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Evaluation of Apple (Malus domestica) Cider Vinegar and Garlic (Allium sativum) Extract as Phytogenic substitutes for growth promoting dietary antibiotics in Sexed Broiler Chickens

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Research Article

Keywords: Antibiotic growth promoters, Phytogenic additives, Broiler Performance

Posted Date: October 10th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2113346/v1

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Abstract

In the poultry industry, growing concern about health risks is tightening regulations on the use of dietary antibiotic growth promoters (AGPs), raising interest in safer substitutes. The study investigated the phytogenic effects of drinking-water supplemented apple cider vinegar (ACV) and garlic extract (GAE) on broilers. The study used 390 Ross 308 broiler chicks reared in a deep litter, open house, within 2.3 m^2 pen partitions. Treatments were randomly allocated in 2 (sex) x 5 (additives) factorial experiment replicated three times. Chicks started (days 1–22) on the same antibiotic starter diet, followed by grower and finisher (29–42 days) phase antibiotic-free diets plus untreated drinking water (Negative control (NC), antibiotic free diet plus ACV treated drinking water (T1), antibiotic free diet plus GAE treated drinking water (T2), antibiotic free diet plus ACV + GAE treated drinking water (T3) or antibiotic diet plus untreated drinking water (positive control (PC). Males had higher ($P < 0.05$) feed intake than females in both growth phases. Birds on the PC gained more ($P < 0.05$) weight than others. Birds on the PC consumed more feed ($P < 0.05$) during the finisher phase than T1, T2 and the NC. Birds on the PC had a lower ($P < 0.05$) grower-phase FCR than others, though with lower ($P < 0.05$) FCR during the finisher phase for birds on T1 and T3. Across sex, birds on the PC had the higher ($P < 0.05$) percent spleen weight than birds on T1. Across the sexes, birds on the PC had smaller proventriculus ($P < 0.05$) than on NC, T1, T2 and T3. Across the sexes, birds on the PC had lower ($P < 0.05$) gizzard weight than birds on the T2 and T3. Birds on the NC exhibited a lower dressing percentage ($P < 0.05$) than all other treatments. Meat pH was higher ($P < 0.05$) in males. In conclusion, in contrast to dietary antibiotics, except for increased dressing percentage, ACV and GAE did not improve broiler performance.

Introduction

Dietary antibiotic growth promoters (AGPs) are traditionally used to increase livestock productivity and efficiency (Suresh *et al.*, 2018). The mechanism of action is through control of gastrointestinal infections by alteration of the gut microbial composition (Singh *et al.*, 2013). A review of recent research on the use of AGPs in food-producing animals (Ronquillo and Hernandez, 2017) revealed a growing awareness to the risks they pose to both animal and human health, and to the environment.

Several alternatives have been proposed and tested, among which are phytogenic feed additives (Amad *et al.*, 2011). Phytogenic additives are defined as plant-derived compounds incorporated into diets to enhance the productivity of livestock (Banerjee *et al.*, 2013). Phytogenics contain complex blends of organic molecules with multiple active components of different modes of antimicrobial action, which makes it difficult for bacteria to develop resistance (Suresh *et al.*, 2018). Mechanisms of action include alteration of the intestinal microbiota, increased digestibility and absorption of nutrients, as well as antioxidative and immunomodulatory activities (Ahmed *et al.*, 2013).

Many natural feeds are known to have beneficial multifunctional properties derived from specific bio-active components (Huyghebaert *et al.*, 2011). One of the natural food condiments known to possess functional properties is apple cider vinegar (ACV) (Bárdos and Bender, 2012). The ACV is a fermented juice from apples of less (< 5%) acidity (Ahmadifar *et al.*, 2019). The growth enhancement effect of ACV is attributed to anti-inflammation (Khan and Iqbal, 2016) and to nutrients, such as organic acids, vitamins and minerals (Pourmozaffar *et al.*, 2017). The organic acids also modulate nutrient digestion (Pourmozaffar *et al.*, 2017), which is linked to trophic effects on the absorptive epithelium, such as increased villi height, which enhance epithelial cell secretions, nutrient digestion and absorption (Khan and Iqbal, 2016). Organic acids are long recognised as safe functional supplements which selectively eliminate harmful intestinal bacteria (Yagnik *et al.*, 2018). The beneficial organic acids include simple monocarboxylic acids such as acetic, formic, butyric and propionic acids (Mehdi *et al.*, 2018). Acetic acid is the predominant acid in ACV (Hayajneh, 2019) which seems most involved in the prebiotic effect (Akanksha and Sunita, 2017).

Similar to ACV, the garlic extract (GAE) has important dietetic and medicinal functionality *(*Hindi, *2013).* Garlic possesses antimicrobial, antioxidant, antithrombotic, anticarcinogenic properties, and exhibits vasodilator characteristics (Issa and Omar, 2012).

Currently, we lack a qualitative, and quantitative understanding of the phytogenic efficacy of ACV and GAE in broilers. Therefore, this study investigated the specific, and potential synergistic/complementary phytogenic activity when these are administered in drinking water for broilers.

