Growth Performance, Behaviour and Post-Administration Effect of Intermittent Apple Cider Vinegar Administration as Alternative Growth Promoter for Broiler Chickens

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Abstract

Organic acids (OAs) are a prospective alternative to antibiotic growth promoters. Apple cider vinegar (ACV) contains a rich blend of some OAs, and in addition, it also has amino acids, enzymes, minerals, vitamins, and phenolic substances, hence its use in this study as an OA source. This study was conducted to investigate the response of broiler chickens to intermittent administration of ACV at two dosages (5 and 10 mL/L ACV) and three frequencies per week (0/week (antibiotic/control), twice/week, and thrice/week). A total of 300 unsexed day-old Arbor acres broiler chicks were allotted to six treatments with five replicates of 10 birds each. The growth performance and behavioural indices including; feeding, drinking, walking, sitting, wing flapping, dustbathing, and stretching behaviours of the chickens were evaluated, as well as the post-administration effect of ACV. Among all growth indices measured in the 2nd and 6th week, only water intake increased in birds offered ACV twice and thrice weekly in the 2nd week. In the 6th week, the frequency of expression of stretching behaviour increased in birds on 5 mL/L ACV. Also, the administration of ACV thrice/week enhanced drinking and dust bathing behaviour while antibiotics administration enhanced walking behaviour. During post-administration, the highest mortality occurred in birds previously on 10 mL/L ACV thrice/week. In conclusion, a dose of 5 mL/L ACV either twice or thrice/week could be incorporated as an antibiotic substitute for broiler chickens without adverse effects on their growth and behaviour. Also, a short administration interval should be exercised when practicing intermittent ACV administration, to avoid mortality.

Keywords: Broiler chickens, apple cider vinegar, growth performance, behaviour, mortality

Running title: Apple cider vinegar as alternative growth promoter for broiler chickens

Performance de Croissance, Comportement et Effet Post-Administration de l'Administration Intermittente de Vinaigre de Cidre de Pomme comme Promoteur de Croissance Alternatif pour les Poulets de Chair

Résumé

Les acides organiques (AO) représentent une alternative prometteuse aux promoteurs de croissance antibiotiques. Le vinaigre de cidre de pomme (VCP) contient un mélange riche de certains AO, et en outre, il possède des acides aminés, des enzymes, des minéraux, des vitamines et des substances phénoliques, ce qui justifie son utilisation dans cette étude en tant que source d'AO. Cette étude a été réalisée pour examiner la réponse des poulets de chair à l'administration intermittente de VCP à deux doses (5 et 10 mL/L de VCP) et trois fréquences par semaine (0/semaine (antibiotique/témoin), deux fois/semaine et trois fois/semaine). Un total de 300 poussins de poulet Arbor Acres non sexés âgés d'un jour ont été répartis en six traitements avec cinq répétitions de 10 oiseaux chacune. Les indices de performance de croissance et de comportement, comprenant l'alimentation, l'hydratation, la marche, le repos, le battement d'ailes, le bain de poussière et les comportements d'étirement des poulets ont été évalués, ainsi que l'effet post-administration du VCP. Parmi tous les indices de croissance mesurés à la 2e et 6e semaine, seule l'ingestion d'eau a augmenté chez les oiseaux recevant du VCP deux ou trois fois par semaine à la 2e semaine. À la 6e semaine, la fréquence d'expression du comportement d'étirement a augmenté chez les oiseaux traités avec 5 mL/L de VCP. De plus, l'administration de VCP trois fois par semaine a amélioré le comportement de boisson et de bain de poussière, tandis que l'administration d'antibiotiques a amélioré le comportement de marche. Après l'administration, la mortalité la plus élevée a été observée chez les oiseaux ayant reçu 10 mL/L de VCP trois fois par semaine. En conclusion, une dose de 5 mL/L de VCP, administrée soit deux fois, soit trois fois par semaine, pourrait être utilisée comme substitut des antibiotiques pour les poulets de chair

sans effets indésirables sur leur croissance et leur comportement. De plus, un intervalle d'administration court devrait être pratiqué lors de l'administration intermittente de VCP pour éviter la mortalité.

***Mots-clés** : Poulets de chair, vinaigre de cidre de pomme, performance de croissance, comportement, mortalité*
***Titre abrégé** : Vinaigre de cidre de pomme comme promoteur de croissance alternatif pour les poulets de chair*

Introduction

The efficiency of modern poultry production could be improved with the use of specific dietary additives (Ndelekwute and Enyenihi, 2017). Antibiotics as an additive have been employed in the commercial poultry industry to promote growth and reduce pathogenic bacteria (Lilly *et al*., 2011). However, consumers' awareness has increased in recent years, on the possible presence of antibiotic-resistant bacteria in meat products (Lilly *et al*., 2011). As a result, many countries have placed restrictions on the use of antibiotic growth promoters. Therefore, the current challenge in the poultry industry is the search for natural additives or supplements to be administered via water and/or diet to improve production efficiency (Shanoon *et al*., 2018).

