Nig. J. Anim. Prod. 2025, 52(2): 96 - 103 https://doi.org/10.51791/njap.v52i2.9198 Nigerian Journal of Animal Production

Productive performance and Caeca microflora of broiler chickens administered blends of ascorbic acid, sodium bicarbonate and sweet citrus peel powder

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Abstract

A 42-day study evaluated the effect of blends of ascorbic acid (AA), sodium bicarbonate, and sweet citrus peel powder on the growth performance and caecal microflora composition of broiler chickens raised in a hot-humid environment. A total of two hundred and forty one-day-old Arbor Acres broiler chicks were randomly allotted to eight treatment groups. Each treatment was replicated three times with 10 birds per replicate in a completely randomised design. The control group received plain drinking water while, the ascorbic acid (AA) group received 300 ppm ascorbic acid per litre of water. The remaining six groups were administered Blend 1 to Blend 6 per litre of water, respectively, throughout the experimental period. No significant (p>0.05) differences were observed in growth performance parameters across the treatment groups. However, numerically lower feed conversion ratios were observed in birds administered AA (1.57), Blends 2 (1.58), 3 (1.66), and 4 (1.60). Higher survivability was recorded in Blend 3 (100%), Blends 2 and 4 (96.70%), and lowest in Blend 5 (80.00%). The highest total bacterial count was observed in birds administered Blend 3 (11.73×10⁶ cfu/mL), which differed significantly (p<0.05) from the control (6.60×10⁶ cfu/mL) and AA (6.87×10⁶ cfu/mL) group, but was similar (p>0.05) to other treatment groups. The lowest bacterial count was observed in the AA group (4 bacteria), while the highest in Blend 5 (8 bacteria). Escherichia coli and Staphylococcus aureus were identified in all treatment groups. Blends 2, 3, and 4 demonstrated potential functional benefits on growth performance and could modulate caecal microbial ecology without adversely affecting the gut health.

Keywords: Survivability, Orange peel, Vitamin C, Caecum, Blend, Chickens

Rendement de production et microflore cæcale chez des poulets de chair ayant reçu des mélanges d'acide ascorbique, de bicarbonate de sodium et de poudre d'écorce d'agrumes doux

Résumé

Une étude de 42 jours a évalué l'effet de mélanges d'acide ascorbique (AA), de bicarbonate de sodium et de poudre d'écorce d'agrumes doux sur les performances de croissance et la composition de la microflore cæcale de poulets de chair élevés dans un environnement chaud et humide. Au total, deux cent quarante poussins de chair Arbor Acres âgés d'un jour ont été répartis aléatoirement dans huit groupes de traitement. Chaque traitement a été reproduit trois fois avec 10 poulets par répétition, selon un plan d'expérimentation entièrement aléatoire. Le groupe témoin a reçu de l'eau potable ordinaire, tandis que le groupe « acide ascorbique » (AA) a reçu 300 ppm d'acide ascorbique par litre d'eau. Les six groupes restants ont reçu respectivement les mélanges 1 à 6 par litre d'eau tout au long de la période expérimentale. Aucune différence significative (p > 0,05) n'a été observée au niveau des paramètres de performance de croissance entre les groupes de traitement. Cependant, des indices de conversion alimentaire numériquement plus faibles ont été observés chez les volailles ayant reçu de l'AA (1,57), les mélanges 2 (1,58), 3 (1,66) et 4 (1,60). Un taux de survie plus élevé a été enregistré avec le mélange 3 (100 %), les mélanges 2 et 4 (96,70 %), et le plus faible avec le mélange 5 (80,00 %). La charge bactérienne totale la plus élevée a été observée chez les oiseaux ayant reçu le mélange 3 (11,73 × 10⁶ ufc/mL), ce qui différait de manière significative (p < 0,05) par rapport au groupe témoin (6,60 × 10⁶ ufc/mL) et au groupe AA (6,87 × 10⁶ ufc/mL), mais était similaire (p > 0,05) à celle des autres groupes de traitement. La charge *bactérienne la plus faible a été observée dans le groupe AA (4 bactéries), tandis que la plus élevée a été constatée dans le groupe « Blend 5 » (8 bactéries). Escherichia coli et Staphylococcus aureus ont été identifiés dans tous les groupes de traitement. Les mélanges 2, 3 et 4 ont démontré des avantages fonctionnels potentiels sur les performances de croissance et pourraient moduler l'écologie microbienne du cæcum sans nuire à la santé intestinale.*