Research Methodology

Description of the study area

The study was conducted at the poultry facility of the experimental farm of the School of Agriculture, University of Venda, Thohoyandou, Limpopo Province, South Africa. Coordinates of the location are: 22.9761° S, 30.4465° E. The study site is characterized by arid and semi-arid conditions and temperature ranges from a minimum of 10°C during winter to a maximum of 40°C during summer (Odhiambo, 2011).

Diets and phytogenic additives

A three-phase feeding regime was implemented, starter (0–15 days), grower (16–28 days of age) and finisher (29–42 days of age). During the starter phase, all chicks were on uniform medicated, 200 g kg^{-1} CP starter diet (Meadow feeds, Product no 11521-000). Additionally, the chicks received poultry stress vitamins (Virbac© Samrand Business Park, Centurion, Pretoria, South Africa) for the first six days following placement. Antibiotic-free (negative control) diets were custom made by Brennco® Feeds, namely, Broiler grower 190 g kg^{-1} CP (Product 424 – 50 PROD 2021/08/30), Broiler Finisher 165 g kg^{-1} CP (Product 428 – 50 PROD 2021/08/30) which were iso-nutrient to antibiotic treated (positive control) standard commercial Meadow® Feeds budget-range diets (Broiler Grower product 115-222-000, Broiler Finisher product 11523-000).

Aqueous GAE was prepared according to (Hayat *et al.*, 2016), with some modifications. Fresh garlic bulbs were purchased from the local market. The bulbs were separated into garlic cloves which were then washed with tap water. Fresh garlic bulbs were loaded into a centrifugal juicer (700W Russell Hobbs centrifugal juice maker Model no. RHJM01) and centrifuged at level 2. The extract was packaged in 750ml glass bottles and stored at 4°C.

To prepare ACV, Golden delicious apples were cut into 2–3 cm cubic chunks. A 3-litre glass jar was half filled with the apple chunks followed by the addition of 8 tablespoons of brown sugar and 1.3 litres of water. The mixture was stirred thoroughly to ensure homogeneity, and the glass jar was covered with a cloth. The jar was kept at room temperature (21–27°C) in a dark cupboard. The mixture was stirred daily over 3 weeks to avoid the development of mold. After 4 weeks, the apple chunks were strained off and the cider retained in the glass jar and left to ferment for another 4 weeks, after which the vinegar was transferred into 750ml glass bottles with an airtight lid and stored at 4°C (Jahantigh *et al*., 2021).

Birds, housing and management

A total of 390-day-old Ross broiler chicks were used in the experiment. The experiment was conducted in a 17.0 m x 9.0 m open broiler house with a section divided into 30 partitions, each measuring 140 cm × 145 cm (2.3 m^2) and stocked at 13 birds per pen. Each pen had one tube feeder (Height 430mm, diameter 390mm) and one water fountain (Height 400 mm, diameter 360 mm, Poltek, Johannesburg, South Africa). The cement floor was covered with wood shavings. The birds were exposed to continuous light throughout the test period, *ad libitum* access to a flaked commercial starter feed and an ad libitum supply of drinking water.

Experimental design

The study design was a randomized 2 (sex) x 5 (additives) factorial experiment replicated 3 times. Within each sex, additive treatments were as follows: NC (Negative control) - Antibiotic free diet plus untreated drinking water; T1- Antibiotic free diet plus ACV treated drinking water; T2- Antibiotic free diet plus GAE treated drinking water; T3- Antibiotic free diet plus ACV + GAE treated drinking water; PC (Positive control)- Antibiotic fortified diet plus untreated drinking water

Data collection

Weights of 3 random birds per replicate were measured every 7-days that is, day 15, day 21, day 28, day 35 and day 42. Feed intake and FCR were calculated for the same 7-day intervals. Mortality was monitored daily and recorded whenever it occurred. On day 42, one (1) random bird per replicate was selected for slaughter. The slaughter protocol followed that described by Benyi *et al*. (2015). Birds to be slaughtered were subjected to 24 hours of feed withdrawal with free access to drinking water after which they were slaughtered and dressed. The viscera (gizzard, heart, liver, small intestine, caecum and abdominal fat) were removed, cleaned (gut) and weighed. The lengths of the small and large intestines were measured (Mabelebele *et al*., 2017). Carcasses were weighed at slaughter and after 5 hours 14^{o}C post-partum to assess drip loss. The pH of small intestines digesta was measured using a portable pH (PH-009(I) meter (Cherian *et al*., 2013). Breast meat pH was measured from 1 g samples cut into small pieces and homogenized in 9 mL of distilled water using a Mettler Toledo pH-meter equipped with a glass electrode (Choo *et al*., 2014).

Statistical analysis

Data was subjected to analysis of variance (ANOVA) for a 2 X 5 factorial experiment using the General Linear Model (GLM) procedures of IBM SPSS Version 26.0 (SPSS, 2019). Significant main effects means were separated using Tukey’s post-hoc test, at $p < 0.05$.