The focus of alternative strategies has been to prevent the proliferation of pathogenic bacteria and the modulation of beneficial bacteria to enhance animal health, immune status, and growth performance (Ravindran, 2006). Zarghi (2018) reported organic acids as a promising alternative to eliminating antibiotics in broiler chicken production. The consumption of organic acids either through food or drinking water reduces the pH of the digestive system, which in turn reduces the accumulation of pathogenic bacteria in the intestinal wall, and stimulates the development of beneficial intestinal microbial flora, resulting in the reduction of toxic metabolites produced by harmful bacteria (Thompson and Hinton, 1997; Alp *et al*., 1999). As reviewed by Khan and Iqbal (2016), the addition of different types of organic acids (e.g. acetic, lactic, formic, fumaric, citric, malic, and tartaric acids) to the drinking water of poultry birds helps to reduce the level of pathogens in the water and the gastrointestinal tract resulting in improved feed digestion and growth performance.

Apple cider vinegar is an organic acid blend rich in various flavonoids and phenolic compounds. As a result, it has been found to possess antioxidant, antifungal and antibacterial properties (Zarghi, 2018). The use of vinegar dates back more than 10,000 years (Tan, 2005). As opined by Shahidi *et al*. (2008), consumption of ACV improves the immune system and helps maintain the blood acid-base balance. Furthermore, a study by Allahdo *et al.* (2018) revealed that the combination of ACV in drinking water with probiotics in feed resulted in a lower feed conversion ratio during 1-10 days of age. Incorporating organic acids into broiler chickens' dietary intake or drinking water improved the beneficial microbial profile, specifically by increasing *Lactobacillus spp*., as observed in previous studies (Emami *et al*., 2017; Elnaggar and El-kelawy, 2024; Islam *et al*., 2024). Unlike pathogenic bacteria, acidophilic bacteria such as *Lactobacilli* can withstand variations in pH levels (Chukwudi *et al.,* 2024). The effects of gut microorganisms on the brain and behaviour are well documented (Cryan and Dinan, 2012; Sarkar *et al*., 2016; Sharon *et al*., 2016). Research has shown that gut bacteria communicate with the host via the immune, endocrine, and neural pathways, known as the microbiota–gut–brain axis (Liang *et al*., 2015). Among the notable strains of bacteria participating in the communication axis between the brain and gut is *Lactobacillus spp.* Various species of this bacteria have been used as dietary probiotic supplements and their positive influence on animal behaviour has been reported (Olorocisimo *et al*., 2023, Pereira *et al*., 2024). However, behavioural changes following supplementation with organic acids or their sources (such as ACV) have not been documented. Behavioural study is an important non-invasive tool for animal welfare assessment; a good welfare status would enhance animal productivity (Dawkins, 1999). Observing animal behaviour during research will provide supplementary insights that can be correlated with other indices measured.

There remains a significant gap in the literature regarding the effects of apple cider vinegar (ACV) in broiler production. Moreover, the few studies conducted did not investigate the potential responses of broiler chickens to intermittent administration of ACV. Applying ACV as an antibiotic alternative in a discontinuous mode would help reduce the cost incurred in the purchase or production of ACV for broiler production. Hence, this study investigated the effect of varying dosages and frequencies of

administration of ACV on the growth performance and behaviour of broiler chickens, as well as the post-administration effect.

Materials and methods

Experimental site

This experiment was conducted at the Poultry Unit of the Directorate of University Farms (DUFARMS) of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State Nigeria. The site is located in the rainforest zone of southwest Nigeria Latitude 7º10'N, Longitude 3º2'E and 98m above sea level (Google Earth, 2020).

Ethical approval

All procedures used in the experiment strictly adhered to the research ethics guidelines of the Federal University of Agriculture, Abeokuta (FUNAAB, 2016). The study was thus approved by the Department of Animal Production and Health, College of Animal Science and Livestock Production, FUNAAB.

Birds, diet and management practices

A total of 300 day-old broiler chicks of the Arbor acres strain were purchased from a reputable breeding farm in Ibadan, Nigeria was used for this study. The chicks were weighed and randomly distributed into six treatment groups and housed in a floor pen with wood shavings as litter materials. Brooding was done for two weeks. Vaccination against infectious bursal disease and Newcastle disease was done on days 7 and 14 and 21 and 28. Following the National Research Council (NRC, 1994) guideline for the nutritional requirement of broiler chickens. The feeding programme consisted of a commercial starter and finisher diets containing 2800 kcal/kgME, 21% crude protein, and 2900 kcal/kgME, 18% crude protein, respectively. Feed and water were offered *ad libitum* throughout the experiment. The nutrient composition of the starter and finisher diets is shown in Table 1.