Mots-clés : Survie, Écorce d'orange, Vitamine C, Cæcum, Mélange, Poulets

Introduction

The poultry industry continues to expand in response to the increasing demand for affordable, high-quality animal protein. However, sustaining optimal broiler performance under intensive production systems remains a significant challenge, particularly in tropical and subtropical environments where heat stress, oxidative imbalance, and enteric disorders combined in compromising productivity and profitability. Heat stress has been consistently reported to impair growth performance, antioxidant status, and physiological stability in broilers (Tavakolinasab and Hashemi, 2025).

The gastrointestinal tract plays a central role in nutrient digestion, absorption, immune modulation, and overall growth performance in broiler chickens. In particular, the caecum harbours a dense and metabolically active microbial population that influences host physiology through fermentation end-products, competitive exclusion of pathogens, and modulation of intestinal integrity. Dietary interventions have been shown to significantly alter the composition and diversity of cecal microbiota in broilers (Lanlan *et al*., 2023). Consequently, natural and functional feed additives with antioxidant, buffering, and antimicrobial properties are increasingly being explored as alternatives to antibiotic growth promoters.

Ascorbic acid (vitamin C) is a potent water-soluble antioxidant known to mitigate oxidative stress, enhance immune competence, and improve performance in poultry, particularly under heat stress conditions (Tavakolinasab and Hashemi, 2025). Although poultry can synthesise ascorbic acid endogenously, dietary inclusion has demonstrated beneficial effects under environmental stress, improving growth performance, physiological responses (Puron *et al*., 1994) and enhancing antioxidant defense systems in broilers exposed to chronic heat stress (Al-Ghoul *et al.*, 2023). Sodium bicarbonate, commonly used as a dietary electrolyte and buffering agent, plays a vital role in maintaining acid–base balance, optimising gut pH, and supporting digestive enzyme activity. Supplementation with sodium bicarbonate has been associated with improved performance and physiological stability in broilers reared under heat stress conditions (Puron *et al*., 1994; Al-Ghoul *et al.,* 2023).

Sweet citrus peel, an agro-industrial by-products have gained attention as a sustainable feed additive, its richness in bioactive compounds, including flavonoids, essential oils, pectins, and phenolic acids, which possess antioxidant, antimicrobial, and prebiotic properties. Supplementation with sweet citrus peel has been shown to improve performance indices and beneficially influence ileal microbial populations in broilers (Majekodunmi *et al*., 2021). Similarly, standardized citrus extracts have demonstrated positive effects on growth performance, gut health, and overall welfare of broiler chickens (Cisse *et al*., 2025). Dietary citrus pulp has also been reported to modulate the diversity and composition of caecal microbiota (Lanlan *et al*., 2023).

While individual effects of ascorbic acid, sodium bicarbonate, and citrus-derived phytogenics on broiler performance have been documented, limited information exists on their combined or synergistic effects on productive performance and caecal microbial ecology. Therefore, this study was designed to evaluate the effects of blended supplementation of ascorbic acid, sodium bicarbonate, and sweet citrus peel powder on growth performance indices and caecal microflora composition of broiler chickens raised in a hot-humid environment.

Materials and Methods

Experimental site

The research was conducted at the Poultry unit of the Teaching and Research farms, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria, in an open-sided, naturally ventilated poultry house during the hot season of the year (late October to November, 2025).

