JWPR
Journal of World's Poultry Research

2026, Scienceline Publication
J. World Poult. Res. 16(2): 168-180, 2026
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2026.16
PII: S2322455X2600016-16

ISSN: 2322-455X

Effects of *Bacillus amyloliquefaciens* and *phytase* Administration on Growth Performance and Organ Weights in Broiler Chickens During the Hot-Humid Season

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Received: March 13, 2026, Revised: April 11, 2026, Accepted: May 15, 2026, Published: June 25, 2026

ABSTRACT

In tropical regions, heat stress reduces broiler chicken productivity, highlighting the need for developing alternative growth promoters to replace antibiotics. The present study aimed to evaluate the effects of dietary phytase (Axtra® Phytase) and the probiotic *Bacillus amyloliquefaciens* (*B. amyloliquefaciens*) on the growth performance and feed intake in broiler chickens during the hot-humid season in Jos, Plateau State, Nigeria. A total of 180 broiler chickens, 15 days old, were randomly allocated to four experimental groups. Each group consisted of 45 chickens with three replicates per group and 15 chickens per replicate. The dietary groups included a control group fed the basal diet without additives (Ctrl), a probiotic group supplemented with *B. amyloliquefaciens* at 1×10^9 CFU/kg (Prob), a phytase group supplemented with Axtra® Phytase enzyme at 200 FTU/kg (Phy), and a combined group that received probiotic and phytase (Phy + Prob). The broiler chickens experienced challenging environmental conditions throughout the study, with average measurements of 29.00 ± 0.40°C for dry-bulb temperature, 68 ± 1.54% for relative humidity, and 27.99 ± 0.50°C for the temperature-humidity index. All these factors were higher than the optimal range for broiler thermal comfort. The live weight, weight gain, feed intake, organ weight, and feed conversion ratio were assessed. On day 42, live and organ weights in the treated groups were significantly higher than those in Ctrl. All supplemented groups indicated increased weight gain. Feed intake was higher in the Ctrl and Prob groups compared to the Phy and Phy + Prob groups. These findings indicated that supplementation with phytase, alone or in combination with *B. amyloliquefaciens*, improved growth performance, feed efficiency, and organ development while modulating feed intake during heat stress. The present results demonstrated that *B. amyloliquefaciens*, phytase, or their combination enhanced growth performance, organ weights, feed efficiency, and overall productivity in broiler chickens subjected to heat stress during the hot-humid season in Nigeria. The current results supported the use of phytase and *B. amyloliquefaciens* in tropical poultry production.

Keywords: *Bacillus amyloliquefaciens*, Broiler chicken, Feed intake, Growth performance, Hot-humid season, Phytase

INTRODUCTION

Poultry production is one of the fastest-growing livestock sectors in Nigeria, driven by increasing demand for affordable and nutritious animal protein (USDA, 2013; Moreki et al., 2025). Over recent decades, the consumption of poultry meat has increased due to Nigeria's growing population and rising per capita income (Heise et al., 2015; Akpan et al., 2024). However, challenges such as heat stress and antibiotic resistance threaten the sustainability and safety of the production process. Prolonged, low-dose inclusion of antibiotics in broiler chickens' diets promotes the development of

To cite this paper: Zakari FO, Okoro P, Agweche OS, Idoga ES, Oladipo OO, Jonathan KB, Adaji OG, Emennaa P, Sati N, Uchendu C, and Aluwong T (2026). Effects of *Bacillus amyloliquefaciens* and *phytase* Administration on Growth Performance and Organ Weights in Broiler Chickens During the Hot-Humid Season. *J. World Poult. Res.*, 16(2): 168-180. DOI: https://dx.doi.org/10.36380/jwpr.2026.16

antibiotic-resistant bacteria (Abreu et al., 2023; Abou-Jaoudeh et al., 2024). These resistant strains can transfer to humans through poultry products, creating a significant global health risk that leads to thousands of deaths annually (Tedersoo et al., 2022; Abreu et al., 2023; Mwansa et al., 2023). Consequently, strategies to minimize antibiotic use in poultry are being developed, with a focus on alternatives such as probiotics and enzymes as growth promoters (Rashid et al., 2023; Shakeri, 2025). *Bacillus amyloliquefaciens* (*B. amyloliquefaciens*) is a spore-forming probiotic bacterium capable of producing multiple extracellular enzymes, such as α-amylases, cellulases, metalloproteases, and proteases (Ngalimat et al., 2021). These enzymes improve nutrient digestibility and absorption while modulating gut immune function (Su et al., 2019; Zhang et al., 2020; Zalila-Kolsi et al., 2023). Additionally, *B. amyloliquefaciens* synthesizes bacteriocins such as *subtilin* and *barnase*, which exhibit bactericidal effects by disrupting cell walls and pathogens' metabolic pathways, targeting species such as *Clostridium perfringens*, *Escherichia coli*, and *Yersinia* spp. (Palacios-Rodriguez et al., 2024; Deng et al., 2025; Jiang et al., 2025). Furthermore, *B. amyloliquefaciens* produces antimicrobial compounds, including Iturin, Fengycin, and Surfactin, which inhibit fungal and bacterial growth, thereby improving intestinal microflora balance and enhancing growth performance in broiler chickens (Lin et al., 2020; Wang et al., 2022). Phytases are enzymes responsible for hydrolyzing phytic acid (myo-inositol hexakisphosphate), releasing inositol and six inorganic phosphate molecules (Yao et al., 2012). Hydrolysis of phytate eliminates its anti-nutritive effects, significantly increasing phosphorus retention, weight gain, feed intake, feed efficiency, metabolizable energy, and calcium retention in poultry, particularly at higher dietary phytase inclusion levels (Kornegay, 2001; Selle and Ravindran, 2007).

The hot and humid seasons, characterized by elevated temperature and humidity, impose considerable stress on livestock, thereby reducing productivity and growth performance (Oke et al., 2021). These environmental factors impose thermal stress on broiler chickens, negatively impacting their productivity by reducing feed intake, feed digestibility, and overall growth performance, while increasing the risk of mortality (Sinha et al., 2017; Goo et al., 2019; Kim et al., 2025). Chronic heat stress disrupts nutrient utilization and energy balance, thereby exacerbating financial losses in the poultry industry (Laganá et al., 2007; Osuji et al., 2024). Moreover, elevated temperatures promote the proliferation of pathogenic bacteria and parasites in the environment and predispose broiler chickens to diseases (Sohail et al., 2012; Wasti et al., 2020; Ghareeb et al., 2022). A temperature-humidity index (THI) above critical thresholds (approximately 26-28°C) leads to reduced feed consumption, body weight, and feed conversion efficiency in broiler chickens (Abu-Dieyeh, 2006; Purswell et al., 2012).

The present study aimed to evaluate the effects of dietary *B. amyloliquefaciens* and phytase supplementation, individually and in combination, on growth performance parameters (feed intake, live body weight, weight gain, and feed conversion ratio [FCR]) and organ weights under the heat stress conditions of the hot-humid season, without the use of antibiotics.

MATERIALS AND METHODS

Ethical approval

The present study was approved by the University of Jos Animal Care and Use Committee (approval number: UJ/FPS/F19-00369), Jos, Nigeria. All experimental procedures involving animals were conducted in strict accordance with the guidelines for the care and use of laboratory and research animals prescribed by the National Institutes of Health (NIH), Bethesda, USA.

Study location and time

The present study was carried out at the Poultry Division of the National Veterinary Research Institute (NVRI) in Vom, Plateau State, Nigeria (9.7301°N, 8.7876°E), located in the Northern Guinea Savannah zone at an elevation of approximately 1,285 m above sea level. The study was conducted during the hot-dry season, from March to April, 2020.

Poultry management

A total of 180 one-day-old Arbor Acre broiler chickens, obtained from Agritec Nigeria Limited (Ibadan, Nigeria), were raised in an intensive deep litter system. This system housed broiler chickens indoors on litter floors, without outdoor access, in a concrete-floored pen measuring 25 m × 19 m × 9 m, which provided sufficient ventilation. Broiler chickens were fed a commercial starter feed for four weeks, followed by a finisher feed until day 42 (Table 1). Broiler chickens had *ad libitum* access to feed and water.