Results

Effects of ACV and or GAE on production performance of male and female Ross 308 broilers are presented in Table 1. During the grower phase, male birds on the PC gained more weight ($P < 0.05$) than birds on T1, and T3, but had a similar weight gain ($P < 0.05$) with birds on the NC and T2. Male birds on the NC, T1, T2 and T3 had similar weight gain ($P > 0.05$). During the grower phase, feed intake, FCR and mortality of male birds were not affected by the additives ($P > 0.05$). Female birds on the PC gained more weight ($P < 0.05$) than those on the T1, T2 and T3 and had a similar weight gain ($P > 0.05$) with birds on the NC. However, female birds on the NC, T1, T2 and T3 had similar weight gain ($P > 0.05$). FCR of birds on the PC was lower ($P < 0.05$) than of birds on the NC, T1, T2 and T3. Feed intake and mortality of female birds were not affected by dietary additives ($P > 0.05$). Across sex, birds on the PC gained more weight and had a better FCR ($P < 0.05$) than birds on the NC, T1, T2 and T3. Feed intake and mortality of birds was not affected by additives ($P > 0.05$). Male birds consumed more feed ($P < 0.05$) and gained more weight ($P < 0.05$) than female birds. However, FCR and mortality ware not influenced ($P > 0.05$) by sex.

During the finisher phase (Table 1), feed intake and weight gain of male birds on the PC were significantly higher ($P < 0.05$) than male birds on the NC, T1, T2 and T3, with the latter four treatments having similar ($P > 0.05$) feed intake. Not surprisingly, weight gain followed a similar pattern with the exception that feed intake of male birds on the NC was higher than of male birds ($P < 0.05$) on T3. Mortality of male birds during the finisher phase ware not affected by dietary additives ($P > 0.05$).

Table 1
Effect of ACV and or GAE on production performance of male and female broiler chickens

		Grower (15 to 28 days of age)				**Finisher (29 to 42 days of age)**				**Overall (15 to 42 days of age)**		
		Weight gain (g/b)	Feed intake (g/b)	FCR	Mortality (%)	Weight gain (g/b)	Feed intake (g/b)	FCR	Mortality (%)	Weight gain (g/b)	Feed intake (Kg)	FCR
Sex	Additives											
Male	NC	903.33^{ab}	1672.05	1.87	0.00	853.33^{a}	1923.85^{b}	2.28	0.00	1757.67^{a}	3595.90^{ab}	2.06
	T1	776.67^{a}	1600.00	2.10	0.00	687.67^{a}	1711.28^{ab}	2.49	0.00	1462.33^{a}	3311.28^{a}	2.28
	T2	858.67^{ab}	1565.90	1.84	0.00	741.00^{a}	1792.56^{ab}	2.48	0.00	1599.00^{a}	3358.46^{a}	2.13
	T3	771.00^{a}	1547.18	2.01	0.00	569.00^{a}	1465.64^{a}	2.74	0.00	1340.67^{a}	3012.82^{a}	2.26
	PC	1051.00^{b}	1609.74	1.54	0.00	1398.67^{b}	2512.48^{c}	1.80	2.67	2449.00^{b}	4122.22^{b}	1.68
	SE	57.509	51.135	0.118	0.000	75.08	96.405	0.272	1.193	1.000	143.739	0.123
Female	NC	804.33^{ab}	1457.44	1.81^{b}	0.00	639.00^{a}	1450.51^{a}	2.35	0.00	1443.33^{a}	2907.95^{a}	2.02
	T1	722.33^{a}	1474.10	2.05^{b}	0.00	684.33^{a}	1574.62^{a}	2.35	0.00	1406.33^{a}	3048.72^{a}	2.17
	T2	681.00^{a}	1403.85	2.06^{b}	0..00	631.00^{a}	1456.56^{a}	2.31	2.67	1312.00^{a}	2860.41^{a}	2.19
	T3	746.67^{a}	1464.61	1.98^{b}	0.00	632.33^{a}	1439.74^{a}	2.31	0.00	1378.67^{a}	2904.36^{a}	2.12
	PC	1032.00^{b}	1520.77	1.48^{a}	0.00	1276.67^{b}	2234.10^{b}	1.76	0.00	2308.67^{b}	3754.87^{b}	2.06
	SE	54.205	73.505	0.069	0.000	70.239	66.773	0.234	1.193	77.979	134.236	0.087
Additives												
	NC	853.83^{a}	1564.74	1.84^{b}	0.00	746.17^{a}	1687.18^{a}	2.31	0.00	1600.00^{a}	3251.92^{a}	2.04^{ab}
	T1	749.00^{a}	1537.05	2.08^{b}	0.00	685.50^{a}	1642.95^{a}	2.42	0.00	1434.33^{a}	3180.00^{a}	2.22^{b}
	T2	769.33^{a}	1484.87	1.95^{b}	0.00	686.00^{a}	1624.56^{a}	2.42	1.33	1455.50^{a}	3109.43^{a}	2.16^{ab}
	T3	758.33^{a}	1505.90	1.99^{b}	0.00	600.67^{a}	1452.69^{a}	2.53	0.00	1358.67^{a}	2958.59^{a}	2.19^{b}
	PC	1041.50^{b}	1565.26	1.51^{a}	0.00	1336.67^{b}	2373.29^{b}	1.78	1.33	2378.33^{b}	3938.55^{b}	1.87^{a}
	SE	39.514	44.771	0.068	0.000	51.407	58.636	0.179	0.843	63.308	98.337	0.075
Sex												
	Male	871.73^{b}	1598.97^{b}	1.867	0.00	849.53	1881.16^{b}	2.36	0.53	1721.33	3480.14^{b}	2.08
	Female	797.07^{a}	1464.15^{a}	1.876	0.00	772.47	1631.11^{a}	2.23	0.53	1569.40	3095.26^{a}	2.11
	SE	24.991	28.316	0.043	0.000	32.512	37.084	0.133	0.533	40.040	62.194	0.048
Significance												
Additives (A)		*	NS	*	NS	*	*	NS	NS	*	*	*
Sex (G)		*	*	NS	NS	NS	*	NS	NS	NS	*	NS
A x G		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different ($P < 0.05$).