Sourcing, processing and preparation of test ingredients

Sodium bicarbonate salt and ascorbic acid were procured from a reputable veterinary store, while sweet orange peels were obtained from orange vendors in open markets within Abeokuta, Ogun state, Nigeria. The peels were sorted out to remove dirt, thereafter sundried until crispy and thereafter milled into powder. Ascorbic acid, sodium bicarbonate and sweet citrus peel powder were mixed at different proportions to formulate the following experimental blends:

Blend 1: 300 ppm ascorbic acid + 0.25% sodium bicarbonate + 3g of SCPP
Blend 2: 300 ppm ascorbic acid + 0.25% sodium bicarbonate + 6g of SCPP
Blend 3: 300 ppm ascorbic acid + 0.25% sodium bicarbonate + 9g of SCPP
Blend 4: 300 ppm ascorbic acid + 0.5% sodium bicarbonate + 3g of SCPP
Blend 5: 300 ppm ascorbic acid + 0.5% sodium bicarbonate + 6g of SCPP
Blend 6: 300 ppm ascorbic acid +0.5% sodium bicarbonate + 9g of SCPP

Experimental Animals, Design and Management

A total of two hundred and forty one-day-old Arbor Acres broiler chicks was used for the experiment. After one week of brooding, birds were weighed and randomly allotted to eight treatment groups. Each treatment was replicated three times with 10 birds per replicate in a completely randomised design (CRD): The control group received plain drinking water throughout the experimental period. Birds in AA group received drinking water supplemented with 300 ppm ascorbic acid per litre. The remaining six groups were administered Blend 1 to Blend 6 per litre of water respectively throughout the study. The birds were reared on a conventional deep litter open sided house. Commercial broiler starter (CP- 22.86%; ME- 2721.05kcal/kg) and finisher (CP- 20.18%; ME- 2739.35kcal/kg) diets were offered to birds *ad libitum*. Fresh drinking water having the respective treatment was prepared daily and given *ad libitum* from day 8 to day 42 of age. Standard routine vaccinations against infectious bursal disease and Newcastle diseases as prescribed by the hatchery were strictly followed.

Data collection

Growth performance parameters

Feed intake was measured as the difference between the feed offered and feed refusals on weekly basis. Body weight gain was monitored weekly. Feed conversion ratio (FCR) was calculated as the ratio of feed intake to the body weight gain. Mortality record was taken daily and survivability was calculated as the percentage of birds that remain alive at the end of the study d. At day 42, four birds per replicate with average weight closest to the mean were selected, and sacrificed by cervical dislocation. Caeca were carefully excised and caeca digesta was collected for the microbial analysis.

Enumeration and isolation of microorganisms

Enumeration of caecal bacteria from the experimental groups was done using pour plate technique. Serial dilution was performed on the caecal digesta samples, Thereafter, which 1mL aliquot was taken from each dilution factor in the test tubes prepared and dispensed into different sterile petri dishes and 18 mL of sterile molten plate count agar (Oxoid). The plates were then swirled gently for proper mixing of the sample with the agar. The agar was allowed to solidify on the bench and then incubated in an inverted position in the incubator at 37^{o}C for 24 hours. After incubation, colonies were counted and recorded. The experiment was performed in triplicates and the mean microbial load was expressed as colony forming unit per milliliter (CFU/mL) (Mongi, 2023). Distinct colonies obtained from the serial dilution were taken using a sterile inoculating loop and sub – cultured onto sterile solidified nutrient agar and incubated for 24 hours at 37^{o}C. The growth obtained was further sub – cultured until a pure culture was obtained.

Identification of Isolates

Bacteria isolated from the samples were identified using cultural (colour, opacity, consistency), morphological test (Gram stain) and biochemical identification techniques (Catalase test, Coagulase, Indole, Urease test, Voges Proskaeur, Methyl red test, Citrate test, Oxidase test) (Cheesebrough, 2000)

Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA) in a completely randomized design (CRD) and analyzed using MINITAB statistical package. Means were separated using Tukey's HSD test of the same software package (at p<0.05).