Table 1: Nutrient composition of the commercial diet as declared by the manufacturer

Ingredient	Starter (%)	Finisher (%)
Crude protein	21.00	18.00
Fats and oil	6.00	6.00
Crude fibre	5.00	6.00
Calcium	1.00	1.00
Available phosphorus	0.45	0.40
Lysine	1.00	0.86
Methionine	0.50	0.30
Salt	0.30	0.30

Test ingredients

Organic, raw, unfiltered apple cider vinegar produced by White House Foods in Winchester, UK, was purchased from a local store in Abeokuta, Ogun State, Nigeria. The pH of raw and diluted ACV was determined prior to the experiment using pH-108A pocket-sized pH meter, and the outcome revealed that the raw ACV had a pH of 3.4 while 5 mL and 10 mL of ACV in 1 L of drinking water had a pH of 4.85 and 4.6, respectively. A broad-spectrum antibiotic containing 20% Enrofloxacin and Bromhexine hydrochloride solution was also procured from the local veterinary store, and was used as a growth promoter for the control treatments.

Experimental design

The experimental layout was a two by three factorial arrangement (two dosages and three frequencies of administration). Birds were randomly assigned to each treatment in a Completely Randomized Design. There were six treatments; each was replicated five times with 10 birds per replicate. Birds in control groups (T1 - Control for 5 mL/L ACV and T4 - Control for 10 mL/L ACV) were administered antibiotics at a dosage of 1 mL in 4 L of drinking water thrice per week while the other four treatments (T2, T3, T5, T6) were administered ACV via drinking water at varying dosages and frequencies. T2 birds were given water containing 5 mL/L ACV (twice weekly), T3 birds were given water containing 5 mL/L ACV (thrice weekly), T5 birds were given water containing 10 mL/L ACV (twice weekly) and T6 birds were given water containing 10 mL/L ACV (thrice weekly). The administration of the ACV was done consecutively, and it commenced on the second day of two weeks of brooding and spanned through the sixth week of the experiment.

To observe the post-administration response of the birds,
ACV was withdrawn at the end of the sixth week and birds were observed for five days in the 7th week. The experimental layout is shown in Table 2.

Table 2: Experimental layout

DOSAGE OF ACV	5mL/L ACV	10mL/L ACV
FREQUENCY OF ADMINISTRATION	0/week (given antibiotics)-Control	0/week (given antibiotics)-Control
	Twice/week	Twice/week
	Thrice/week	Thrice/week

Data collection

Growth performance: Data was collected on the following growth performance parameters; feed intake, water intake, weight gain, Feed Conversion Ratio (FCR), and mortality.

$$Average\ feed\ intake\ (g) = \frac{Total\ feed\ given\ (g) - Total\ feed\ leftover(g)}{Total\ number\ of\ birds}$$

$$Average\ water\ intake\ (l) = \frac{Total\ water\ given\ (l) - Total\ water\ leftover(l)}{Total\ number\ of\ birds}$$

$$Average\ weight\ gain\ (g) = \frac{Final\ weight\ (g) - Initial\ weight\ (g)}{Total\ number\ of\ birds}$$

$$FCR = \frac{\text{Feed intake (g)}}{Weight\ gain\ (g)}$$

$$Mortality\ (\%) = \frac{Number\ of\ dead\ birds}{Total\ number\ of\ birds\ stocked} \times 100$$

***Behavioural observation*:** Closed-Circuit Television (CCTV) cameras model Chloride UK CSC – 3020W were installed in the brooding and rearing pens for behavioural recordings. The cameras were mounted so that birds in two replicates per treatment (20 birds/treatment) were captured. The recording was done consecutively thrice weekly for eight hours (24 hours/week recording) between 8 a.m. and 4 p.m. and later watched to extract the number of visitations to the feeders and drinkers, and frequency of occurrence of walking, sitting, stretching, wing flapping, and dust bathing. A continuous or all occurrence sampling technique described by Lehner (1992) was used to gather the behavioural data. The 8 hours daily behavioural recordings (24 hours/week) of 20 birds/treatment were watched, and all the numbers of times each bird expressed the behaviours of interest were added up, and the average per bird was calculated. The ethogram used for this behavioural study was adopted from Villagrá *et al*. (2014) and it is provided in Table 3.

