (Abdulhasan, 2018; Abdel-Wareth et al., 2019; Agbai et al., 2022). However, other studies had contradictory findings for growth performance (Salehimanesh et al., 2016; Sarangi et al., 2016; Nisar et al., 2021) and immunity (Salehimanesh et al., 2016; Nisar et al., 2021; Salehimanesh et al., 2016; Sarangi et al., 2016; Nisar et al., 2021). These inconsistent findings could be attributed to differences in synbiotic compositions and dosages (Jin et al., 1997; Patterson and Burkholder, 2003). For example, the effects of synbiotic can vary due to differences in strains of bacteria and their concentrations in the diet (Jin et al., 1997). Besides that, how bacteria regulate changes in the gastrointestinal tract also differs depending on the prebiotic and probiotic introduced (Patterson and Burkholder, 2003). For example, mannanoligosaccharides (MOS) used as the prebiotic in a synbiotic mixture (Sohail et al., 2012, 2013) act by binding and removing pathogens from the intestinal tract (Spring et al., 2000), unlike fructooligosaccharides, which selectively enrich beneficial bacterial populations (Mohammed et al., 2018). The exact mechanisms by which specific combinations of probiotics and prebiotics control growth performance must be identified and warrant further research.

In the present study, synbiotic supplementation did not affect the growth performances of Cobb 500 and Ross 308 broilers, which is consistent with previous studies (Sarangi et al., 2016; Salehimanesh et al., 2016; Nisar et al., 2021). Similar to the earlier study, broilers fed a synbiotic-supplemented diet and reared under thermoneutral conditions did not affect the growth performance of the broilers compared to control-fed broilers (Sarangi et al., 2016; Salehimanesh et al., 2016), which aligned with our results. However, our findings on growth performance did not concur with those of Mohammed et al. (2019) who also utilised the same type of synbiotic and dosage (0.5 g/kg). While our research found no effect, their study suggested that synbiotic supplementation positively impacted broilers' growth performance. There is no clear explanation for these inconsistent findings; only a speculative one can be offered at this stage.

Multiple studies have shown significant performance indicator differences among commercial broiler strains (Woyengo et al., 2010; Lopez et al., 2011; Olanrewaju et al., 2014; Bedford et al., 2016). In this study, we focused on Cobb 500 and Ross 308 broilers, while Mohammed et al. (2018) studied Ross 708 broilers. It has been observed that Ross 308 showed better growth performance and meat yield than Ross x Ross 708 (Olanrewaju et al., 2014). Additionally, Bedford et al. (2016) found significant metabolic performance differences between Ross 308 and Ross 708 birds, especially in response to dietary tributyrin supplementation. Gous et al. (2005) also noted that Cobb is an early-developing strain, while Ross is a late-developing strain.

Substantial evidence indicates that the breeder's age can impact the hatching rate, egg and hatchling weight, and final broiler performance. Hatchlings from older breeders tend to have higher body weight and body weight relative to yolk compared to those from younger breeders

Table 7
Effect of diet[a] and strain on the caecal bacteria of broiler chickens (Means ± SE).

Item	Diet			Strain		p-value		
	C	V	PS	Cobb 500	Ross 308	Diet	Strain	Diet x Strain
Escherichia coli	5.08 ± 0.110	5.10 ± 0.110	6.00 ± 0.120	5.11 ± 0.120	5.11 ± 0.120	0.730	0.640	0.590
Clostridium spp.	5.74 ± 0.100	5.63 ± 0.110	5.89 ± 0.120	6.29 ± 0.090	6.09 ± 0.100	0.180	0.180	0.280
Lactobacillus spp.	6.09 ± 0.130	5.56 ± 0.090	6.11 ± 0.130	5.63 ± 0.180	5.85 ± 0.180	0.490	0.180	0.330
Total Bacteria	5.78 ± 0.13	6.05 ± 0.130	6.45 ± 0.120	6.08 ± 0.110	6.24 ± 0.090	0.520	0.450	0.470
Salmonella spp.	ND	ND	ND	ND	ND	–	–	–

Means within a row-subgroup do not significantly differ at ($p \leq 0.05$).
ND: Not detected.
[a] C: Control; V: Antibiotic; PS: Synbiotic.