Results and Discussion

The growth performance of broiler chickens administered blends of ascorbic acid, sodium bicarbonate and sweet citrus peels is presented in Table 1. No significant (p>0.05) variations were observed in the initial weight, final weight, weight gain, total feed intake, feed conversion ratio and survivability among the treatment groups. However, numerically improved FCR was observed in birds administered Blend 2 (1.58) and ascorbic acid (1.57). Birds administered Blend 3 had 100% survivability, followed by Blends 2 and 4 with 96.70% survivability. Lowest survivability was recorded in birds on Blend 5 (80.00%), followed by Blend 6 (83.30%) and the control group (85.20%). The absence of significant differences in growth performance indices among treatment groups indicates that the inclusion of blends of ascorbic acid, sodium bicarbonate and sweet citrus peel did not adversely affect nutrient utilization or growth efficiency of broiler chickens under hot–humid environmental conditions. Heat stress is known to impair growth performance, reduced feed intake, impaired nutrient digestion and altered endocrine regulation (Lara and Rostagno, 2013; Nawab *et al*., 2018). Therefore, the comparable body weight, weight gain, feed intake and survivability observed across treatments suggest that the dietary blends were physiologically tolerable and capable of maintaining metabolic homeostasis under thermal condition.

Survivability patterns further support the protective role of selected blends. Birds administered Blend 3, and to a lesser extent Blends 2 and 4, exhibited higher survivability than the control and higher-dose blends. Heat stress–related mortality is frequently associated with dehydration, electrolyte imbalance and oxidative tissue damage (Renaudeau *et al*., 2012; Nawab *et al*., 2018). The improved survivability observed in some blends suggests that moderate inclusion levels of ascorbic acid, sodium bicarbonate and sweet citrus peel may enhance resilience to thermal stress by stabilizing physiological and metabolic functions. Conversely, the reduced survivability observed in Blends 5 and 6 may indicate that higher inclusion rates did not confer additional benefits and may have imposed mild metabolic or osmotic stress.

Table 1. Growth performance of broiler chickens administered blends of Ascorbic acid, Sodium bicarbonate and Sweet citrus peel powder

Treatments	Initial weight (g/bird)	Final weight (g/bird)	Weight gain (g/bird)	Totalfeed intake (g/bird)	FCR	Survivability (%)
Control	433.33	2670.00	2236.70	3713.00	1.67	85.20
AA	431.11	2690.00	2258.90	3542.00	1.57	93.30
Blend1	427.78	2513.30	2085.60	3604.00	1.73	93.30
Blend 2	437.78	2804.40	2366.70	3742.00	1.58	96.70
Blend 3	434.81	2556.70	2121.90	3523.00	1.66	100.00
Blend 4	434.44	2586.70	2152.20	3444.00	1.60	96.70
Blend 5	431.48	2546.70	2115.20	3817.00	1.79	80.00
Blend 6	424.44	2446.70	2022.20	3474.00	1.74	83.30
SEM	4.44	82.70	82.40	213.00	0.11	11.20
P-value	0.525	0.137	0.156	0.884	0.753	0.876

Control = ordinary water, AA = Ascorbic acid, SEM = Standard error of mean

Table 2 presents the average water intake of broiler chickens administered blends of ascorbic acid, sodium bicarbonate, and sweet citrus peel in a hot, humid environment. No significant differences (p>0.05) in average water intake were observed across treatment groups. However, numerically higher intake was recorded in the ascorbic acid (279.90 ml/bird), Blend 4 (278.20 ml/bird), Blend 5 (274.70 ml/bird), and Blend 2 (273.50 ml/bird) groups compared with the control, Blends 1, 3, and 6. The similarity in average water intake across groups suggests that the blends did not adversely affect the birds' hydration behaviour or water metabolism. Water intake is a critical adaptive response in poultry exposed to heat stress, as increased consumption supports evaporative cooling, blood volume maintenance, and electrolyte balance (Lin *et al.*, 2006). Although statistical differences were not observed, the numerically higher water intake recorded in birds administered AA and some blends, particularly Blends 4, 5 and 2, may indicate enhanced thermoregulatory responses