NS = non-significant, SE = Standard error. *=$P < 0.05$

NC- Antibiotic free diet plus untreated drinking water,

T1- Antibiotic free diet plus ACV treated drinking water,

T2- Antibiotic free diet plus GAE treated drinking water,

T3- Antibiotic free diet plus ACV + GAE treated drinking water,

PC- Antibiotic fortified diet plus untreated drinking water

The pattern in feed intake and weight gain for female birds was similar to that observed in their male contemporaries. Female birds on the PC consumed more feed and gained more weight ($P < 0.05$) than the latter four treatments which had similar feed intake ($P < 0.05$) and weight gain ($P < 0.05$). FCR and mortality of female birds during the finisher phase ware not affected by dietary feed additives ($P > 0.05$). Across sex, all treatments with the exception of PC, (which had a significantly higher ($P < 0.05$) feed consumption and weight gain) had similar feed consumption and weight gain ($P > 0.05$). FCR and mortality of birds across sex was not affected by additives ($P > 0.05$). Male birds consumed more feed ($P < 0.05$) than female birds. However, weight gain, FCR and mortality ware not influenced ($P > 0.05$) by sex during finisher phase.

Overall but within sex group, female birds on the PC consumed more feed ($P < 0.05$) than those on the other treatments. A similar pattern was observed for feed consumption by male birds with the exception that feed consumption for males on the PC were similar to those on the NC ($P > 0.05$). Overall, both male and female birds on the PC gained more weight ($P < 0.05$) than those on the other four treatments and no significant differences were found among these four treatments. FCR and mortality for both male and female birds were not influenced ($P > 0.05$) by the different treatments. A similar pattern in feed consumption and weight gain was observed across sex. Birds on the PC had a lower FCR than birds on the T1 and T3 ($P < 0.05$), and had similar FCR with birds on the NC and T2. However, birds on the NC, T1, T2 and T3 had similar FCR ($P > 0.05$). Despite consumption of significantly more feed ($P < 0.05$) by male birds compared to female birds, FCR, weight gain, and mortality statistics were not affected by sex ($P > 0.05$).

Table 2
Effect of ACV and or GAE on digestive organs, digesta pH, dressing percentage and meat pH of male and Female Ross 308 broiler chickens

		Gut digesta pH	**Liver weight (%)**	**Spleen weight (%)**	**Proventriculus weight (%)**	**Gizzard weight (%)**	**Gastrointestinal tract length (cm)**	**Dressing %**	**Meat pH**
Sex	Additives								
Male	NC	5.50	2.10	0.11	0.45 [ab]	1.46	1.77	56.11[a]	5.16
	T1	5.37	2.13	0.14	0.56 [b]	1.77	1.94	69.88[b]	4.95
	T2	5.50	1.57	0.10	0.46 [ab]	2.01	1.84	76.96[b]	5.02
	T3	5.53	1.78	0.12	0.48 [b]	2.05	1.65	66.94[ab]	4.81
	PC	5.73	1.71	0.10	0.29 [a]	0.87	1.77	73.98[b]	5.07
	SE	0.155	0.183	0.009	0.039	0.254	0.110	2.652	0.081
Female	NC	5.53	1.76	0.13	0.47 [ab]	1.73	1.86	69.01[ab]	4.79
	T1	5.67	1.97	0.13	0.57 [b]	1.86	1.66	70.93[ab]	4.86
	T2	5.43	1.63	0.11	0.50 [ab]	2.02	1.89	63.93[a]	4.89
	T3	5.47	1.89	0.14	0.53 [ab]	1.80	1.81	74.46[ab]	4.83
	PC	5.63	1.76	0.10	0.32 [a]	1.13	1.65	75.98[b]	4.97
	SE	0.115	0.204	0.013	0.049	0.338	0.148	2.512	0.070
Additives									
	NC	5,52	1.93	0.12[ab]	0.46[b]	1.60 [ab]	1,82	62,56[a]	4,97
	T1	5,52	2.05	0.14[b]	0.56[b]	1.81 [ab]	1,80	70,41[b]	4,90
	T2	5,47	1.60	0.11[ab]	0.48[b]	2.02 [b]	1,86	70,45[b]	4,96
	T3	5,50	1.83	0.13[ab]	0.50[b]	1.92 [b]	1,73	70,70[b]	4,82
	PC	5,68	1.74	0.10[a]	0.31[a]	1.00 [a]	1,71	74,98[b]	5,02
	SE	0.096	0.137	0.008	0.031	0.211	0.092	1.826	0.054
Sex									
	Male	5.53	1.86	0.11	0.45	1.63	1.79	68.77	5.00[a]
	Female	5.55	1.80	0.12	0.48	1.71	1.77	70.86	4.87[b]
SE		0.061	0.086	0.005	0.020	0.134	0.058	1.155	0.034
Significance									
Additives (A)		NS	NS	*	*	*	NS	*	NS
Sex (G)		NS	NS	NS	NS	NS	NS	NS	*
A x G		NS	NS	NS	NS	NS	NS	*	NS