(Gous et al., 2005). Therefore, the differences in breeder age may have also contributed to the disparities between the current findings and those of Mohammed et al. (2018). Furthermore, the concentration of synbiotic can affect the composition of bacteria, and different dosages can have distinct effects on broiler performance. Mohammed et al. (2018) reported that under normal temperature conditions (day 14), the broiler chickens' feed intake and body weight increased when 0.5 g/kg and 1.0 g/kg of synbiotic feed were provided compared to a basal diet. However, the feed intake and body weight gain under heat stress were only higher in the 1.0 g/kg feed group than in the control group on day 42 (Mohammed et al., 2018). This study suggests that using a dietary synbiotic concentration of 1.0 g/kg of feed may be beneficial for achieving higher yields throughout the broiler's life.

Although we noted that synbiotic supplementation didn't improve the body weight of broiler chickens, it altered duodenal morphology, characterised by longer villi and shallower crypts compared to those fed a basal diet. Similarly, work on probiotics suggests improved intestinal morphology but not growth performance (Song et al., 2014; Olnood et al., 2015; Li et al., 2020).

On the contrary, Younis et al. (2024) reported that synbiotic supplementation improved jejunal villus height and weight gain in broilers. These discrepancies in findings could be associated with differences in the feed ingredients and diet composition (Jansseune et al., 2024). Furthermore, it has been documented that biotic supplementation is less beneficial in chickens raised in laboratory conditions, which are more hygienic than those in a commercial setting (Olnood et al., 2015).

The intensive genetic selection programs for modern broiler strains have resulted in rapid growth rates and high feed efficiency (Sakomura et al., 2006; Hartcher and Lum, 2020; Guevara-Torres et al., 2021). However, this focus on production traits may lead to negative consequences such as lameness, reduced movement, and increased sensitivity to stress (Mohammed et al., 2021). The gut microbiota of commercial broilers can be targeted with probiotics (Kridtayopas et al., 2019; Mohammed et al., 2021), prebiotics (Javid et al., 2022), and synbiotics (Yan et al., 2019; Hu et al., 2022) to help mitigate leg problems in fast-growth broilers (Kridtayopas et al., 2019; Mohammed et al., 2021). Because synbiotic supplementation did not produce observable shifts in gut microflora, it may not have induced the physiological changes to affect leg health. The failure to observe increases in beneficial bacteria could be attributable to an inadequate synbiotic dose, as similar limitations have been documented previously (Mohammed et al., 2022; Younis et al., 2024).

5. Conclusion

The study found that a synbiotic supplement at 0.5 g/kg increased duodenal villus height in broiler chickens. However, it had little impact on growth performance, tibia strength, and caecal microflora. Research on biotics supplementation in broilers should consider several interacting factors, such as feed ingredients, diet composition, dosage, and rearing environment, which can markedly influence the outcomes.

Acknowledgements

This study was partly funded by the Inisiatif Putra Muda (GP-IPM) research grant (9475500).

References

Abdel-Wareth, A.A., Hammad, S., Khalaphallah, R., Salem, W.M., Lohakare, J., 2019. Synbiotic as eco-friendly feed additive in diets of chickens under hot climatic conditions. Poult. Sci. 98, 4575–4583. https://doi.org/10.3382/ps/pez115.

Abdulhasan, S., 2018. Research article effect of digestrom® and poultry star® on the body performance and immunity status of broiler chickens. Int. J. Poultry Sci. 17, 385–391. https://doi.org/10.3923/ijps.2018.385.391.

Agbai, K., Afolayan, M., Obianwuna, U., 2022. Effect of commercially blended synbiotic product as a replacement for antibiotic growth promoters on growth performance and villi morphology of broiler chicks in dry hot season. Niger. J. Anim. Prod. 49, 13–19. https://doi.org/10.1016/2Fj.psj.2022.101769.

Alshamy, Z., Richardson, K.C., Hünigen, H., Hafez, H.M., Plendl, J., Al Masri, S., 2018. Comparison of the gastrointestinal tract of a dual-purpose to a broiler chicken line: a qualitative and quantitative macroscopic and microscopic study. PLoS One 13, e0204921. https://doi.org/10.1371/journal.pone.0204921.