Table 2: Water intake (mL/bird) of broiler chickens administered blends of ascorbic acid, sodium bicarbonate and sweet citrus peel in a hot humid environment

Treatment	Average water intake (mL/bird)
Control	269.10
AA	279.90
Blend 1	249.40
Blend 2	273.50
Blend 3	249.50
Blend 4	278.20
Blend 5	274.70
Blend 6	250.80
SEM	10.900
P-value	0.160

[abc]*Means within same column bearing different subscripts differ significantly (p<0.05).*
SEM = Standard error of mean.

The effect of dietary treatments on the caeca microbial count is depicted in Figure 1. The highest total bacterial count was recorded in birds administered Blend 3 (11.73×10^6 cfu/mL), which differed significantly ($p<0.05$) from the control (6.60×10^6 cfu/mL) and ascorbic acid (6.87×10^6 cfu/mL) group, but was similar ($p>0.05$) to other treatment groups. The bacterial diversity across groups is indicated in Table 2. The increase in total bacterial load may reflect enhanced microbial proliferation due to increased availability of fermentable substrates from citrus peel pectins and not necessarily indicate dysbiosis. Dietary fibre and pectin-rich agro-indusrial by-products are known to stimulate microbial fermentation in the caecum therefore, increasing bacterial density and metabolic activity (Bampidis and Robinson, 2006). Modulation of gut microbiota by phytogenic additives has been widely reported, with responses depending on inclusion level, bioactive composition, and host physiological status (Mountzouris *et al*., 2011).

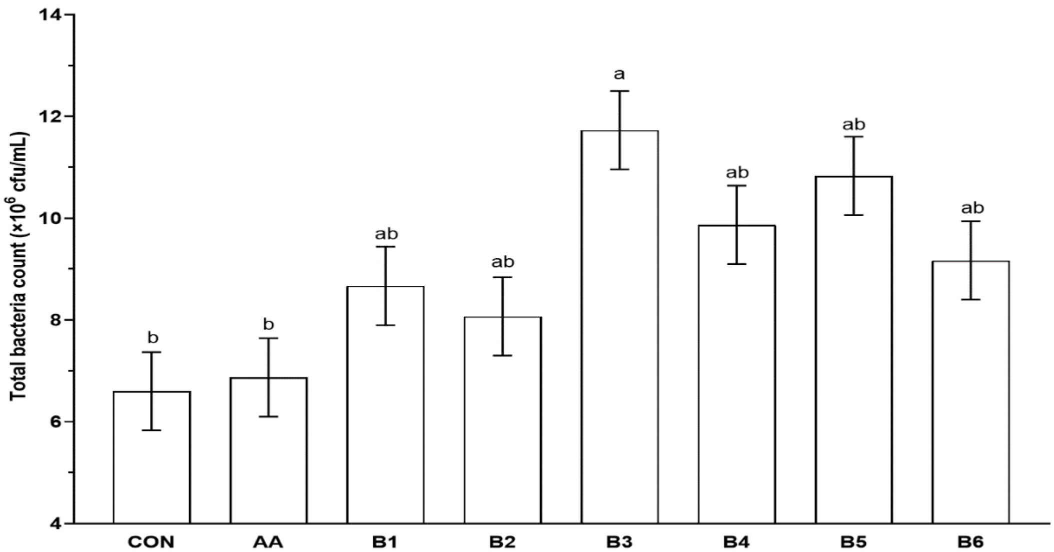

Figure 1: Total bacteria count in the caecum of broiler chickens administered blends of ascorbic acid, sodium bicarbonate and sweet citrus peel powder