Broiler chickens were vaccinated against Marek’s disease after hatching via a subcutaneous injection. Live vaccines against Newcastle disease (LaSota strain) on days

7 and 21 and a primary vaccination for infectious bursal disease (Gumboro) on day 14, followed by a booster on day 17, all administered through drinking water to minimize stress and ensure widespread application during their early growth, following the protocols of the National Veterinary Research Institute (NVRI), Nigeria. The vaccines used in the study were manufactured at NVRI, Nigeria. This schedule follows common poultry health guidelines aimed at providing early and continuous protection against major viral infections. Therapeutic antibiotics were not required during the trial. Biosecurity measures were ensured by providing foot baths, footwear, and clothing for all individuals assisting with the experiment. The pen was inaccessible to non-essential personnel, animals, other birds, and rodents.

Table 1. Ingredients and nutrient composition of the starter and finisher diets during the 42 days of study

Ingredient (%)	**Starter (1-35 days)**	**Finisher (42 days)**
Maize	61.25	67.78
Soybean meal	28	22
Groundnut cake	3	3
Vegetable oil	3	2.5
Fishmeal	1	0.5
Limestone	1.2	0.8
Dicalcium phosphate	1.5	1
Salt	0.3	0.3
Methionine	0.15	0.12
Lysine	0.1	0.08
*Premix (vit/min)	0.5	0.5
Nutrient composition	**Starter (days 1-35)**	**Finisher (days 35-42)**
Moisture (%)	11.7	9.20
Crude protein (g)	15.9	16.51
Crude fibre (g)	8.81	9.01
Lipids (g)	2.45	3.40
Ash (g)	7.25	8.35
Nitrogen fibre extract (g)	54.09	53.53
Calcium (g)	0.93	1.55
Phosphorus (g)	0.06	0.42
Metabolizable energy (kcal/kg)	3,308	3,250

Analyzed in the biochemistry laboratory, National Veterinary Research Institute, Vom, Jos, Nigeria. *Premix contains vitamin A: 2.0 mg (≈ 6,000 IU), vitamin D3: 0.5 mg (≈ 200 IU), vitamin E: 5.0 mg (≈ 7-10 IU), vitamin K: 0.5 mg, thiamine (B1): 0.2 mg, riboflavin (B2): 0.5 mg, pyridoxine (B6): 0.2 mg, niacin: 6.0 mg, pantothenic acid: 2.0 mg, folic acid: 0.1 mg, biotin: 0.02 mg, vitamin B1: 20.003 mg, choline chloride: 1000 mg (1.00 g), and zinc (Zn, as sulfate): 60 mg.

Thermal environmental parameters

Thermal conditions in the pen were recorded every 2 hours between 07:00 and 19:00 using a Brannan dry-bulb and wet-bulb thermometer (Cumbria, England). Relative humidity (RH) was derived from the dry-bulb and wet-bulb readings, and the temperature-humidity index (THI) was computed according to the method described by Tao and Xin (2003).

$$THI = 0.85 \times DBT + 0.15 \times WBT$$

where DBT is the dry-bulb thermometer, and WBT is the wet-bulb thermometer in °C.

Experimental design and treatments

A total of 180 broiler chickens were randomly assigned to four treatment groups, each consisting of three replicates of 15 chickens. The groups received the following dietary treatments from day 1 to day 42. The first received the basal commercial diet without supplementation (control). From day 1 to day 42, broilers were assigned to four dietary treatments. The first group received the basal commercial diet without supplementation (control). The second group was fed the basal diet supplemented with *B. amyloliquefaciens* (BPD 1; Vetnostrum Animal Health Co., Ltd., Xinfeng Township, Hsinchu County 304, Taiwan) at 1×10^9 CFU/g of feed, consistent with the standardized concentration reported by Diaz (2007). The probiotic product (1×10^9 CFU/g) was incorporated at 1 kg/ton of complete feed (1 g/kg), delivering 1×10^9 CFU/kg of finished diet. The third group received the basal diet containing Axtra® phytase (Danisco Animal Nutrition, PO Box 777, Marlborough, Wiltshire, SN8 1XN, United Kingdom) at 200 FTU/kg of feed. This enzyme preparation, with a specific activity of approximately 10,000 FTU/g, was administered at a dosage designed to deliver 200 FTU per kilogram of the final feed mix (approximately 0.02 g/kg). This dosage aligned with the enzyme activity and inclusion recommendations provided by Coppedge et al. (2011). This phytase level (200 FTU/kg) has previously been shown to enhance growth performance, feed efficiency, and bone mineralization. The fourth group was fed the basal diet containing probiotic and phytase (Phy + Prob).

Production performance parameters

Feed intake and body weight were recorded weekly over a 6-week period using a digital weighing scale (Model WT20001KF, Changzhou, China). Carcass weight and the weights of internal organs were measured using the same methods described above. Body weight gain and FCR were calculated according to the procedures described by Avci et al. (2005). The number of broiler chickens that died or were humanely euthanized throughout the experimental period was recorded.

Statistical analysis

All statistical analyses were performed using R software (R Core Team, 2025). Values were presented as mean ± standard error of the mean (SEM). Live weight, weight gain, feed intake, and FCR were analyzed using a two-way repeated-measures ANOVA to evaluate the main effects of day, dietary treatment, and their interaction (day × treatment). Organ weights and carcass component traits (kidney, breast muscle, back muscle, neck, drumstick, thigh, shank, lung, heart, spleen, liver, gall bladder, abdominal fat, gizzard, proventriculus, and crop) were analyzed using one-way analysis of variance (ANOVA) with dietary treatment as the fixed effect. Significant treatment effects were followed by Tukey's honest significant difference (HSD) post hoc test. Mortality was analyzed using the Chi-square test (dead/total broiler chickens), with statistical significance set at a p-value less than 5% ($p < 0.05$).

RESULTS

Thermal environmental parameters

The mean dry-bulb temperature, RH, and THI during the study were 29.00 ± 0.40°C, 68 ± 1.54%, and 27.99 ± 0.50°C, respectively (Figure 1). Dry-bulb temperature ranged from 25 to 33°C, RH from 49.00 to 85.00%, and THI from 22.10 to 32.38°C.

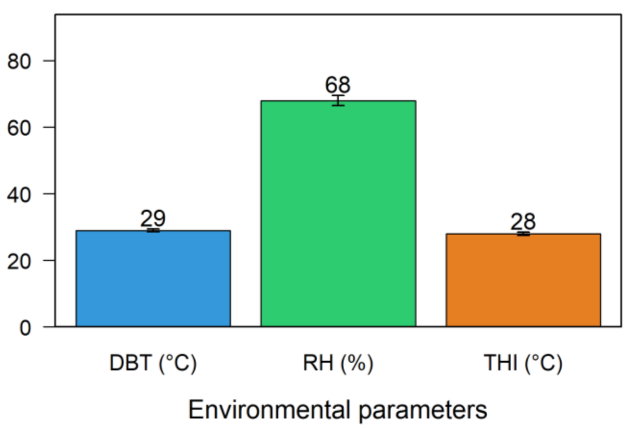

Figure 1. Environmental parameters of the poultry pen during a 42-day hot-humid season. DBT: Dry-bulb temperature, RH: Relative humidity, and THI: Temperature-humidity index

Live weights and weight gain

The present results indicated highly significant effects of experimental days, dietary treatments, and their interaction on live body weight ($p < 0.05$; Figure 2; Table 2). On day 14, broiler chickens in the Prob, Phy, and Prob + Phy groups had significantly higher live weights than the Ctrl group ($p < 0.05$). From days 21 to 42, Prob, Phy, and the Prob + Phy groups had significantly higher live weights than the Ctrl group ($p < 0.05$). On days 21 and 28, broiler chickens in the Phy and Prob + Phy groups had significantly higher live weights than the Ctrl group ($p < 0.05$). Live weights on days 35 and 42 in the Prob, Phy, and Prob + Phy groups remained significantly higher than those in the Ctrl group ($p < 0.05$).