Awad, E., 2016. Effects of Feeding low-protein Diets Fortified with Amino Acids on Broiler Chickens Under High Environmental Temperatures. Universiti Putra Malaysia Serdang, Malaysia.

Awad, E.A., Najaa, M., Zulaikha, Z.A., Zulkifli, I., Soleimani, A.F., 2020. Effects of heat stress on growth performance, selected physiological and immunological parameters, caecal microflora, and meat quality in two broiler strains. AJAS (Asian-Australas. J. Anim. Sci.) 33, 778. https://doi.org/10.5713/2Fajas.19.0208.

Badar, I., Jaspal, M., Yar, M., Ijaz, M., Khalique, A., Zhang, L., Manzoor, A., Ali, S., Rahman, A., Husnain, F., 2021. Effect of strain and slaughter age on production performance, meat quality and processing characteristics of broilers reared under tropical climatic conditions. Earth Planets Space 85. https://doi.org/10.1399/eps.2021.326.

Baurhoo, B., Ferket, P., Zhao, X., 2009. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. Poult. Sci. 88, 2262–2272. https://doi.org/10.3382/ps.2008-00562.

Baurhoo, B., Phillip, L., Ruiz-Feria, C., 2007. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poult. Sci. 86, 1070–1078. https://doi.org/10.1093/ps/86.6.1070.

Bedford, A., Yu, H., Hernandez, M., Squires, J., Leeson, S., Hou, Y., Gong, J., 2016. Different responses of Ross 308 and 708 broiler strains in growth performance and related properties to diet treatment with or without tributyrate glycerides. J. Anim. Sci. 94, 452. https://doi.org/10.2527/jam2016-0939.

Berg, C., Sanotra, G., 2003. Can a modified latency-to-lie test be used to validate gait-scoring results in commercial broiler flocks? Anim. Welf. 12, 655–659. https://doi.org/10.1017/S0962728600026336.

Biesek, J., Banaszak, M., Kadziolka, K., Wlazlak, S., Adamski, M., 2022. Growth of Broiler chickens, and physical features of the digestive system, and leg bones after aluminosilicates used. Sci. Rep. 12, 20425. https://doi.org/10.1038/s41598-022-25003-w.

Cai, H., Luo, S., Liu, Q., Zhou, Q., Yan, Z., Kang, Z., Liao, S., Li, J., Lv, M., Lin, X., 2023. Effects of a complex probiotic preparation, Fengqiang Shengtai and coccidiosis vaccine on the performance and intestinal microbiota of broilers challenged with Eimeria spp. Parasites Vectors 16, 253. https://doi.org/10.1186/s13071-023-05855-5.

Cao, C., Chowdhury, V.S., Cline, M.A., Gilbert, E.R., 2021. The microbiota-gut-brain axis during heat stress in chickens: a review. Front. Physiol. 12, 752265. https://doi.org/10.3389/2Ffphys.2021.752265.

Chambers, J.M., Freeny, A.E., Heiberger, R.M., 2017. Analysis of variance; designed experiments. In: Statistical Models in S. Routledge, pp. 145–193.

De Cesare, A., Sirri, F., Manfreda, G., Moniaci, P., Giardini, A., Zampiga, M., Meluzzi, A., 2017. Effect of dietary supplementation with Lactobacillus acidophilus D2/CSL (CECT 4529) on caecum microbioma and productive performance in broiler chickens. PLoS One 12, e0176309. https://doi.org/10.1371/journal.pone.0176309.g002.

De Verdal, H., Mignon-Grasteau, S., Jeulin, C., Le Bihan-Duval, E., Leconte, M., Mallet, S., Martin, C., Narcy, A., 2010. Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. Poult. Sci. 89, 1955–1961. https://doi.org/10.3382/ps.2010-813.

Do Nascimento, D.C.N., Dourado, L.R.B., De Siqueira, J.C., De Lima, S.B.P., da Silva, M.d. C.M., Da Silva, G.G., Sakomura, N.K., de Campos Ferreira, G.J.B., Biagiotti, D., 2018. Productive features of broiler chickens in hot weather: effects of strain and sex. Semina Ciências Agrárias 39, 731–745. https://doi.org/10.5433/1679-0359.2018v39n2p731.