The following microorganisms were identified in the caecal samples (Table 3): *Escherichia coli*, *Staphylococcus aureus,* Salmonella spp, Listeria spp, Enterobacter spp, *Campylobacter jejuni*, *Pseudomonas aeruginosa, Klebsiella pneumonia*, Shigella spp, Clostridium spp, Proteus spp. The lowest number of bacterial species was observed in the ascorbic acid **(**AA) group (4 bacteria), while the highest was observed in birds administered Blend 5 (8 bacteria species). *Escherichia coli* and *Staphylococcus aureus* were identified in all the treatment groups. Listeria spp and Clostridium spp were only observed in Blends 1 and 2, respectively. Enterobacter spp was observed only in Blends 2 and 3. Proteus spp was absent in all the treatment groups except in Blends 3, 4 and 5.

The presence of *Escherichia coli* and *Staphylococcus aureus* across treatments is consistent with their status as common commensal or opportunistic organisms in the poultry gut (Oakley *et al.*, 2014). The lower number of bacterial species identified in the AA group suggests suppressive effect of ascorbic acid on certain opportunistic pathogens, potentially mediated through enhanced host immune competence and reduced oxidative stress. On the contrary, the higher bacterial diversity observed in Blend 5 may reflect either increased substrate availability or insufficient antimicrobial potency of that particular combination. As observed in this study, certain genera exhibited treatment-specific distribution pattern, Listeria spp. and Clostridium spp. were detected only in Blends 1 and 2, respectively, while *Enterobacter spp*. appeared only in Blends 2 and 3, such selective microbial shifts may result from the antimicrobial properties of citrus bioactives, which have been shown to disrupt bacterial cell membranes and inhibit specific Gram-negative and Gram-positive organisms (Ghasemi *et al*., 2010).

In conclusion, supplementation of broiler chickens' drinking water with blends of ascorbic acid, sodium bicarbonate, and sweet citrus peel powder did not significantly influence growth performance indices, including body weight, weight gain, feed intake, feed conversion ratio, and survivability under the conditions of this study. However, the numerically improved feed conversion ratios and enhanced survivability observed in Blends 2, 3 and 4 suggest potential functional benefits that may become more pronounced under environmental or pathogenic stress conditions the dietary blends exerted measurable effects on caecal microbial populations, as evidenced by variations in total bacterial counts and variations in bacterial distribution among treatment groups. The findings of this study demonstrate that these additive combinations can modulate gut microbial ecology without adversely affecting growth performance in Arbor Acres broiler chickens.

Table 3: Caecal microbial distribution in broiler chickens administered blends of ascorbic acid, sodium bicarbonate and sweet citrus peel powder

Treatment	A	B	C	D	E	F	G	H	I	J	K
Control	+	+	-	-	-	+	+	+	+	-	-
AA	+	+	+	-	-	-	-	-	+	-	-
Blend 1	+	+	-	+	-	+	+	+	-	-	-
Blend 2	+	+	-	-	+	-	+	-	-	+	-
Blend 3	+	+	+	-	+	+	-	+	-	-	+
Blend 4	+	+	-	-	-	-	+	+	+	-	+
Blend 5	+	+	+	-	-	+	+	+	+	-	+
Blend 6	+	+	+	-	-	-	-	+	+	-	-

+: growth observed, -: no growth observed, A: *Escherichia coli*, B: *Staphylococcus aureus*, C: Salmonella spp, D: Listeria spp, E: Enterobacter spp, F: *Campylobacter jejuni*, G: *Pseudomonas aeruginosa*, H: *Klebsiella pneumonia*, I: Shigella spp, J: Clostridium spp, K: Proteus spp

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Date received: 14th May, 2026
Date accepted: 22nd June, 2026