The present results revealed highly significant effects of days, treatment, and their interaction on weight gain in broiler chickens ($p < 0.05$; Figure 2; Table 3). Weight gain significantly increased from day 14 to 42 in the Prob, Phy, and Prob + Phy groups ($p < 0.05$), with the highest weight gains observed in broiler chickens in the Prob + Phy group ($p < 0.05$). On day 42, weight gain in the Prob group (590.10 ± 30.66 g), the Phy group (549.00 ± 29.88 g), and the Prob + Phy group (629.40 ± 36.28 g) was significantly higher than in the Ctrl group (404.50 ± 39.95 g; $p < 0.05$).

Production performance parameters

The present results revealed highly significant effects of day, treatment, and their interaction on feed intake in broiler chickens ($p < 0.05$). On days 21, 28, 35, and 42, feed intake was significantly higher in the Ctrl group than in the Prob and Phy groups ($p < 0.05$; Table 4). Overall, broiler chickens in the Phy group (994.80 ± 42.50 g) and Prob + Phy group (976.10 ± 42.78 g) significantly consumed less feed than those in the Ctrl group at 1,106.00 ± 50.90 g ($p < 0.05$; Table 4; Figure 2). The current results indicated a strong effect of day on FCR (Table 5). While treatment groups significantly affected FCR compared to the control group ($p < 0.05$), the interaction between day and treatment was not significant ($p > 0.05$). The present results demonstrated that the impact of treatments on FCR remained relatively consistent across different ages.

Carcass weight

Dietary treatment significantly affected carcass weight compared to the control group ($p < 0.05$). Broiler chickens fed the Phy + Prob diet had the highest carcass weight (1,590.96 g), followed by Phy (1,481.20 g) and Prob (1,365.57 g) groups. The control group exhibited the lowest carcass weight (1,299.24 g). The current results indicated that carcass weight was significantly higher in Phy and Phy + Prob groups than in the control group ($p < 0.05$). Furthermore, the carcass weight in the Phy + Prob group was significantly higher than that in the Prob group ($p < 0.05$; Table 6).

Table 2. Weekly live weights of broiler chickens administered probiotic (*Bacillus amyloliquefaciens*) and Axtra phytase during a 42 days of hot-humid season

Age (days)	Ctrl (g)	Prob (g)	Phy (g)	Phy+Prob (g)
1	39.07 ± 0.67[a]	39.24 ± 0.56[a]	39.78 ± 0.63[a]	39.49 ± 0.63[a]
7	93.91 ± 1.82[a]	94.42 ± 1.99[a]	97.73 ± 1.90[a]	98.24 ± 2.12[a]
14	218.70 ± 6.21[a]	242.80 ± 4.02[a]	243.10 ± 4.18[a]	245.60 ± 3.87[a]
21	439.50 ± 9.92[a]	468.00 ± 6.27[b]	479.10 ± 6.69[b]	490.50 ± 7.07[b]
28	714.80 ± 16.56[a]	761.50 ± 11.12[b]	820.80 ± 14.49[c]	856.50 ± 14.46[c]
35	1,105.00 ± 23.80[a]	1,184.00 ± 18.17[b]	1,269.00 ± 18.28[c]	1,306.00 ± 22.69[c]
42	1,509.00 ± 31.94[a]	1,774.00 ± 23.06[b]	1,818.00 ± 28.51[c]	1,932.00 ± 34.65[c]

Values are presented as mean ± SEM. [a,b,c]Means within a row with different superscript letters differ significantly ($p < 0.05$). Ctrl: Control, Prob: Probiotic, Phy: Phytase, Prob + Phy: Combined supplementation of probiotic and phytase

Table 3. Weekly weight gain of broiler chickens given probiotic (*Bacillus amyloliquefaciens*) and Axtra phytase during a 42-day hot-humid season

Age (Days)	Ctrl (g)	Prob (g)	Phy (g)	Phy+Prob (g)
1	NA	NA	NA	NA
7	54.84 ± 2.04[a]	55.18 ± 2.19[a]	57.96 ± 2.09[a]	58.76 ± 2.13[a]
14	124.80 ± 5.85[a]	148.40 ± 3.95[b]	145.40 ± 4.75[b]	147.30 ± 4.08[b]
21	220.80 ± 13.17[a]	225.20 ± 8.03[b]	236.00 ± 7.56[b]	244.90 ± 8.09[b]
28	275.40 ± 16.93[a]	293.60 ± 13.01[b]	341.70 ± 14.76[b]	366.00 ± 13.99[b]
35	389.70 ± 26.71[a]	422.10 ± 19.86[b]	447.90 ± 22.58[b]	450.80 ± 25.27[b]
42	404.50 ± 39.95[a]	590.10 ± 30.66[b]	549.00 ± 29.88[b]	629.40 ± 36.28[b]

[a,b] Means within a row with different superscript letters differ significantly ($p < 0.05$). Ctrl: Control, Prob: Probiotic (*Bacillus amyloliquefaciens*), Phy: Phytase, Prob + Phy: Combined supplementation of probiotic and phytase, NA: Not applicable.

Table 4. Weekly feed intake of broiler chickens administered with Probiotic (*Bacillus amyloliquefaciens*) and Axtraphytase during a 42-day hot-humid season

Age (Days)	Ctrl (g)	Prob (g)	Phy (g)	Phy+Prob (g)
7	226.00 ± 18.28[a]	221.50 ± 18.24[a]	209.10 ± 16.87[a]	202.10 ± 17.14[a]
14	628.10 ± 25.78[a]	618.50 ± 25.58[a]	627.40 ± 21.01[a]	582.00 ± 25.77[a]
21	1061.00 ± 38.82[a]	1029.00 ± 39.87[b]	985.20 ± 31.36[b]	962.70 ± 32.68[b]
28	1320.00 ± 41.44[a]	1291.00 ± 48.25[b]	1180.00 ± 48.25[b]	1196.00 ± 15.73[b]
35	1682.00 ± 46.68[a]	1712.00 ± 49.32[b]	1424.00 ± 37.02[b]	1399.00 ± 36.57[b]
42	1721.00 ± 52.56[a]	1769.00 ± 52.56[b]	1543.00 ± 26.64[b]	1515.00 ± 26.64[b]

[a,b] Means within a row with different superscript letters differ significantly ($p < 0.05$). Ctrl: Control, Prob: Prob (*Bacillus amyloliquefaciens*), Phy: Phytase, Prob + Phy: Probiotic + phytase