Table 3: Ethogram of behaviour recorded in chickens offered Apple cider vinegar

Activity	**Description**
Sitting	Behaviour of sitting or lying on the litter material without performing any other activity.
Walking	When the bird walks in the pen.
Feeding	When the bird eats, regardless of whether it is standing or sitting.
Drinking	When the bird drinks, regardless of whether it is standing or sitting.
Dust bathing	Behaviour of burrowing, sitting and then throwing litter materials over the body.
Stretching	Behaviour of stretching a wing and/or a foot of the same hemisphere.
Wing flapping	Movement by which the bird flaps both wings energetically.

Villagrá *et al*., 2014

Statistical analysis

Data obtained were subjected to one-way Analysis of Variance using General Linear Model (GLM) of MINITAB 2018 statistical package, and all significant means were separated using Tukey Krammer's Test of the same statistical package at P < 0.05 significant level. In addition, bar graphs of the post-administration mortality of the birds during the 7th week were plotted using GraphPad Prism 8 software (GraphPad Software Inc., San Diego, USA) after statistical analysis.

Results and discussion

Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the growth performance of broiler chickens at two weeks of age

The main effects of dosage and frequency of administration of ACV on the growth performance of broiler chickens at two weeks of age are presented in table 4. Water consumption increased in birds offered ACV twice and thrice weekly compared to the control, while all other parameters were not significantly (P<0.05) influenced by the frequency of administration of ACV. No significant (P>0.05) difference was also observed in all growth parameters with regard to the dosage of ACV.

Table 4: Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the growth performance of broiler chickens at two weeks of age

Parameter	Dosage of ACV (mL/L)				Frequency of administration/week				
	5	10	SEM	P-value	0	Twice	Thrice	SEM	P-value
IW (g/bird)	42.55	44.55	2.22	0.53	43.27	44.18	43.18	2.72	0.96
FW (g/bird)	269.33	281.68	4.55	0.07	268.70	277.93	279.89	5.57	0.33
TWG (g/bird)	226.79	237.13	5.41	0.19	225.43	233.75	236.71	6.63	0.47
TFI (g/bird)	374.31	387.48	9.02	0.31	360.50	398.90	383.30	11.00	0.07
FCR	1.66	1.64	0.05	0.72	1.60	1.72	1.63	0.06	0.39
TWI (l/bird)	0.30	0.31	0.01	0.27	0.27^{b}	0.32^{a}	0.33^{a}	0.01	<0.01
Mortality (%)	0.67	0.00	0.47	0.33	0.00	1.00	0.00	0.58	0.38

^{a,b}: Means in a row with different superscripts by factor are significantly (P<0.05) different SEM: Standard error of mean

FCR: Feed conversion ratio; IW: Initial weight, FW: Final weight, TWG: Total weight gain, TFI: Total feed intake, TWI: Total water intake

Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the growth performance of broiler chickens at two weeks of age

The interactive effects of dosage and frequency of ACV administration on broiler chickens' growth performance are presented in Table 5. All growth indices measured were not significantly (P>0.05) affected by the administration of ACV at the tested dosages and frequencies of administration.

Table 5: Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the growth performance of broiler chickens at two weeks of age

Parameters	5 mL/L ACV			10 mL/L ACV			SEM	P-value
	0/ week	Twice/ week	Thrice/ week	0/ week	Twice/ week	Thrice/ week		
IW (g/bird)	43.27	43.27	41.09	43.27	45.09	45.27	3.85	0.86
FW (g/bird)	269.00	265.00	274.00	268.40	290.86	285.78	7.88	0.26
TWG (g/bird)	225.73	221.73	232.91	225.13	245.77	240.51	9.37	0.42
TFI (g/bird)	360.50	386.90	375.50	360.50	410.90	391.00	15.60	0.74
FCR	1.60	1.77	1.63	1.61	1.67	1.64	0.09	0.30
TWI (l/bird)	0.27	0.30	0.32	0.27	0.33	0.34	0.02	0.61
Mortality (%)	0.00	2.00	0.00	0.00	0.00	0.00	0.82	0.38

^{a,b}: Means in a row with different superscripts by factor are significantly (P<0.05) different

SEM: Standard error of mean

IW: Initial weight, FW: Final weight, TWG: Total weight gain, TFI: Total feed intake, TWI: Total water intake

Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the growth performance of broiler chickens at six weeks of age

The main effects of dosage and frequency of administration of ACV on the growth performance of broiler chickens at six weeks of age are shown in Table 6. All growth parameters were not significantly (P>0.05) affected by both the dosage and frequency of administration of ACV.