For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different (P < 0.05).

NS = non-significant, SE = Standard error. *=P < 0.05

NC- Antibiotic free diet plus untreated drinking water,

T1- Antibiotic free diet plus ACV treated drinking water,

T2- Antibiotic free diet plus GAE treated drinking water,

T3- Antibiotic free diet plus ACV + GAE treated drinking water,

PC- Antibiotic fortified diet plus untreated drinking water

Effects of ACV and or GAE on digestive organs measurement, digesta pH, dressing percentage and meat pH of male and female Ross 308 broilers are presented in Table 2. Gut digesta pH, relative weight of liver, gastrointestinal tract length and meat pH of both male and female Ross 308 broiler chickens were not affected by ACV and or GAE administered through drinking water (P > 0.05). Across sex, birds on the PC had a higher relative weight of spleen (P > 0.05) than birds on T1, but was similar with birds on the NC, T2 and T3 (P > 0.05). Male birds on the PC exhibited a lower relative weight of proventriculus (P < 0.05) than male birds on the T1 and T3 and had a similar (P > 0.05) relative weight of proventriculus with male birds on the NC and T2. However, male birds on the NC, T2 and T3 had a similar (P > 0.05) relative weight of proventriculus. Female birds on the PC exhibited a lower relative proventriculus weight (P < 0.05) than female birds on the T1 and had a similar (P > 0.05) relative proventriculus weight with female birds on the NC, T2 and T3. However, female birds on the NC, T1, T2 and T3 had a similar (P > 0.05) relative weight of proventriculus. Across sex, birds on the PC had lower relative weight of proventriculus (P < 0.05) than birds on NC, T1, T2 and T3. Across sex, birds on the PC had lower relative weight of gizzard (P < 0.05) than birds on the T2 and T3, but was similar with birds on the NC and T1 (P > 0.05). Dressing percentage of male birds on the T2 was higher than of birds on the NC but was similar (P > 0.05) to male birds on the T1, T3 and PC. However, male birds on the NC had a similar (P > 0.05) dressing percentage with birds on the T3. Dressing percentage of female birds on the PC was higher than of female birds on the T2 but was similar (P > 0.05) with birds on the NC, T1, T3 and PC. Across sex, birds on the NC had a lower dressing percentage (P < 0.05) than birds on the PC, T1, T2 and T3. A significant interaction (P < 0.05) between sex and diet was noted for dressing percentage, whereby female birds on the negative control exhibited a higher dressing percentage than male birds. Gut digester pH, relative weight of the; liver, spleen, gizzard, proventriculus, gastrointestinal tract length and dressing percentage of Ross 308 broiler chickens was not influenced (P > 0.05) by sex. Male broiler chicken exhibited a higher (P < 0.05) breast meat pH compared females. Meat pH was not affected by additives (P > 0.05).

Discussion

In the present study, male broiler chickens gained more weight than females during the grower phase. However, during the finisher phase and throughout the trial, sex did not influence weight gain. Male broiler chickens typically have higher growth rates than female chickens (Benyi *et al*., 2015; Eid and Iraqi, 2014; Osei-Amponsah *et al*., 2012; Abdullah Y. Abdullah, 2010; Sam *et al*., 2010). Contrary to the present study, Ikusika *et al.* (2020) reported a heavier and higher feed intake in females than male Aboaca, Ross and Anak chickens. The higher body weight gain for males reflects the male's genetic metabolic advantage due to a number of factors (Madilindi *et al*., 2018), including greater competition for food, aggressive behaviour by the male, differences in nutritional requirements for growth and fatness (Zerehdaran *et al*., 2005).