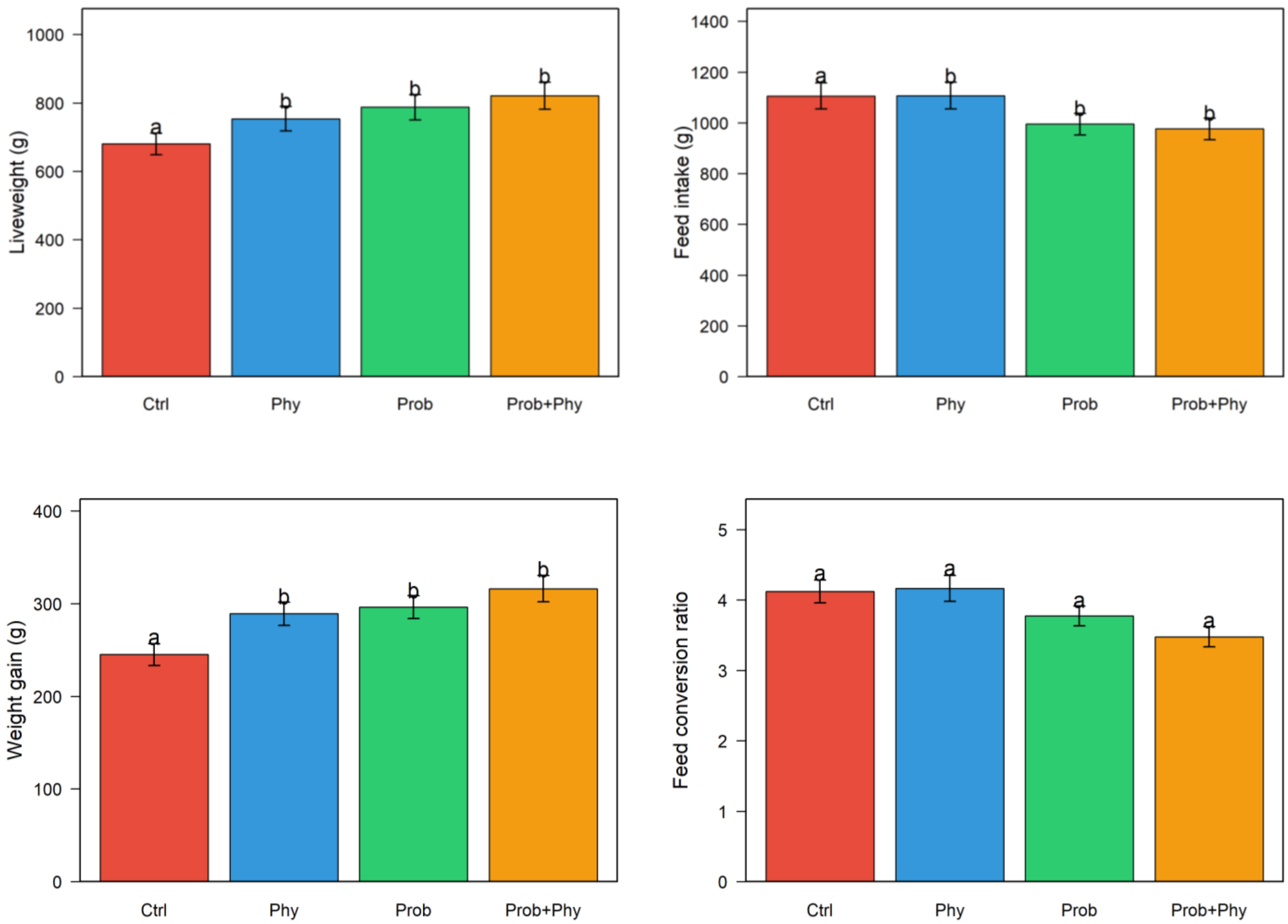

Figure 2. Growth performance parameters (live weight, weight gain, feed intake, and feed conversion ratio) of Arbor Acre broilers fed different dietary treatments over a 42-day period. Values are means ± SEM (n = 45 per treatment). [a, b] Means with different superscript letters differ significantly ($p < 0.05$)

Table 5. Weekly feed conversion ratio of broiler chickens administered with probiotic (*Bacillus amyloliquefaciens*) and axtraphytase during a 42-day hot-humid season.

Age (Days)	Ctrl (g)	Prob (g)	Phy (g)	Phy+Prob (g)
7	4.01 ± 0.13[a]	3.99 ± 0.18[a]	3.58 ± 0.07[a]	3.62 ± 0.18[a]
14	4.47 ± 0.45[a]	4.58 ± 0.41[a]	4.43 ± 0.25[a]	3.93 ± 0.13[a]
21	4.63 ± 0.24[a]	4.52 ± 0.16[a]	4.29 ± 0.14[a]	3.99 ± 0.18[a]
28	4.07 ± 0.33[a]	4.50 ± 0.66[a]	3.87 ± 0.08[a]	3.49 ± 0.31[a]
35	4.05 ± 0.36[a]	3.95 ± 0.34[a]	3.41 ± 0.37[a]	3.30 ± 0.24[a]
42	3.48 ± 0.66[a]	3.43 ± 0.63[a]	3.06 ± 0.28[a]	2.53 ± 0.34[a]

[a]Means within a row with the same superscript letter are not significantly different ($p > 0.05$). Ctrl: Control, Prob: Prob (*Bacillus amyloliquefaciens*), Phy: Phytase, Prob + Phy: Probiotic + phytase

Table 6. Effects of probiotic (*Bacillus amyloliquefaciens*) and axtraphytase on carcass weight of broiler chickens and different body parts during a 42-day hot-humid season

Organ weight (g)	Ctrl	Prob	Phy	Phy + Prob
Kidney	12.66 ± 0.37 [b]	10.64 ± 0.81[c]	13.29 ± 0.51[b]	14.46 ± 0.52[a]
Breast muscle	426.90 ± 19.59[c]	436.94 ± 12.57[c]	485.28 ± 37.05[b]	517.13 ± 20.32[a]
Back muscle	214.27 ± 4.02 [c]	231.31± 4.66[b]	256.61 ± 9.66[a]	268.71 ± 13.72 [a]
Neck	83.80 ± 2.00[c]	91.33 ± 2.11[b]	104.01 ± 3.48[a]	111.56 ± 2.95 [a]
Carcass weight	1,299.24 ± 40.48[c]	1,365.57 ± 21.53[b]	1,481.20 ± 48.45[a]	1,590.96 ± 58.37[a]
Drumstick	182.27 ± 4.65[c]	186.13 ± 4.21[c]	206.53 ± 9.05[b]	224.31 ± 11.18[a]
Thigh	215.63 ± 5.93[c]	220.86 ± 4.86[c]	233.87 ± 9.17[b]	264.22 ± 12.70 [a]
Shank	73.23 ± 2.96[b]	70.12 ± 1.80[b]	76.98 ± 2.68[a]	82.42 ± 3.00[a]
Lung	10.46 ± 1.05[b]	12.14 ± 0.54[a]	11.18 ± 0.53[b]	13.40 ± 0.64 [a]
Heart	9.18 ± 0.38[b]	8.99 ± 0.65[b]	11.67 ± 0.85[a]	11.37 ± 0.48[a]
Spleen	2.78 ± 0.26[b]	2.23 ± 0.31[b]	3.63 ± 0.45[a]	3.57 ± 0.34 [a]
Liver	43.38 ± 1.69[c]	45.58 ± 2.34[c]	59.20 ± 3.44[a]	47.79 ± 2.00[b]
Gall bladder	1.70 ± 0.08[b]	1.31 ± 0.14[c]	1.93 ± 0.12[a]	2.02 ± 0.15 [a]
Abdominal fat	21.60 ± 1.08[c]	28.13 ± 3.10[b]	35.56 ± 3.87 [a]	25.28 ± 4.09 [bc]
Gizzard	28.70 ± 1.50[a]	30.34 ± 1.28[a]	31.82 ± 1.60[a]	32.09 ± 1.00[a]
Proventriculus	8.83 ± 0.42[a]	9.30 ± 1.03[a]	10.93 ± 0.60[a]	10.39 ± 0.51 [a]
Crop	7.12 ± 0.83[b]	6.39 ± 0.96[b]	9.47 ± 0.80[a]	9.47 ± 0.75[a]

[a,b,c] Means within a row with different superscript letters are significantly different ($p < 0.05$). Ctrl: control, Prob: probiotic (*Bacillus amyloliquefaciens*), Phy: Phytase, Prob + Phy: Probiotic and Xtrap phytase

Organ weight

Mean breast muscle weights significantly differed by dietary treatment ($p < 0.05$), ranging from 426.90 g in the Ctrl group to 517.13 g in the Phy + Prob group, 436.94 g in the Prob group, and 485.28 g in the Phy group. Broiler chickens fed Phy and Phy + Prop diets had significantly higher breast muscle weights than the Ctrl group, with Phy + Prob producing the greatest increase ($p < 0.05$; Table 6).

The Phy + Prob group indicated the highest drumstick weight (224.31 g), followed by Phy (206.53 g), Prob (186.13 g), and Ctrl (182.27 g) groups. Drumstick weights were significantly higher in broiler chickens fed Phy and Phy + Prob diets compared to those fed Ctrl and Prob diets ($p < 0.05$; Table 6).

Abdominal fat weight was significantly affected by diet ($p < 0.05$). Broiler chickens fed Phy had the highest abdominal fat weight (35.56 g), followed by Prob (28.13 g), Phy + Prob (25.28 g), and Ctrl (21.60 g) groups. The current results indicated significant differences between the Phy and Ctrl groups ($p < 0.05$). Furthermore, the Phy + Prob group demonstrated significantly lower abdominal fat deposition compared to the Phy group ($p < 0.05$; Table 6).