Table 6: Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the growth performance of broiler chickens at six weeks of age

	Dosage of ACV (mL/L)				Frequency of administration/week				
Parameter	**5**	**10**	**SEM**	**P-value**	**0**	**Twice**	**Thrice**	**SEM**	**P-value**
IW (g/bird)	42.55	44.55	2.22	0.53	43.27	44.18	43.18	2.72	0.96
FW (g/bird)	1517.20	1474.70	23.60	0.22	1496.40	1476.10	1515.20	28.90	0.64
TWG (g/bird)	1474.60	1430.10	23.50	0.19	1453.10	1432.00	1472.00	28.80	0.62
TFI (g/bird)	2984.00	2924.40	34.90	0.24	2976.60	2950.70	2935.40	42.80	0.79
FCR	2.02	2.04	0.04	0.67	2.05	2.06	1.99	0.05	0.58
TWI (L/bird)	8.67	8.40	0.13	0.14	8.55	8.52	8.53	0.16	0.99
Mortality (%)	0.67	0.67	0.67	1.00	2.00	0.00	0.00	0.82	0.16

SEM: Standard error of mean
IW: Initial weight, FW: Final weight, TWG: Total weight gain, TFI: Total feed intake, TWI: Total water intake

Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the growth performance of broiler chickens at six weeks of age

Table 7 shows the interactive effects of dosage and frequency of administration of ACV on the growth performance of broiler chickens at six weeks of age. The interaction between the dosages and frequencies of administration of ACV did not significantly (P>0.05) influence all the growth indices.

Table 7: Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the growth performance of broiler chickens at six weeks of age

	5 mL/L ACV			10 mL/L ACV				
Parameters	**0/week**	**Twice/ week**	**Thrice/ week**	**0/week**	**Twice/ week**	**Thrice/ week**	**SEM**	**P-value**
IW (g/bird)	43.27	43.27	41.09	43.27	45.09	45.27	3.85	0.86
FW (g/bird)	1496.90	1490.00	1564.70	1495.90	1462.30	1465.80	40.90	0.48
TWG (g/bird)	1453.60	1446.70	1523.60	1452.60	1417.20	1420.50	40.70	0.45
TFI (g/bird)	2976.60	3021.70	2953.80	2976.60	2879.60	2917.00	60.50	0.49
FCR	2.05	2.09	1.94	2.05	2.03	2.06	0.07	0.49
TWI (l/bird)	8.55	8.69	8.76	8.54	8.35	8.29	0.22	0.57
Mortality (%)	2.00	0.00	0.00	2.00	0.00	0.00	1.15	1.00

SEM: Standard error of mean
IW: Initial weight, FW: Final weight, TWG: Total weight gain, TFI: Total feed intake, TWI: Total water intake

Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the behaviour of broiler chickens at two weeks of age

In Table 8, the main effects of dosage and frequency of administration of ACV on the behaviour of broiler chickens at two weeks of age are presented. No significant difference (P>0.05) exists among all behavioural parameters considered. Also, dust bathing behaviour was absent in all birds across the treatment groups.

Table 8: Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the behaviour of broiler chickens at two weeks of age

	Dosage of ACV (mL/L)				Frequency of administration/week				
Parameter	**5**	**10**	**SEM**	**P-value**	**0**	**Twice**	**Thrice**	**SEM**	**P-value**

Feeding	9.28	10.42	1.18	0.52	8.70	11.00	9.85	1.44	0.56
Drinking	8.12	7.77	0.53	0.66	8.10	8.05	7.68	0.65	0.88
Walking	21.25	20.95	1.38	0.88	22.18	21.08	20.05	1.69	0.69
Sitting	3.50	3.32	0.53	0.82	2.63	4.38	3.23	0.65	0.23
Wing flapping	2.72	2.18	0.35	0.32	2.63	2.83	1.90	0.42	0.33
Stretching	2.20	2.30	0.32	0.83	2.33	2.75	1.68	0.39	0.22
Dust bathing	0.00	0.00	0.00	-	0.00	0.00	0.00	0.00	-

SEM: Standard error of mean

Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the behaviour of broiler chickens at two weeks of age

Table 9 shows the interactive effects of dosage and frequency of administration of ACV on the behaviour of broiler chickens at two weeks of age. There was no significant difference (P>0.05) among all behavioural parameters considered. In addition, there were zero occurrences of dustbathing behaviour in all birds studied.