Farouk, M.M., Al-Mazeedi, H.M., Sabow, A.B., Bekhit, A.E.D., Adeyemi, K.D., Sazili, A.Q., Ghani, A., 2014. Halal and kosher slaughter methods and meat quality: a review. Meat Sci. 98, 505–519. https://doi.org/10.1016/j.meatsci.2014.05.021.

Fornazier, R., Junior, V.R., Albino, L.F., Rodrigues, D., Tavernari, F.d., da Silva, D., Rostagno, H., Serafini, S., 2019. A symbiotic improves performance and carcass yield of broilers. JAPR 28, 383–389. https://doi.org/10.3382/japr/pfy082.

Gous, R., Fisher, C., Berhe, E., 2005. Genetic variation in broilers and predicted response. In: Proceedings of the 15th European Symposium on Poultry Nutrition, Balatonfüred, Hungary.

Guevara-Torres, B.L., Landin-Grandvallet, L.A., Tirado-Madrid, A., Villagómez-Cortés, J. A., 2021. Comparison of locomotion problems and its economic impact on cobb and Ross Broiler strains. Vet. Sci. Res. J. 3, 40–46. https://doi.org/10.30564/vsr.v3i2.4126.

Gyawali, R., Nwamaioha, N., Fiagbor, R., Zimmerman, T., Newman, R.H., Ibrahim, S.A., 2019. The role of prebiotics in disease prevention and health promotion. In: Ronald Ross Watson, V.R.P. (Ed.), Dietary Interventions in Gastrointestinal Diseases. Elsevier, pp. 151–167. https://doi.org/10.1016/B978-0-12-814468-8.00012-0.

Hamasalim, H.J., 2016. Synbiotic as feed additives relating to animal health and performance. Adv. Microbiol. 6, 288–302. https://doi.org/10.4236/aim.2016.64028.

Hammemi, C., Askri, A., Létourneau-Montminy, M.-P., Alnahhas, N., 2024. Current state of breast meat quality in standard-yielding broiler strains. JAPR 33, 100383. https://doi.org/10.1016/j.japr.2023.100383.

Hartcher, K., Lum, H., 2020. Genetic selection of broilers and welfare consequences: a review. Worlds Poult. Sci. J. 76, 154–167. https://doi.org/10.1080/00439339.2019.1680025.

Hassan, F., Atallah, S., Reda, R., 2021. Comparison of performance, meat quality, and profitability of Cobb, Hubbard, and Ross broiler strains. Earth Planets Space 85. https://doi.org/10.1399/eps.2021.332.

Houshmand, M., Azhar, K., Zulkifli, I., Bejo, M., Kamyab, A., 2012. Effects of prebiotic, protein level, and stocking density on performance, immunity, and stress indicators of broilers. Poult. Sci. 91, 393–401. https://doi.org/10.3382/ps.2010-01050.

Hu, J., Mohammed, A., Murugesan, G., Cheng, H., 2022. Effect of a synbiotic supplement as an antibiotic alternative on broiler skeletal, physiological, and oxidative parameters under heat stress. Poult. Sci. 101, 101769. https://doi.org/10.1016/j.psj.2022.101769.

Jansseune, S.C., Lammers, A., van Baal, J., Blanc, F., van der Laan, M.-H.P., Calenge, F., Hendriks, W.H., 2024. Diet composition influences probiotic and postbiotic effects on broiler growth and physiology. Poult. Sci. 103, 103650.

Javid, M.A., Masood, S., Zaneb, H., Rehman, H.U., Nazir, M.M., Waqas, M.Y., Asif, M., Basit, M.A., Abbas, G., Sufyan, A.B., 2022. Effect of dietary growth promoter supplementations on the serum biochemistry and morphometric characteristics of tibia bone in broiler chicken. Braz. J. Poult. Sci. 24. https://doi.org/10.1590/1806-9061-2021-1454 eRBCA-2021-1454.

Jin, L., Ho, Y., Abdullah, N., Jalaludin, S., 1997. Probiotics in poultry: modes of action. Worlds Poult. Sci. J. 53, 351–368. https://doi.org/10.1079/WPS19970028.