Spleen weight varied significantly among treatments ($p < 0.05$), ranging from 2.78 g in the Ctrl group, 2.23 g in the Prob group, 3.63 g in the Phy group, and 3.57 g in the Phy + Prob group. Spleen weight was significantly greater in broiler chickens fed Phy and Phy + Prob diets compared to the Ctrl groups ($p < 0.05$), while no difference in spleen weight was observed between Phy and Phy + Prob treatment groups ($p > 0.05$; Table 6).

Dietary treatments significantly influenced liver weight ($p < 0.05$). Broiler chickens that received the Phy diet exhibited the highest liver weight (59.20 g), followed by those on Phy + Prob (47.79 g), Prob (45.58 g), and Ctrl (43.38 g) diets. Liver weight was significantly higher in the Phy than in the Ctrl and Prob group ($p < 0.05$). In contrast, the weight of the proventriculus was not significantly affected by dietary treatments ($p < 0.05$), ranging from 8.83 g in the Ctrl group to 10.93 g in the Phy group, with no significant differences among treatment groups ($p > 0.05$; Table 6).

Mortality rate

Mortality rates in broiler chickens were not significantly affected by supplementation with Prob, Phy, or their combination compared to the control group ($p < 0.05$). In the 42-day trial with 45 broiler chickens per treatment, the mortality counts were 6 in the Ctrl group, 2 in the Prob group, 2 in the Prob + Phy group, and 4 in the Phy group. These numerical differences were not statistically significant ($p < 0.05$; Table 7).

Table 7. The mortality rate of broiler chickens administered with probiotic and Axtraphytase during a 42-day hot-humid season

Age (Days)	Ctrl	Prob	Prob + Phy	Phy
7	1	1	1	1
14	2	1	0	1
21	1	0	1	1
28	1	0	0	0
35	1	0	0	0
42	0	0	0	0

Treatment	Deaths (O)	Survivors (O)	Deaths (E)	Survivors (E)	(χ^2)
Ctrl	6	39	3.5	41.5	1.945
Prob	2	43	3.5	41.5	1.166
Prob + Phy	2	43	3.5	41.5	1.166
Phy	4	41	3.5	41.5	0.077

Crtl: Control, Prob: Probiotic, Phy: Phytase, Prob + Phy: Probiotic and phytase, O: Observed, E: Expected, χ^2: Chi-squared distribution

DISCUSSION

Thermal environment and growth performance of broiler chickens under heat stress

The mean dry-bulb temperatures recorded during the present study were largely outside the recommended thermoneutral range of 18-24°C for mature broiler chickens aged 28-42 days reared under tropical conditions (Dei and Bumbie, 2011). While these temperatures were consistent with the 29-33°C range for broiler chickens aged 1-3 weeks, as described by Meltzer (1983), the high THI and different RH during the finishing phase indicated persistent heat stress (Purswell et al., 2012). Prolonged exposure to such thermal loads typically impairs metabolic efficiency, nutrient digestibility, and body weight gain, as chickens expend more energy on thermoregulation instead of growth.

Growth and feed efficiency responses

Live weight, weight gain, and FCR indicated that although dietary probiotic, phytase, and their combination improved growth performance, the overall efficiency of the flock remained below the commercial standards for Ross 308/Arbor Acres broiler chickens (Derakhshan et al., 2023). Typically, FCR for Ross 308/Arbor Acres broiler chickens aged 21-42 days is between 1.2 and 1.7 (Derakhshan et al., 2023). Supplementation with AxtraPhytase, either alone or combined with *B. amyloliquefaciens*, notably improved live weight and weight gain compared to the probiotic-only and control groups. These findings align with those reported by Flores et al. (2019) and Maas et al. (2021), who found that enzyme-probiotic combinations enhanced nutrient utilization and broiler chickens' performance. Similarly, Selle et al. (2003) and Singh and Kim (2021) observed that multienzyme preparations containing phytase (500 FTU/kg) and xylanase (4,400 EXU/kg) improved weight gain and nutrient availability. These improvements might be due to efficient phytate hydrolysis, increased phosphorus release, and enhanced amino acid and energy absorption (Yao et al., 2012; Scholey et al., 2018).

Dietary supplementation with *B. amyloliquefaciens* TL106 (7.5×10^9 CFU/kg) improved growth, immune response, gut health, and microbiota balance in broiler chickens, suggesting its potential as an antibiotic alternative (Bao et al., 2022). Similarly, Ahmat et al. (2021) indicated that the inclusion of *B. amyloliquefaciens* LFB112 at 5×10^8 CFU/kg remarkably increased body weight and daily weight gain, especially by day 39, in comparison to both control and antibiotic-treated groups. Several studies reported that multiple *Bacillus* strains or *B. coagulans* supplementation considerably improved live weight and weight gain between days 1 and 42 of production (Khajeh Bami et al., 2020). Furthermore, Zhang et al. (2013) reported that *Bacillus*-based probiotics at 10^5-10^8 CFU/kg enhanced body weight gain in broiler chickens.

Despite these relative improvements, overall FCR values remained above commercial Ross 308 benchmarks, likely due to chronic thermal stress. Elevated environmental temperatures increased maintenance energy requirements and reduced feed efficiency, as broiler chickens limit voluntary intake to minimize metabolic heat production (Herd and Arthur, 2009; Vale et al., 2010). Additionally, heat stress elevates oxidative stress and corticosterone levels, impairing protein synthesis and nutrient utilization (Caffrey et al., 2017; Quinteiro-Filho et al., 2017). Due to these physiological limitations, the dietary additives may not have achieved their maximum effectiveness.

Role of phytase supplementation

In-feed supplementation with novel phytase improved growth and reduced woody breast severity by enhancing glucose uptake, glycolysis, and intracellular ATP generation, thereby supporting muscle glycogenesis and protein synthesis (Walk et al., 2024). Broader physiological benefits include improved digestion and absorption of phytate-bound phosphorus (Ma et al., 2025), enhanced amino acid absorption (Martínez-Vallespín et al., 2022), improved energy utilization (Bernardes et al., 2022), strengthened intestinal integrity (Nari et al., 2020), and reduced oxidative stress (Lee et al., 2019). Moreover, phytase supported bone development by controlling the release of phosphorus (P), calcium (Ca), zinc (Zn), and magnesium (Mg; Moradi et al., 2023; Philippi et al., 2023). Consistent with these findings, the inclusion of 500-1000 FTU of phytase in broiler chicken (Cobb 500) diets led to notable improvements in weight gain and final body weight (Borges et al., 2025). Similar to the progressive weight gain trends observed from day 14 onward in the present study, Derakhshan et al. (2023) found that Ross 308 broiler chickens receiving phytase (5000 FTU) or a combination of probiotic (2×10^9 CFU/g) and phytase exhibited remarkably higher weight gain and feed intake at days 21 and 42, compared to control and probiotic-only groups after a 42-day trial. The present study demonstrated that dietary supplementation with phytase, either alone or in combination with a probiotic, notably enhanced carcass yield and muscle accretion. Broiler chickens fed phytase with probiotics exhibited the greatest carcass weight and muscle yields, particularly in the breast and drumstick, indicating a synergistic enzyme-probiotic interaction. The superior muscle deposition reflected enhanced protein utilization and nutrient bioavailability. Phytase-mediated hydrolysis of phytate complexes increased the accessibility of phosphorus, amino acids, and energy, key nutrients for muscle protein synthesis (Kriseldi et al., 2021; Bernardes et al., 2022; Walk et al., 2024). The probiotic component likely enhanced this process by optimizing gut microbial balance and intestinal function, leading to more effective nutrient uptake and a greater allocation towards lean tissue growth.