Table 9: Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the behaviour of broiler chickens at two weeks of age

	5 mL/L ACV			10 mL/L ACV				
Parameters	**0/week**	**Twice/ week**	**Thrice/ week**	**0/ week**	**Twice/ week**	**Thrice/ week**	**SEM**	**P-value**
Feeding	8.15	10.30	9.40	9.25	11.70	10.30	2.04	0.99
Drinking	7.85	8.50	8.00	8.35	7.60	7.35	0.92	0.73
Walking	22.50	21.95	19.30	21.85	20.20	20.80	2.40	0.80
Sitting	2.50	3.85	4.15	2.75	4.90	2.30	0.92	0.33
Wing flapping	2.45	3.45	2.25	2.80	2.20	1.55	0.60	0.45
Stretching	2.15	2.80	1.65	2.50	2.70	1.70	0.55	0.92
Dust bathing	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-

SEM: Standard error of mean

Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the behaviour of broiler chickens at six weeks of age

The main effects of dosage and frequency of administration of ACV on the behaviour of broiler chickens at six weeks of age are shown in Table 10. Birds administered 5 mL/L ACV dosage expressed stretching behaviour more than those given 10 mL/L. The administration of ACV thrice/week increased (P<0.05) the frequency of drinking and dust bathing behaviour in the birds, while antibiotics administration increased (P<0.05) the expression of walking behaviour.

Table 10: Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the behaviour of broiler chickens at six weeks of age

	Dosage of ACV (mL/L)				Frequency of administration/week				
Parameter	**5**	**10**	**SEM**	**P-value**	**0**	**Twice**	**Thrice**	**SEM**	**P-value**
Feeding	46.32	45.03	1.77	0.61	45.86	45.37	45.80	1.95	0.98
Drinking	29.73	27.24	1.22	0.18	30.01^{ab}	24.79^{b}	30.67^{a}	1.40	0.03
Walking	46.80	45.41	3.81	0.81	57.71^{a}	38.61^{b}	42.00^{ab}	4.37	0.04
Sitting	22.23	20.40	1.33	0.36	24.41	19.84	19.70	1.53	0.14
Wing flapping	7.26	6.46	5.63	0.34	5.95	7.17	7.45	0.65	0.35
Stretching	5.80^{a}	4.37^{b}	0.37	0.02	4.63	4.83	5.80	0.42	0.20
Dust bathing	2.44	2.50	0.20	0.83	1.88^{b}	2.66^{ab}	2.87^{a}	0.22	0.04

[a,b]: Means in a row with different superscripts by factor are significantly (P<0.05) different
SEM: Standard error of mean

Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the behaviour of broiler chickens at six weeks of age

The interactive effects of dosage and frequency of ACV administration on broiler chickens' behaviour at six weeks of age are presented in Table 11. The birds given 5 mL/L ACV thrice/week exhibited stretching behaviour most, while those in the antibiotics (ACV 0/week), 5 mL/L ACV twice/week and 10 mL/L ACV thrice/week groups exhibited it least. Dust bathing behaviour was recorded most among birds given 5 mL/L ACV thrice/week (3.57), while the lowest was from birds given 5 mL/L ACV twice/week (1.85).

Table 11: Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the behaviour of broiler chickens at six weeks of age

Parameters	5 mL/L ACV			10 mL/L ACV			SEM	P-value
	0/week	Twice/ week	Thrice/ week	0/week	Twice/ week	Thrice/ week		
Feeding	46.65	46.18	46.13	45.07	44.57	45.47	2.55	0.98
Drinking	31.05	26.25	31.90	28.97	23.33	29.43	1.83	0.98
Walking	61.35	38.63	40.43	54.07	38.60	43.57	5.72	0.76
Sitting	25.35	21.65	19.70	23.47	18.03	19.70	2.31	0.73
Wing flapping	6.40	6.40	8.97	5.50	7.93	5.93	0.85	0.09
Stretching	4.95^{b}	4.05^{b}	8.40^{a}	4.30^{b}	5.60^{ab}	3.20^{b}	0.55	<0.01
Dust bathing	1.90^{abc}	1.85^{c}	3.57^{a}	1.87^{bc}	3.47^{ab}	2.17^{abc}	0.29	<0.01

[a,b,c]: Means in a row with different superscripts by factor are significantly (P<0.05) different
SEM: Standard error of mean

Main effect of dosage and frequency of administration of apple cider vinegar (ACV) on post-administration mortality percentage of broiler chickens at days 4 and 5 of the 7th week

Figure 1 shows the main effect of dosage and frequency of administration of ACV on post-administration mortality percentage of broiler chickens at days 4 and 5 of the 7th week. The dosage and frequency of administration of ACV significantly affected the birds' mortality percentage. Broiler chickens on 10 mL/L ACV dosage had the highest mortality (11.33%), and the mortality also increased with increasing frequency of administration.