Khalid, N., Ali, M.M., Ali, Z., Amin, Y., Ayaz, M., 2021. Comparative productive performance of two broiler strains in open housing system. Adv. Life Sci. 8, 124–127. https://doi.org/10.62940/als.v8i2.879.

Kridtayopas, C., Rakangtong, C., Bunchasak, C., Loongyai, W., 2019. Effect of prebiotic and synbiotic supplementation in diet on growth performance, small intestinal morphology, stress, and bacterial population under high stocking density condition of broiler chickens. Poult. Sci. 98, 4595–4605. https://doi.org/10.3382/ps/pez152.

Kuzmuk, K.N., Swanson, K.S., Tappenden, K.A., Schook, L.B., Fahey Jr, G.C., 2005. Diet and age affect intestinal morphology and large bowel fermentative end-product concentrations in senior and young adult dogs. J. Nutr. 135, 1940–1945. https://doi.org/10.1093/jn/135.8.1940.

Li, Q., Wan, G., Peng, C., Xu, L., Yu, Y., Li, L., Li, G., 2020. Effect of probiotic supplementation on growth performance, intestinal morphology, barrier integrity, and inflammatory response in broilers subjected to cyclic heat stress. Anim. Sci. J. 91, e13433. https://doi.org/10.1111/asj.13433.

Liu, K.-l., He, Y.-f., Xu, B.-w., Lin, L.-x., Chen, P., Iqbal, M.K., Mehmood, K., Huang, S.-C., 2023. Leg disorders in broiler chickens: a review of current knowledge. Anim. Biotechnol. 34, 5124–5138. https://doi.org/10.1080/10495398.2023.2270000.

Lopez, K., Schilling, M., Corzo, A., 2011. Broiler genetic strain and sex effects on meat characteristics. Poult. Sci. 90, 1105–1111. https://doi.org/10.3382/ps.2010-01154.

Marcato, S., Sakomura, N., Kawauchi, I., Barbosa, N., Freitas, E., 2006. Growth of body parts of two broiler chicken strain. XII European Poultry Conference.

Mohammed, A., Hu, J., Murugesan, R., Cheng, H.-W., 2022. Effects of a synbiotic as an antibiotic alternative on behavior, production performance, cecal microbial ecology, and jejunal histomorphology of broiler chickens under heat stress. PLoS One 17, e0274179. https://doi.org/10.1371/journal.pone.0274179.

Mohammed, A., Jacobs, J., Murugesan, G., Cheng, H., 2018. Effect of dietary synbiotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. Poult. Sci. 97, 1101–1108. https://doi.org/10.3382/ps/pex421.

Mohammed, A., Jiang, S., Jacobs, J., Cheng, H., 2019. Effect of a synbiotic supplement on cecal microbial ecology, antioxidant status, and immune response of broiler chickens reared under heat stress. Poult. Sci. 98, 4408–4415. https://doi.org/10.3382/ps/pez246.

Mohammed, A., Mahmoud, M., Murugesan, R., Cheng, H.-w., 2021. Effect of a synbiotic supplement on fear response and memory assessment of broiler chickens subjected to heat stress. Animals 11, 427. https://doi.org/10.3390/ani11020427.

Mookiah, S., Sieo, C.C., Ramasamy, K., Abdullah, N., Ho, Y.W., 2014. Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. J. Sci. Food Agric. 94, 341–348. https://doi.org/10.1002/jsfa.6365.

Naghi Shokri, A., Ghasemi, H.A., Taherpour, K., 2017. Evaluation of Aloe vera and synbiotic as antibiotic growth promoter substitutions on performance, gut morphology, immune responses and blood constitutes of broiler chickens. Anim. Sci. J. 88, 306–313. https://doi.org/10.1111/asj.12629.

Navidshad, B., Liang, J.B., Jahromi, M.F., 2012. Correlation coefficients between different methods of expressing bacterial quantification using real time PCR. Int. J. Mol. Sci. 13, 2119–2132. https://doi.org/10.3390/ijms13022119.