Similar to the present findings, Madilindi *et al.* (2018) did not detect significant sex effects on FCR and mortality in Cobb Avian48 broiler chickens. Contrary to current findings, Novele *et al.* (2008) and Trocino *et al.* (2015) reported a better FCR in female than male broiler chickens. The disparity in experimental findings can be attributed to different or interaction of environmental and genetic effects. In the present study, across sexes, birds on the positive control gained more weight than birds on the negative control, and all the test treatments, with the birds on negative control achieving similar weight gain to the test treatments in all the growth phases. Similar findings were previously reported for garlic by Fayed *et al.* (2011) and Jakubcova *et al.* (2014). The results of the present study imply that ACV and/ or GAE supplementation do not influence weight gain of broiler chickens. In contrast, Mahmood *et al.* (2009), Dieumou *et al.* (2012) and Al-Rabadi *et al.* (2020) reported a higher weight gain in birds supplemented with garlic. Garlic supplementation of the diet of broiler chickens resulted in higher live weight compared to control diet (Stanacev *et al*., 2011; Onibi *et al*., 2009). Different findings may be caused by additive preparation in relation to functional characteristics (powder, flour, extracts, concentration of active compounds) and or from the dosage. Similar to the present finding, Allahdo *et al.* (2018) did not detect significant difference in weight gain of broiler chickens supplemented with ACV in drinking water compared to the control. Contrary to the present study, broiler chickens supplemented with ACV had a higher weight gain than the control from 1–28 days of age (Jahantigh *et al*., 2021).

The results of this study indicate that across sexes, the positive control had a higher feed intake than birds on other treatments, with the birds on the negative control achieving similar feed intake to the test treatments during the finisher and throughout the trial which implies that ACV and/ or GAE supplementation did not influence feed intake. During the grower phase, feed intake was not influenced by additives, these results mean that ACV and/ or GAE supplementation does not have any significant effect on broiler feed intake. As in the current study, several studies have reported that dietary garlic supplementation did not significantly influence the feed intake of broiler chickens (Umatiya *et al*., 2018; Issa and Omar, 2012; Dieumou *et al*., 2009; Onibi *et al*., 2009). The present results are different to the findings of Eid and Iraqi (2014) who reported a significant decrease in cumulative feed intake in broiler chickens that received a diet containing garlic compared to the control. Additionally, Raeesi *et al.* (2010) found that food consumption was significantly higher for the control group than for groups fed garlic-containing diets. Contrary to the present study, Allahdo *et al.* (2018) reported lower feed intake by birds which consumed water supplemented with ACV compared to birds that consumed additives free water during the starter (1–10 days of age) and grower (11–24 days of age) periods.

The results of the present study indicate that across sexes, birds on the positive control had a better FCR than birds on the negative control, and all the test treatments throughout the trial. This means that ACV and/or GAE fortified water did not have a positive effect on the FCR of broiler chickens. Birds on negative control achieved similar FCR to the test treatments during the grower, finisher and throughout the trial. This implies that ACV, GAE and the combination of ACV and GAE did not influence FCR of Ross 308 broiler chickens. The lack of significant effects on FCR during the grower phase is consistent with the results of Dieumou *et al.* (2012) and Umatiya *et al.* (2018) who reported no significant difference between the FCR of birds supplemented with garlic and birds on the control. Contrary to these observations, Raeesi *et al.* (2010) reported that supplementation of garlic powder had a considerable effect on improvement of FCR. Fayed *et al.* (2011) also reported significantly lower FCR for birds fed on a ration supplemented with garlic. The authors postulated that better FCR by birds on the garlic fortified diet could be attributed to garlic antibacterial properties which lead to better nutrients absorption and hence an improved FCR.

The current study found no significant differences in FCR between birds receiving ACV in their drinking water and the other treatment groups. Similarly, Jahantigh *et al.* (2021) reported no significant changes in the FCR between the ACV treated group and control groups. However, Allahdo *et al.* (2018) reported that during the starter (1 to 10 days) and grower phase (11 to 24 days) birds which consumed water supplemented with ACV had significantly lower FCR compared to those that consumed water without ACV. The growth enhancement effect of ACV is attributed to its high contents of certain nutrients, such as vitamins, organic acids and minerals and its role in nutrient digestibility (Pourmozaffar *et al*., 2017). In the present study, male and female broiler chickens had similar mortality rate. This agrees with findings by Beg *et al.* (2016), who reported similar mortality rate in male and female chickens.

The current study showed no statistically significant differences in mortality of birds supplemented with GAE and the combination of ACV and GAE compared to those on the negative and positive controls. This implies that GAE and the combination of ACV and GAE supplementation did not affect mortality of broiler chickens and therefore they may not a suitable alternative for antibiotics. Dieumou *et al.* (2012) and Fayed *et al.* (2011) reported similar findings indicating no significant difference between the mortality rate of birds supplemented with garlic and birds on the control diet. Al-Rabadi *et al.* (2020) reported that feeding different garlic powder levels at different feeding stages significantly eliminated any mortality incidence compared to broilers fed control diets during the same stages. Notably, Eid and Iraqi (2014) and Onibi *et al.* (2009) reported significant reduction in mortality for birds fed garlic containing feed whereas the current study did not find any significant differences in mortality between the treatments. Eid and Iraqi (2014) claims that garlic powder had a positive effect on the immune response of chickens which might have decreased infection with diseases which is then reflected on livability and performance of birds which received garlic powder.

The current study found no significant differences in mortality between birds receiving ACV in their drinking water and the other treatment groups. This implies that ACV and the combination of ACV and GAE supplementation does not affect mortality of broiler chickens and therefore they may not be a suitable alternative to antibiotics. Similarly, Allahdo *et al.* (2018) reported that mortality of birds which consumed water supplemented with ACV was not significantly different from that of the control and other treatment groups.