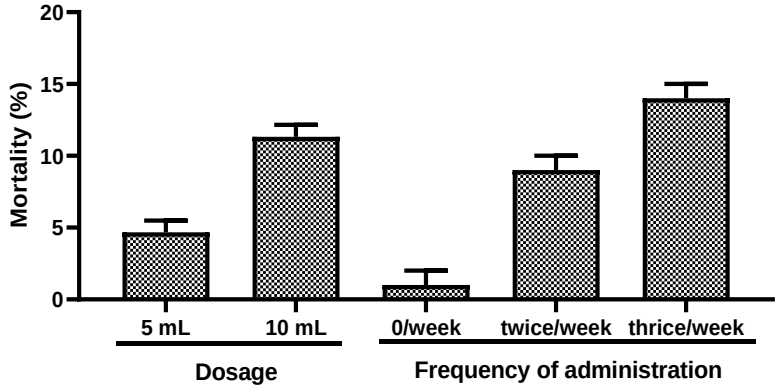

Figure 1: Graph showing the main effect of dosages and frequencies of administration of ACV on post-administration mortality percentage of broiler chickens at days 4 and 5 of the 7th week

Interactive effect of dosage and frequency of administration of apple cider vinegar (ACV) on post-administration mortality percentage of broiler chickens at days 4 and 5 of the 7th week

Figure 2 shows the interactive effect of dosage and frequency of administration of ACV on post-administration mortality percentage of broiler chickens at days 4 and 5 of the 7th week. Birds offered 10 mL/L ACV thrice weekly had the highest (24.00%) mortality percentage, while those groups offered antibiotics had the lowest (1.00%) mortality rate.

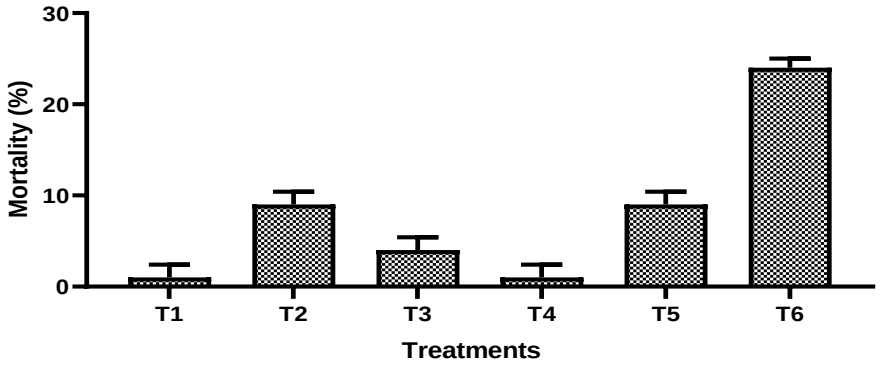

Figure 2: Graph showing the interactive effect of dosages and frequencies of administration of ACV on post-administration mortality percentage of broiler chickens at days 4 and 5 of the 7th week

T1: 5 mL/L ACV control group (administered antibiotics); T2: 5 mL/L ACV twice weekly; T3: 5 mL/L ACV thrice weekly; T4: 10 mL/L ACV control group (administered antibiotics); T5: 10 mL/L ACV twice weekly; T6: 10 mL/L ACV thrice weekly

Discussion

For an alternative to antibiotic growth promoter to be classified as effective, it should have a substantial, sustainable and favourable impact on animal production and health, be safe, not just for the animals, but also for the consumers, and as well be easy to administer, store and should provide a significant return on investment (Collett, 2004). Short-chain fatty acids (SCFAs) have been considered a potential alternative to antibiotics (Van Immerseel *et al*., 2004). Apple cider vinegar (ACV) is abundant in acetic acid, a form of SCFAs. The overall results of the growth performance of birds in this study suggest the beneficial effect of ACV because most growth indices such as feed intake, weight gain, and feed conversion ratio (FCR) evaluated were similar to the control treatment administered antibiotics; this shows the tendency of ACV to favourably compete with antibiotics as a growth promoter for broiler birds. This finding disagrees with Araujo *et al*. (2018), who observed that only antibiotics stimulated better weight gain, feed intake and feed conversion rate when used compared to those of organic acids and probiotics. However, an earlier report by Hayajneh (2019) corroborated the result of this research. Hayajneh (2019) examined the growth-promoting effect of ginger powder and ACV in comparison with flavomycin, an antibiotic growth promoter, and concluded that there was no significant difference in the feed intake, feed conversion ratio and weight gain of broiler chickens administered the test ingredients.