Abdominal fat deposition was strongly influenced by dietary treatment. Broiler chickens supplemented with phytase had the highest abdominal fat weight, whereas those fed the combination of phytase and probiotic had lower fat accumulation, suggesting that probiotic inclusion redirected nutrients toward lean tissue accretion by improving metabolic efficiency. Spleen weight, indicative of immune organ health, was notably higher in broiler chickens receiving phytase or a phytase-probiotic combination compared to control and probiotic diets. This splenomegaly likely signified heightened immune competence, potentially due to improved nutrient availability, especially P and essential amino acids vital for lymphoid tissue development. The absence of differences between phytase and phytase-probiotic combination suggested that phytase is the primary contributor to immune organ development. Previous studies confirmed that *Bacillus*-based probiotics modulated immune function, as *B. subtilis* supplementation increased spleen size (Zhang et al., 2013) and bursa weight (Hossain et al., 2016), while *B. amyloliquefaciens* similarly improved spleen and bursa indices (Luan et al., 2019). Additionally, Soliman et al. (2021) indicated that *B. subtilis* supplementation, at doses of 5×10^6 CFU/g per liter of water, led to higher carcass and immune organ weights. Furthermore, *B. amyloliquefaciens* LFB112 notably improved thymus and spleen indices, which were comparable to mechanisms involving B-cell stimulation and increased immunoglobulin production.

CONCLUSION

Phytase, particularly when combined with a *Bacillus*-based probiotic, improved body weight, carcass yield, nutrient digestibility, muscle growth, and immune organ development in broiler chickens under tropical heat stress, thereby partially mitigating the detrimental effects of high temperatures. However, the persistence of poor feed conversion across all treatment groups highlighted that severe chronic heat stress and suboptimal environmental factors remained significant constraints on overall production efficiency. Future studies should evaluate higher phytase doses, optimized *Bacillus* strains, and improved housing such as cooling systems or enhanced ventilation to further mitigate heat-stress-induced reductions in feed efficiency. Growth performance was suboptimal compared to Arbor Acre standards due to heat stress and management factors, so the observed improvements represent relative gains under challenging conditions rather than absolute benchmarks.

DECLARATIONS

Acknowledgements

The authors extended their gratitude to the staff of the National Veterinary Research Institute, Vom, Nigeria, for their contributions to this experiment.

Authors' contributions

Tagang Aluwong conceptualized the study, performed statistical analysis and sample collection, and led writing and drafting. Promise Okoro, Ocholi Gideon Adaji, Agweche Shalom Onuche, Friday Ocheja Zakari, and Enokela Shaibu Idoga were responsible for sample collection. Friday Ocheja Zakari and Kolawole Bamidele Jonathan supported the statistical analysis and participated in the design, administration, and sample collection. Paulinus Emenna, Chidiebere Uchendu, Tagang Aluwong, Olusola Olalekan Oladipo, and Nancy Sati helped with design, administration, and manuscript drafting. All authors reviewed and approved the final version.

Availability of data and materials

The data and materials are available upon reasonable request from the corresponding author.

Competing interests

All authors declared that there is no conflict of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors confirm that no AI tools were used for writing and preparing the present study.

Funding

The research was funded by the Tertiary Education Trust Fund, Nigeria.

REFERENCES

Abou-Jaoudeh C, Andary J, and Abou-Khalil R (2024). Antibiotic residues in poultry products and bacterial resistance: A review in developing countries. Journal of Infection and Public Health, 17(12): 102592. DOI: https://www.doi.org/10.1016/j.jiph.2024.102592

Abreu R, Semedo-Lemsaddek T, Cunha E, Tavares L, and Oliveira M (2023). Antimicrobial drug resistance in poultry production: Current status and innovative strategies for bacterial control. Microorganisms, 11(4): 953. DOI: https://www.doi.org/10.3390/microorganisms11040953

Abu-Dieyeh ZHM (2006). Effect of high temperature per se on growth performance of broilers. International Journal of Poultry Science, 5(1): 19-21. DOI: https://www.doi.org/10.3923/ijps.2006.19.21

Ahmat M, Cheng J, Abbas Z, Cheng Q, Fan Z, Ahmad B, Hou M, Osman G, Guo H, Wang J et al. (2021). Effects of *Bacillus amyloliquefaciens* LFB112 on growth performance, carcass traits, immune and serum biochemical response in broiler chickens. Antibiotics, 10(11): 1427. DOI: https://www.doi.org/10.3390/antibiotics10111427

Akpan S, Udoh E, and Nkanta VS (2024). The per capita meat consumption: The trend and macroeconomic determinants in Nigeria. Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature, 27(4): 973-983. DOI: https://www.doi.org/10.3390/antibiotics10111427

Avci M, Yerturk M, and Kaplan O (2005). Effects of ascorbic acid on the performance and some blood parameters of Japanese quails reared under hot climate conditions. Turkish Journal of Veterinary and Animal Sciences, 29(5): 829-833. Available at: http://www.dergipark.gov.tr/tbtkveterinary/issue/12544/151332

Bernardes RD, de Oliveira CH, Calderano AA, de Sousa Ferreira R, Dias KMM, de Almeida BF, Alexio PE, and Albino LFT (2022). Effect of phytase and protease combination on performance, metabolizable energy, and amino acid digestibility of broilers fed nutrient-restricted diets. Revista Brasileira de Zootecnia, 51: e20210211. DOI: https://www.doi.org/10.37496/rbz5120210211

Bao C, Zhang W, Wang J, Liu Y, Cao H, Li F, Liu S, Shang Z, Cao Y, and Dong B (2022). The effects of dietary *Bacillus amyloliquefaciens* TL106 supplementation, as an alternative to antibiotics, on growth performance, intestinal immunity, epithelial barrier integrity, and intestinal microbiota in broilers. Animals, 12(22): 3085. DOI: https://www.doi.org/10.3390/ani12223085

Borges SO, Petrolli TG, Hannas MI, Bernardes RD, de Oliveira CH, Dias KMM, de Almeida BF, da Fonseca L, and Calderano AA (2025). Effects of phytase supplementation on broiler chickens fed nutrient-deficient diets. Livestock Science, 301: 105823. DOI: https://www.doi.org/10.1016/j.livsci.2025.105823

Caffrey NP, Dohoo IR, and Cockram MS (2017). Factors affecting mortality risk during transportation of broiler chickens for slaughter in Atlantic Canada. Preventive Veterinary Medicine, 147(1): 199-208. DOI: https://www.doi.org/10.1016/j.prevetmed.2017.09.011

Coppedge J, Klein J, Brown B, Ratliff B, Ruch F, and Lee JT (2011). Effects of co-administration of phytase and NSPase on broiler performance and bone ash. International Journal of Poultry Science, 10(10): 933-939. DOI: https://www.doi.org/10.3923/ijps.2011.933.939

Dei HK and Bumbie GZ (2011). Effect of wet feeding on growth performance of broiler chickens in a hot climate. British Poultry Science, 52(1): 82-85. DOI: https://www.doi.org/10.1080/00071668.2010.540230

Deng H, Cheng S, Fan J, Hao H, Fang D, Li W, and Wang Q (2025). *Bacillus amyloliquefaciens* SC06 ameliorated intestinal mucosal injury by regulating intestinal stem cell proliferation and differentiation via activating Wnt/β-catenin signaling pathway in Clostridium perfringens-challenged mouse. Microorganisms, 13(9): 2136. DOI: https://www.doi.org/10.3390/microorganisms13092136

Derakhshan M, Ghasemian SO, and Gholami-Ahangaran M (2023). Effects of probiotic and phytase on growth performance, biochemical parameters and antioxidant capacity in broiler chickens. Veterinary Medicine and Science, 9(2): 860-866. DOI: https://www.doi.org/10.1002/vms3.1075

Diaz D (2007). Effect of *Bacillus amyloliquefaciens* CECT-5940 spores on broiler performance and digestibility. Engormix Poultry Industry. Online Article. Available at: https://en.engormix.com/poultry-industry/poultry-nutrition-other-additives/effect-bacillus-amyloliquefaciens-cect_a33979

Flores CA, Duong T, Askelson TE, Dersjant-Li Y, Gibbs K, Awati A, and Lee JT (2019). Effects of direct-fed microorganisms and enzyme blend co-administration on growth performance in broilers fed diets with or without antibiotics. Journal of Applied Poultry Research, 28(4): 1181-1188. DOI: https://www.doi.org/10.3382/japr/pfz084

Ghareeb AF, Schneiders GH, Richter JN, Foutz JC, Milfort MC, Fuller AL, Yuan J, Rekaya R, and Aggrey SE (2022). Heat stress modulates the disruptive effects of Eimeria maxima infection on ileum nutrient digestibility, molecular transporters, and tissue morphology in meat-type chickens. PLoS One, 17(6): e0269131. DOI: https://www.doi.org/10.1371/journal.pone.0269131