Sex had no significant effect on intestine digesta pH in Ross 308 broiler chicken. There is limited research on the effect of broiler sex in relation to intestine digesta pH. The present study did not find any statistically significant differences between the intestine digesta pH of birds supplemented with ACV, GAE and ACV and GAE combination and those on the negative and positive control diets. Sunu *et al.* (2021) reported different results indicating that garlic symbiotic significantly reduces the pH of the duodenum, jejunum, and ileum. Garlic symbiotic benefits the host as it provides a specific substrate for fermentation, promoting the growth of probiotics which improve the health of lactic acid bacteria (Adil and Magray, 2012). The fermentation of probiotics in the intestine produces a high concentration of lactic acid that causes a drop in pH (Nkukwana *et al.*, 2015). Dono *et al.* (2014) suggests that any condition that promotes a lower pH in the gut, often associated with the colonization of beneficial microbes, may also be correlated with a higher efficiency of energy and nutrient use and growth.

The current study found no significant differences in gut digesta pH between birds receiving ACV in their drinking water and the other treatment groups. On the other hand, Abbas *et al.* (2011) found that adding various levels of acetic acid (Organic acid found in ACV) to drinking water reduced the pH of the intestine in broiler chickens. In addition, Ndelekwute *et al.* (2019) reported that acetic acid reduces the pH in the duodenum. Broiler chickens utilize minerals better because of organic acid which reduce intestinal pH that leads to an increase in the activity of digestive enzymes (Swiatkiewicz *et al.*, 2010).

The results of the present study indicate that sex does not influence the relative weight of the liver in Ross 308 broiler chickens. Similarly, several studies found that relative liver weights of male and female broiler chickens did not differ significantly between the two sexes (Dieumou *et al.*, 2012; Pires *et al.*, 2007; Peebles *et al.*, 1997; Plavnik and Hurwitz, 1982). Other researchers obtained different findings reporting that the relative weight of liver of male broiler chickens was significantly higher than that of female chickens (Madilindi *et al.*, 2018; Benyi *et al.*, 2015; Novele *et al.*, 2008). Brake *et al.* (1993) found that female birds had a greater percentage of liver weight compared to male birds.

The results of this study indicate that ACV and/or GAE supplementation did not influence relative weight of liver. Several researchers agree with the findings of the current study indicating that relative weight of liver of broiler chickens were unaffected by garlic dietary supplements (Aydogan *et al.*, 2020; Enoka *et al.*, 2020; Samanthi *et al.*, 2015; Dieumou *et al.*, 2012; Issa and Omar, 2012; Mahmood *et al.*, 2009). Heidari *et al.* (2018) also reported that relative liver weights of broiler chickens were not significantly affected by Acidifier supplementation. ACV has a protective effect on the liver and improves liver function (Bouazza *et al.*, 2016; Naziroğlu *et al.*, 2014). There is limited to no pre-existing research regarding the effect of ACV on the weight of the liver in broiler chickens.

In the present study, relative weight of spleen was not influenced by sex. A similar finding was reported by Pires *et al.* (2007) who found that sex did not affect relative spleen weights. There is limited research about the effect of sex on spleen weight. The results of this study indicate that birds on the positive control had significantly lower relative weight of spleen than birds on ACV supplemented drinking water, and relative weight of the spleen of birds on garlic supplemented drinking water was similar to that of birds on the negative control and all the other treatments. Other researchers have obtained similar findings indicating that the relative weight of the spleen was unaffected by garlic supplementation in broiler chickens (Aydogan *et al.*, 2020; Enoka *et al.*, 2020; El-katcha *et al.*, 2016; Lee *et al.*, 2016). However, Elagib *et al.* (2013) reported that relative spleen weights decreased significantly with feeding diets containing garlic powder compared to a control diet. The current study shows no significant differences in relative spleen weights between birds that received ACV in their drinking water and the other treatment groups. Like in the current study findings, Jahantigh *et al.* (2021) reported that ACV intake through diet did not change the relative weight of spleen.

Similar to the present study finding, sex did not have an effect on the relative weight of proventriculus (Stęczny and Kokoszyński, 2020; Tesfaye *et al.*, 2013). In disagreement with the present study, Leksrisompong *et al.* (2007) found different results stating that the relative proventriculus weight was significantly larger in female than in male birds. Zhao *et al.* (2012) also reported that female birds had higher relative weight of proventriculus than males. The results of this study indicate that birds on the positive control had significantly lower relative weight of proventriculus than birds on negative control, ACV and/or GAE supplemented drinking water which had significantly similar relative weight of proventriculus.

Similar to the findings of the present study, Vishwas *et al.* (2020) and Kırkpınar *et al.* (2011) found that the supplementation of garlic in broiler diets did not have significant effects on relative weight of proventriculus. In contrast, Al-Massad *et al.* (2018) and Kafı *et al.* (2017) state that garlic supplementation in broiler diets increased proventriculus weight. The current study found no significant differences in proventriculus weight between birds that received ACV in their drinking water and the other treatment groups. There is a dearth of information about the effect of ACV on relative weight of proventriculus.