At two weeks of age, water consumption by birds administered ACV was higher than that of birds on antibiotics. The numerically higher feed intake by birds in those treatments might be responsible for the increase in water consumption. According to NRC (1994), a positive correlation usually exists between feed and water intake of animals under optimal environmental conditions and health status. This higher water intake by birds on ACV may also indicate the acceptability of acidified water by birds since they possess taste buds. Although, according to Roura *et al*. (2008), the taste buds of chickens are only a fraction of the number of taste buds found in other animals. In a trial conducted by Watkins *et al*. (2004) to study the impact of acidification of drinking water to different pH (3, 4 or 5) on broiler weight gain, FCR, water consumption, and liveability, no significant improvement was found in

all growth parameters. It was concluded that birds are very tolerant to a wide range of water pH.

As part of the factors affecting animal behaviour, the microbiota-gut-brain axis is a well-established concept that showcases the influence intestinal bacteria have on neural processes, hormone production, and behaviour expressions, through a bidirectional communication between the gut bacteria and the brain (Kraimi *et al*., 2019). To the best of our knowledge, there are currently no reports on the influence of organic acids on the behaviour of poultry birds. However, other feed additives that can modulate intestinal microbiota, such as prebiotics, synbiotics, and probiotics, have been shown to affect the behaviour of poultry birds positively (Hassan *et al*., 2019; Mohammed *et al*., 2022; Zammit and Park, 2023; Johnson *et al*., 2024). The interactive effect of dosage and frequency of administration of ACV in this current research was evident in birds' expression of comfort/maintenance behaviours (dust bathing and stretching) at 6 weeks of age. Administration of 5 mL/L ACV thrice/week to birds increased the frequency of dustbathing and stretching behaviour. According to Duncan and Mench (1993), comfort behaviours are performed after completing basic needs. Natural behaviour such as dustbathing has also been found to help prevent frustration (Cindy *et al*., 2011). Similar to our finding, previous research on the use of other gut-influencing feed additives such as probiotics and synbiotics, reported an increase in the expression of comfort-related behaviour by broiler chickens (Fayed and Tony, 2008; Mohammed *et al*., 2018). Also, the sequence of movement involved in the exhibition of both stretching and dust bathing behaviour in the ACV group might help strengthen the leg muscles of the birds, thereby preventing leg deformities and walking incapacities (Shields *et al*., 2004). Alternatively, birds on antibiotics were observed to express walking behaviour more, as a form of physical activity to enhance their leg strength.

Apple cider vinegar administration was terminated at the birds' sixth week after all treatment groups had been served for their assigned frequencies, either twice or thrice per week consecutively. To observe the post-administration response, chickens were maintained on *ad libitum* feeding and drinking in their respective treatment groups. Surprisingly, mortality of the birds increased significantly in the seventh week, on days 4 and 5 post-administration, whereas, during the six weeks of ACV administration, only 3.7% of mortality was recorded in the entire flock. Most chickens lost during this post-administration period were those previously administered 10 mL/L ACV, particularly those in the thrice per week group (given the highest ACV concentration). Furthermore, minimal mortality was observed in the antibiotic groups (1%) compared to the ACV groups (4 - 24%) on both days. The reason for the high post-administration mortality in the ACV group is unclear, but it may be related to the mode of action of ACV on GIT (gastrointestinal tract) microbiota. Kalaba *et al*. (2019) reported that ACV could have either bactericidal or bacteriostatic effects on pathogenic bacteria, depending on the concentration of acetic acid in the vinegar, the population and the type of bacteria present. Yagnik *et al*. (2018) also reported that one of the mechanisms of action of ACV on bacteria is by reducing the GIT pH thereby limiting the propagation of pathogenic bacteria. (Yagnik *et al*., 2018). Further study should be conducted to investigate chicken gut microbiota during and after ACV administration, to investigate how it impacts the bacteria population. This current study hypothesized that the mortality might have stemmed from the worn-off inhibitory effect of the ACV on the GIT pathogenic bacteria, leading to their overpopulation. Hence, apple cider vinegar might be short-acting compared to antibiotics. A withdrawal period is usually stated for antibiotics due to their long-acting effect when administered to animals. The withdrawal period reflects the amount of time necessary for an animal to metabolise an administered product until it is no longer functional or present or has reduced to a safe, acceptable level in the case of drugs (Mund *et al*., 2017).

Conclusion and recommendation

As a replacement for antibiotic growth promoters, administration of apple cider vinegar at a dose of 5 mL per litre of drinking water either twice or thrice/week can be incorporated into the medication schedule of broiler birds without adverse effects on their growth and behaviour. A short administration interval should be exercised when practising intermittent administration of ACV due to its short-acting effect.

Acknowledgement
We acknowledge efforts of Adelaja Folarin, Adeoye Jumoke, Adekunle Afolarin, Akinade Azeez, and Enitan Adebayo during the fieldwork and data collection.

Conflicts of interest statement
The authors declare no conflict of interest.

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Date received: 23rd October, 2024
Date accepted: 12th January, 2025