Nisar, H., Sharif, M., Rahman, M., Rehman, S., Kamboh, A., Saeed, M., 2021. Effects of dietary supplementations of synbiotics on growth performance, carcass characteristics and nutrient digestibility of broiler chicken. Braz. J. Poult. Sci. 23. https://doi.org/10.1590/1806-9061-2020-1388.

Olanrewaju, H., Miller, W., Maslin, W., Collier, S., Purswell, J., Branton, S., 2014. Effects of strain and light intensity on growth performance and carcass characteristics of broilers grown to heavy weights. Poult. Sci. 93, 1890–1899. https://doi.org/10.3382/ps.2013-03806.

Olnood, C.G., Beski, S.S., Choct, M., Iji, P.A., 2015. Novel probiotics: their effects on growth performance, gut development, microbial community and activity of broiler chickens. Anim. Nutr. 1, 184–191. https://doi.org/10.1016/j.aninu.2015.07.003.

Patterson, J., Burkholder, K., 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82, 627–631. https://doi.org/10.1093/ps/82.4.627.

Petričević, V., Pavlovski, Z., Škrbić, Z., Lukić, M., 2011. The effect of genotype on production and slaughter properties of broiler chickens. Biotechnol. Anim. Husb. 27, 171–181. https://doi.org/10.2298/BAH1102171P.

Popov, I., Skripkin, V., Mazanko, M., Epimakhova, E., Prazdnova, E., Dilekova, O., Dannikov, S., Trukhachev, V., Rastovarov, E., Derezina, T., 2024. Effects of spore-forming bacillus probiotics on growth performance, intestinal morphology, and immune system of broilers housed on deep litter. JAPR 33, 100396. https://doi.org/10.1016/j.japr.2023.100396.

Rabia, J., Abbas, T.H.K., 2021. Effect of adding different levels of grapes (Vitis vinifera L.) seeds and leaf powder or their extracts on some bone characteristics and total ash content in broiler chickens. AJDFR 40, 341–344. https://doi.org/10.18805/ajdfr.DR-220.

Rezaei, S., Jahromi, M.F., Liang, J.B., Zulkifli, I., Farjam, A.S., Laudadio, V., Tufarelli, V., 2015. Effect of oligosaccharides extract from palm kernel expeller on growth performance, gut microbiota and immune response in broiler chickens. Poult. Sci. 94, 2414–2420. https://doi.org/10.3382/ps/pev216.

Richards, P., Fothergill, J., Bernardeau, M., Wigley, P., 2019. Development of the caecal microbiota in three broiler breeds. Front. Vet. Sci. 6, 201. https://doi.org/10.3389/fvets.2019.00201.

Sakomura, N., Marcato, S., Munari, D., Freitas, E., Es, J.F., 2006. Growth curves and body nutrients deposition on two broiler chickens strains. Proc. XII Eur. Poult. Conf. WPSA, Verona, Italy.

Salehimanesh, A., Mohammadi, M., Roostaei-Ali Mehr, M., 2016. Effect of dietary probiotic, prebiotic and synbiotic supplementation on performance, immune responses, intestinal morphology and bacterial populations in broilers. J. Anim. Physiol. Anim. Nutr. 100, 694–700. https://doi.org/10.1111/jpn.12431.

Santos, M.N., Widowski, T.M., Kiarie, E.G., Guerin, M.T., Edwards, A.M., Torrey, S., 2022. In pursuit of a better broiler: walking ability and incidence of contact dermatitis in conventional and slower growing strains of broiler chickens. Poult. Sci. 101, 101768. https://doi.org/10.1016/j.psj.2022.101768.

Sarangi, N.R., Babu, L., Kumar, A., Pradhan, C., Pati, P., Mishra, J., 2016. Effect of dietary supplementation of prebiotic, probiotic, and synbiotic on growth performance and carcass characteristics of broiler chickens. Vet. World 9, 313. https://doi.org/10.14202/2Fvetworld.2016.313-319.

Schokker, D., Veninga, G., Vastenhouw, S.A., Bossers, A., de Bree, F.M., Kaal-Lansbergen, L.M., Rebel, J.M., Smits, M.A., 2015. Early life microbial colonization of the gut and intestinal development differ between genetically divergent broiler lines. BMC Genom. 16, 1–13. https://doi.org/10.1186/s12864-015-1646-6.