Goo D, Kim JH, Park GH, Delos Reyes JB, and Kil DY (2019). Effect of heat stress and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. Animals, 9(3): 107. DOI: https://www.doi.org/10.3390/ani9030107

Heise H, Crisan A, and Theuvsen L (2015). The poultry market in Nigeria: Market structures and potential for investment. International Food and Agribusiness Management Review, 18(2): 197-222. DOI: https://www.doi.org/10.22004/ag.econ.207011

Herd RM and Arthur PF (2009). Physiological basis for residual feed intake. Journal of Animal Science, 87(2): 64-71. DOI: https://www.doi.org/10.2527/jas.2008-1345

Hossain MM, Begum M, and Kim IH (2016). Effect of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on growth performance, nutrient digestibility, meat quality and microbial shedding in broilers. Veterinary Medicine, 60(10): 77-86. DOI: https://www.doi.org/10.17221/7981-VETMED

Jiang Z, Su W, Yang M, Fu J, Gong T, Li W, Wen C, Wang X, Wang F, Jin M et al. (2025). Integrated multi-omics reveals *Bacillus amyloliquefaciens* BA40 against *Clostridium perfringens* infection in weaned piglets. Journal of Advanced Research, 77: 75-90. DOI: https://www.doi.org/10.1016/j.jare.2025.01.033

Khajeh Bami M, Afsharmanesh M, and Ebrahimnejad H (2020). Effect of dietary *Bacillus coagulans* and different forms of zinc on performance, intestinal microbiota and meat quality of broiler chickens. Probiotics and Antimicrobial Proteins, 12(2): 461-472. DOI: https://www.doi.org/10.1007/s12602-019-09558-1

Kim YB, Yeom GL, Park JY, Lee HN, Park GY, Shin JW, Lee SY, and Kim JH (2025). Nutritional strategies to alleviate heat stress in broiler chickens and laying hens. Journal of Animal Science and Technology, 67(5): 949. DOI: https://www.doi.org/10.5187/jast.2500205

Kornegay ET (2001). Digestion of phosphorus and other nutrients: The role of phytases and factors influencing their activity. In: M. R. Bedford and G. G. Partridge (Editors), Enzymes in farm animal nutrition. CABI Publishing, New York, pp. 237-271. Available at: https://www.cabidigitallibrary.org/doi/10.1079/9780851993935.0237

Kriseldi R, Bedford MR, Dilger RN, Foradori CD, MacKay L, and Dozier III WA (2021). Effects of phytase supplementation and increased nutrient density on growth performance, carcass characteristics, and hypothalamic appetitive hormone expression and catecholamine concentrations in broilers from 1 to 43 days of age. Poultry Science, 100(12): 101495. DOI: https://www.doi.org/10.1016/j.psj.2021.101495

Laganá C, Ribeiro AML, Kessler ADM, Kratz LR, and Pinheiro CC (2007). Effects of reduction of dietary heat increment on performance and digestibility of broilers under heat stress. Brazilian Journal of Poultry Science, 9(1): 45-51. DOI: https://www.doi.org/10.1590/S1516-635X2007000100007

Lee YJ, Kim BK, Lee BH, Jo KI, Lee NK, Chung CH, Lee YC, and Lee JW (2008). Purification and characterization of cellulase produced by *Bacillus amyloliquefaciens* DL-3 utilizing rice hull. Bioresource Technology, 99(2): 378-386. DOI: https://www.doi.org/10.1016/j.biortech.2006.12.013

Lee SA, Nagalakshmi D, Raju MVR, Rao SVR, Bedford MR, and Walk CL (2019). Phytase as an alleviator of high-temperature stress in broilers fed adequate and low dietary calcium. Poultry Science, 98(5): 2122-2132. DOI: https://www.doi.org/10.3382/ps/pey566

Lin LZ, Zheng QW, Wei T, Zhang ZQ, Zhao CF, Zhong H, Xu XY, Lin JF, and Guo LQ (2020). Isolation and characterization of fengycins produced by *Bacillus amyloliquefaciens* JFL21 and its broad-spectrum antimicrobial potential against multidrug-resistant foodborne pathogens. Frontiers in Microbiology, 11: 579621. DOI: https://www.doi.org/10.3389/fmicb.2020.579621

Luan SJ, Sun YB, Wang Y, Sa RN, and Zhang HF (2019). *Bacillus amyloliquefaciens* spray improves growth performance, immune status and respiratory mucosal barrier in broiler chickens. Poultry Science, 98(4): 1403-1409. DOI: https://www.doi.org/10.3382/ps/pey478

Ma B, Zhang Y, Liu M, Shi C, Zhou Y, Chen M, Chang Y, Qu H, Zhang L, Zhang C et al. (2025). Dietary non-phytate phosphorus and phytase interaction affects growth performance, mineral transportation and metabolism in broiler chickens. Poultry Science, 104(6): 105108. DOI: https://www.doi.org/10.1016/j.psj.2025.105108

Maas RM, Verdegem MC, Debnath S, Marchal L, and Schrama JW (2021). Effect of enzymes (phytase and xylanase), probiotics (*B. amyloliquefaciens*) and their combination on growth performance and nutrient utilisation in Nile tilapia. Aquaculture, 533: 736226. DOI: https://www.doi.org/10.1016/j.aquaculture.2020.736226

Martínez-Vallespín B, Ader P, and Zentek J (2025). Effect of increasing levels of phytase on performance, prececal nutrient digestibility, intestinal mucosa physiology and immune response in broiler chickens from 1 to 21 days of age. Poultry Science, 104(9): 105473. DOI: https://www.doi.org/10.1016/j.psj.2025.105473

Meltzer A (1983). Thermoneutral zone and resting metabolic rate of broilers. British Poultry Science, 24(4): 471-476. DOI: https://www.doi.org/10.1080/00071668308416763

Moreki JC, Bhawa S, Moreki MI, and Adesehinwa AOO (2025). Poultry production in Africa: Present status, challenges, opportunities and prospects. Egyptian Journal of Veterinary Sciences, Article is in Press, pp. 1-15. DOI: https://www.doi.org/10.21608/ejvs.2025.352822.2604

Moradi S, Abdollahi MR, Moradi A, and Jamshidi L (2023). Effect of bacterial phytase on growth performance, nutrient utilization, and bone mineralization in broilers fed pelleted diets. Animals, 13(9): 1450. DOI: https://www.doi.org/10.3390/ani13091450

Mwansa M, Mukuma M, Mulilo E, Kwenda G, Mainda G, Yamba K, Bumbangi FN, Muligisa-Muonga E, Phiri N, Silwamba I et al. (2023). Determination of antimicrobial resistance patterns of *Escherichia coli* isolates from farm workers in broiler poultry production and assessment of antibiotic resistance awareness levels among poultry farmers in Lusaka, Zambia. Frontiers in Public Health, 10: 998860. DOI: https://www.doi.org/10.3389/fpubh.2022.998860

Nari N, Ghasemi HA, Hajkhodadadi I, and Farahani AK (2020). Intestinal microbial ecology, immune response, stress indicators and gut morphology of male broiler chickens fed low-phosphorus diets supplemented with phytase, butyric acid or *Saccharomyces boulardii*. Livestock Science, 234: 103975. DOI: https://www.doi.org/10.1016/j.livsci.2020.103975

Ngalimat MS, Yahaya RSR, Baharudin MMAA, Yaminudin SM, Karim M, Ahmad SA, and Sabri S (2021). A review on the biotechnological applications of the operational group Bacillus amyloliquefaciens. Microorganisms, 9(3): 614. https://www.doi.org/10.3390/microorganisms9030614

Oke OE, Uyanga VA, Iyasere OS, Oke FO, Majekodunmi BC, Logunleko MO, Abiona JA, Nwosu EU, Abiaja MO, Daramola JO et al. (2021). Environmental stress and livestock productivity in hot-humid tropics: Alleviation and future perspectives. Journal of Thermal Biology, 100: 103077. DOI: https://www.doi.org/10.1016/j.jtherbio.2021.103077