The results of the present study reveal that relative gizzard weight was not influenced by ACV and/or GAE supplemented drinking water. Similar to the present study, other researchers reported a no significant difference in relative gizzard weight in chickens supplemented with garlic (Islam *et al.*, 2017; El-katcha *et al.*, 2016; Dieumou *et al.*, 2012; Mahmood *et al.*, 2009). In contrast, Raeesi *et al.* (2010) reported that higher relative gizzard weight in control compared to the diet supplemented with garlic. There is a dearth of information about the effect of ACV on relative gizzard weight.

The results of the present study reveal that gastrointestinal tract length was not influenced by sex. However, Mabelebele *et al.* (2017) found that male chickens exhibited longer gastrointestinal tracts than female chickens. Gonzales *et al.* (2003) and Novel *et al.* (2009) also reported longer intestines in males than female broiler chickens. However, other researchers obtained results that are in agreement with the current study indicating that garlic supplementation does not have an effect on gastrointestinal tract length of broiler chickens (Patel *et al.*, 2017; Daneshmand *et al.*, 2012; Tatara *et al.*, 2008). In contrast, Al-Massad *et al.* (2018) reported that powdered garlic promotes small intestine length compared to groups on garlic free diets. As in the current study, Jahantigh *et al.* (2021) reported that dietary ACV did not significantly affect the length of intestines.

The current study shows no significant differences in the dressing percentages between male and female broilers. Similarly, Novel *et al.* (2009) and Olawumi and Fagbuaro (2011) reported no significant differences in dressing percentages of males and female broiler chickens. However, Hussein *et al.* (2019) reported a higher dressing percentage in male than female broiler chickens. The results of the present study indicate that birds on the negative control had a lower dressing percentage than birds on the positive control, and all the test treatments, dressing percentages for latter two groups were statistically similar. Regarding the increased dressing percentage of GAE supplementation in diet of broiler chickens several researchers obtained results that confirm the current study (Eltazi *et al.*, 2014; Oleforuh-Okoleh *et al.*, 2014; Fayed *et al.*, 2011). However, other researchers reported that garlic supplementation does not influence the dressing percentage of broiler chickens (Patel *et al.*, 2017; Kharde and Soujanya, 2014; Fadlalla *et al.*, 2010; Dieumou *et al.*, 2009). The present study shows an improvement in dressing percentage in birds supplemented with GAE when compared to the negative control, however it is statistically similar with the positive control. There is limited research regarding the effect of ACV on the dressing percentage in broiler chickens.

As in the present study, Schneider *et al.* (2012) found that male birds had higher breast meat pH than female birds. However, Hussein *et al.* (2019) reported that muscles of female birds had a higher pH than that of male birds. Other researchers found no significant difference in the breast meat pH value of male and female broiler chickens (Kirkpinar *et al.*, 2014; Lopez *et al.*, 2011; Musa *et al.*, 2006). Kirkpinar *et al.*, (2014) reported results similar to those of the current study. However, Choi *et al.* (2010) and Abdullah *et al.* (2010) reported a decrease in the breast meat pH in birds supplemented with garlic. The current study established that ACV supplementation in drinking water for broiler chickens did not affect breast meat pH. There is limited previous research on the effect of ACV on breast meat pH in chickens.

Overall, disparity among studies in findings may be attributed to additive preparation, physical form (powder, organic or aqueous extracts) and route (dietary, drinking water) of administration, in relation to the stability, and therefore critical dosages of the functional compounds. Phytogenic efficacy may also depend on the production environment. For example, in poorly resourced tropical production systems, apart from potentially high disease burden coupled to poor biosecurity, thermal stress also disrupts gut health, and, depending on the thermal discomfort, the bird's thermoregulation involves differentiated changes in feed and water intake (Rostagno, 2020), with implications on the intake of dietary, as opposed to drinking water additives.

In conclusion, dietary antibiotics improved growth performance. However, except for increased dressing percentage, ACV and GAE administered through drinking water did not express any phytogenic benefit to justify substitution for growth promoting dietary antibiotics. To address inconsistent findings on efficacy of these products among studies, further research is recommended to standardize the methods used in preparation, identify the ideal physical forms (powder, organic or aqueous extracts) and routes (dietary, drinking water) of administration, in relation to the stability, and therefore critical dosages of functional compounds, with consideration of production factors critical to broiler gut health such as heat stress, the disease burden and biosecurity.

Declarations

Acknowledgement The authors wish to acknowledge financial support by the Department of Agriculture, Land Reform and Rural Development, and by the University of Venda Research and Innovation Grant.

Authors' Contributions All authors participated in the conception, design of the experiment, interpretation of results, writing, and integration of the manuscript. Critical revision and final approval of version to be published was also done by all authors.

Data availability Data will be made available on request.

Ethical statement All procedures of this study were in accordance with the standards that comply with the animal welfare requirements of the University of Venda Animal Research Ethics Committee (Certificate number: SARDF/19/ANS/20/2011) and the South African Poultry Association Code of Practice (SAPA, 2012).

Conflict of interest Authors confirm that there were no conflicts of interest, this document has been read and approved by all authors.

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