Sohail, M., Hume, M., Byrd, J., Nisbet, D., Ijaz, A., Sohail, A., Shabbir, M., Rehman, H., 2012. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult. Sci. 91, 2235–2240. https://doi.org/10.3382/ps.2012-02182.

Sohail, M., Ijaz, A., Younus, M., Shabbir, M., Kamran, Z., Ahmad, S., Anwar, H., Yousaf, M., Ashraf, K., Shahzad, A., 2013. Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers. JAPR 22, 485–491. https://doi.org/10.3382/japr.2012-00682.

Song, J., Xiao, K., Ke, Y., Jiao, L., Hu, C., Diao, Q., Shi, B., Zou, X., 2014. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult. Sci. 93, 581–588. https://doi.org/10.3382/ps.2013-03455.

Spring, P., Wenk, C., Dawson, K., Newman, K., 2000. The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poult. Sci. 79, 205–211. https://doi.org/10.1093/ps/79.2.205.

Sterling, K., Pesti, G., Bakalli, R., 2006. Performance of different broiler genotypes fed diets with varying levels of dietary crude protein and lysine. Poult. Sci. 85, 1045–1054. https://doi.org/10.1093/ps/85.6.1045.

Tainika, B., Şekeroğlu, A., Akyol, A., Waithaka Ng'ang'a, Z., 2023. Welfare issues in broiler chickens: overview. Worlds Poult. Sci. J. 79, 285–329. https://doi.org/10.1080/00439339.2023.2175343.

Team, R.C., 2024. RA language and environment for statistical computing. R Foundation for Statistical. Computing.

Tuohy, K., Rouzaud, G., Bruck, W., Gibson, G., 2005. Modulation of the human gut microflora towards improved health using prebiotics-assessment of efficacy. Curr. Pharm. Des. 11, 75–90. https://doi.org/10.2174/1381612053382331.

Waldenstedt, L., 2006. Nutritional factors of importance for optimal leg health in broilers: a review. Anim. Feed Sci. Technol. 126, 291–307. https://doi.org/10.1016/j.anifeedsci.2005.08.008.

Wijtten, P., Langhout, D., Verstegen, M., 2012. Small intestine development in chicks after hatch and in pigs around the time of weaning and its relation with nutrition: a review. Acta Agriculturae Scandinavica, Section A–Animal Science 62, 1–12. https://doi.org/10.1080/09064702.2012.676061.

Wilcox, C.H., Sandilands, V., Mayasari, N., Asmara, I.Y., Anang, A., 2024. A literature review of broiler chicken welfare, husbandry, and assessment. Worlds Poult. Sci. J. 80, 3–32. https://doi.org/10.1080/00439339.2023.2264824.

Woyengo, T., Golian, A., Bennett, C., Muc, H., Guenter, W., 2010. Performance of two 1970s and Ross 308 broiler strains fed drug-free low-protein and recommended-protein diets. JAPR 19, 101–111. https://doi.org/10.3382/japr.2009-00042.

Yan, F., Mohammed, A., Murugesan, G., Cheng, H., 2019. Effects of a dietary synbiotic inclusion on bone health in broilers subjected to cyclic heat stress episodes. Poult. Sci. 98, 1083–1089. https://doi.org/10.3382/ps/pey508.

Younis, J.H., Karadas, F., Beski, S.S.M., 2024. The effects of different level of synbiotic supplementation in diet of broiler on growth performance, intestinal histology and

microbial colony. Arch. Razi Inst. 79, 1227. https://doi.org/10.32592/ARI.2024.79.6.1227.

Zhao, L., Wang, G., Siegel, P., He, C., Wang, H., Zhao, W., Zhai, Z., Tian, F., Zhao, J., Zhang, H., 2013. Quantitative genetic background of the host influences gut microbiomes in chickens. Sci. Rep. 3, 1163. https://doi.org/10.1038/srep01163.

Zulkifli, I., Nor, S., 2004. Fear and stress reactions, and the performance of commercial broiler chickens subjected to regular pleasant and unpleasant contacts with human being. Appl. Anim. Behav. Sci. 88, 77–87. https://doi.org/10.1016/j.applanim.2004.02.014.