Osuji EE, Onyeneke RU, Nkwocha GA, and Olaolu MO (2024). Exploring impact of climate change on poultry production in Nigeria. Agricultural Research, 13(3): 613-621. DOI: https://www.doi.org/10.1007/s40003-024-00708-8

Palacios-Rodriguez AP, Espinoza-Culupú A, Durán Y, and Sánchez-Rojas T (2024). Antimicrobial activity of *Bacillus amyloliquefaciens* BS4 against Gram-negative pathogenic bacteria. Antibiotics, 13(4): 304. DOI: https://www.doi.org/10.3390/antibiotics13040304

Purswell JL, Dozier CD, Olarenwaju AA, Davis JD, Yin H, and Gates RS (2012). Effect of temperature humidity index on live performance in broiler chickens grown from 49 to 63 days of age. ASABE conference presentation. 9th International Livestock Environment Symposium, Valencia, Spain, pp. 1-9. Available at: https://dr.lib.iastate.edu/bitstreams/aef8485c-d2de-46bb-9154-1b85c4b40514/download

Philippi H, Sommerfeld V, Windisch W, Olukosi OA, Monteiro A, and Rodehutscord M (2023). Interactions of zinc with phytate and phytase in the digestive tract of poultry and pigs: A review. Journal of the Science of Food and Agriculture, 103(15): 7333-7342. DOI: https://www.doi.org/10.1002/jsfa.12879

Quinteiro-Filho WM, Calefi AS, Cruz DSG, Aloia TPA, Zager A, Astolfi-Ferreira CS, Pinantino-Ferreira JA, Sharif S, and Palermo-Neto J (2017). Heat stress decreases expression of cytokines, avian β-defensins 4 and 6 and Toll-like receptor 2 in broiler chickens infected with Salmonella Enteritidis. Veterinary Immunology and Immunopathology, 186(1): 19-28. DOI: https://www.doi.org/10.1016/j.vetimm.2017.02.006

Rashid S, Alsayeqh AF, Akhtar T, Abbas RZ, and Ashraf R (2023). Probiotics: Alternative to antibiotics in poultry production. International Journal of Veterinary Science, 16(1): 45-51. DOI: https://www.doi.org/10.47278/journal.ijvs/2022.175

R Core Team (2025). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. Available at: https://www.R-project.org/

Scholey DV, Morgan NK, Riemensperger A, Hardy R, and Burton EJ (2018). Effect of supplementation of phytase to diets low in inorganic phosphorus on growth performance and mineralization of broilers. Poultry Science, 97(7): 2435-2440. DOI: https://www.doi.org/10.3382/ps/pey088

Selle PH, Ravindran V, Ravindran G, Pittolo PH, and Bryden WL (2003). Influence of phytase and xylanase supplementation on growth performance and nutrient utilization of broilers offered wheat-based diets. Asian-Australasian Journal of Animal Sciences, 16(3): 394-402. DOI: https://www.doi.org/10.5713/ajas.2003.394

Selle PH and Ravindran V (2007). Microbial phytase in poultry nutrition. Animal Feed Science and Technology, 135(1): 1-41. DOI: https://www.doi.org/10.1016/j.anifeedsci.2006.06.010

Shakeri M (2025). Lactic acid bacteria as a substitute for antibiotics in broiler chickens raised under colored environment. Farm Animal Health and Nutrition, 4(2): 28-31. DOI: https://www.doi.org/10.58803/fahn.v4i2.76

Singh AK and Kim WK (2021). Effects of dietary fiber on nutrient utilization and gut health of poultry: A review of challenges and opportunities. Animals, 11(1): 181. DOI: https://www.doi.org/10.3390/ani11010181

Sinha R, Ranjan A, Lone S, Rahim A, Devi I, and Tiwari S (2017). Impact of climate change on livestock production and reproduction: ameliorative management. International Journal of Livestock Research, 7(1): 1-8. DOI: https://www.doi.org/10.5455/ijlr.20170417042102

Sohail MU, Hume ME, Byrd JA, Nisbet DJ, Ijaz A, Sohail A, Shabbir MZ, and Rehman H (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poultry Science, 91(10): 2235-2240. DOI: https://www.doi.org/10.3382/ps.2012-02182

Soliman ES, Hamad RT, and Abdallah MS (2021). Preventive antimicrobial action and tissue architecture ameliorations of *Bacillus subtilis* in challenged broilers. Veterinary World, 14(2): 523. DOI: http://www.doi.org/10.14202/vetworld.2021.523-536

Su YT, Liu C, Long Z, Ren H, and Guo XH (2019). Improved production of spores and bioactive metabolites from *Bacillus amyloliquefaciens* in solid-state fermentation by a rapid optimization process. Probiotics and Antimicrobial Proteins, 11(3): 921-930. DOI: https://www.doi.org/10.1007/s12602-018-9474-z

Tao X and Xin H (2003). Acute synergistic effects of air temperature, humidity, and velocity on homeostasis of market-size broiler. Transactions of the American Society of Agricultural Engineers, 46(2): 491-497. DOI: https://www.doi.org/10.13031/2013.12971

Tedersoo T, Roasto M, Mäesaar M, Häkkinen L, Kisand V, Ivanova M, Valli MH, and Meremäe K (2022). Antibiotic resistance in *Campylobacter* spp. isolated from broiler chicken meat and human patients in Estonia. Microorganisms, 10(5): 1067. DOI: https://www.doi.org/10.3390/microorganisms10051067

United States Department of Agriculture (USDA) (2013). International egg and poultry report. USDA, Washington, D.C, pp. 1-10. Available at: https://www.ams.usda.gov/mnreports/pywintlpoultryandegg.pdf

Vale MM, Moura DS, Naas IA, and Pereira DF (2010). Characterization of heat waves affecting mortality rates of broilers between 29 days and market age. Brazilian Journal of Poultry Science, 12(4): 279-285. DOI: https://www.doi.org/10.1590/S1516-635X2010000400010

Walk CL, Mullenix GJ, Maynard CW, Greene ES, Maynard C, Ward N, and Dridi S (2024). Novel fourth-generation phytase improves broiler growth performance and reduces woody breast severity through modulation of muscle glucose uptake and metabolism. Frontiers in Physiology, 15: 1376628. DOI: https://www.doi.org/10.3389/fphys.2024.1376628

Wang S, Wang R, Zhao X, Ma G, Liu N, Zheng Y, Tan J, and Qi G (2022). Systemically engineering *Bacillus amyloliquefaciens* for increasing antifungal activity and green antifungal lipopeptide production. Frontiers in Bioengineering and Biotechnology, 10: 961535. DOI: https://www.doi.org/10.3389/fbioe.2022.961535

Wasti S, Sah N, and Mishra B (2020). Impact of heat stress on poultry health and performance and potential mitigation strategies. Animals, 10(8): 1266. DOI: https://www.doi.org/10.3390/ani10081266

Yao MZ, Zhang YH, Lu WL, Hu MQ, Wang W, and Liang AH (2012). Phytases: Crystal structures, protein engineering and potential biotechnological applications. Journal of Applied Microbiology, 112(1): 1-14. DOI: https://www.doi.org/10.1111/j.1365-2672.2011.05181.x

Zalila-Kolsi I, Ben-Mahmoud A, and Al-Barazie R (2023). *Bacillus amyloliquefaciens*: Harnessing its potential for industrial, medical and agricultural applications—A comprehensive review. Microorganisms, 11(9): 2215. DOI: https://www.doi.org/10.3390/microorganisms11092215

Zhang F, Huo K, Song X, Quan Y, Wang S, Zhang Z, and Yang C (2020). Engineering of a genome-reduced strain *Bacillus amyloliquefaciens* for enhancing surfactin production. Microbial Cell Factories, 19(1): 223. DOI: https://www.doi.org/10.1186/s12934-020-01485-z

Zhang ZF, Cho JH, and Kim IH (2013). Effects of *Bacillus subtilis* UBT-MO2 on growth performance, immune organ weight, gas concentration in excreta and intestinal microbial shedding in broiler chickens. Livestock Science, 155(1): 343-347. DOI: https://www.doi.org/10.1016/j.livsci.2013.05.021