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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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ABSTRACT

Tightening global regulations on the use of subclinical dietary antibiotics to enhance broiler growth are in response to increasing concern about the risk of resistance and their residues in animal products. The study evaluated the potential of apple cider vinegar (ACV) and garlic extract (GAE) as safer, phytogenic alternatives. A batch of 390 mixed-sex Ross 308-d-old broiler chicks was received into an open, deep litter house, and feather sexed in the second week into 30 experimental units of 13 birds per 2.03 m^2 pen. From days 1 to 22, all chicks were on a 200 g/kg crude protein, coccidiostat-treated commercial starter diet. During the grower (16 to 28 days) and finisher (29 to 42 days) phases, chick pens were assigned treatments in a 2 (sex) × 5 (additives) factorial experiment replicated three times. The GAE was a pure extract, while ACV was produced by fermenting 1,000 g fresh apple and 80 g supplementary brown cane sugar in 1.3 liters of water for 4 wk. The five treatments comprised antibiotic (15% granular zinc bacitracin and 12% valinomycin sodium, each at 500 g/tonne) grower (190 g/kg crude protein, 13.0 MJ ME/kg) and finisher (165 g/kg crude protein, 13.2 MJ ME/kg) commercial diets with untreated drinking water as positive controls (PC), antibiotic-free duplicates of the PC diets with untreated drinking water as the negative controls (NC), with 3 mL/L filtered ACV in drinking water (T1), 2 mL/L filtered GAE-treated drinking water (T2), or mixed (3 mL/L ACV + 2mL/L GAE) additive drinking water (T3). 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 feed convesion (feed:gain) ratio (FCR) than others, and lower ($P < 0.05$) FCR during the finisher phase than birds on T1 and T3. Birds on the PC had higher ($P < 0.05$) percent spleen weight than birds on T1, with smaller proventriculi ($P < 0.05$) than on NC, T1, T2, and T3, and smaller gizzard weight than birds on the T2 and T3. Birds on the NC exhibited less 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 improved dressing percentage, the ACV and GAE did not express phytogenic benefit at the experimental dosage.

Key words: antibiotic resistance, growth promoters, phytofeed additives

INTRODUCTION

Dietary antibiotic growth promoters (**AGPs**) are traditionally used to increase livestock productivity and efficiency (Suresh et al., 2018). The mechanism of action of AGPs is through control of gastrointestinal infections (Singh et al., 2013). A review of recent research on the use of AGPs in food-producing animals (Ronquillo and Hernandez, 2017) revealed that subtherapeutic use of antibiotics to promote growth is increasingly controlled in many countries because of the risk associated with their misuse and development of antibiotic microbial resistant strains (Landy et al., 2011).

Several alternatives to AGPs have been proposed and tested, among which are phytogenic feed additives (Amad et al., 2011). Phytogenic additives are defined as plant-derived extracts that contain functional compounds which 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 have beneficial multifunctional properties derived from specific bioactive 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 low pH (<0.05) fermented apple juice (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 modulate nutrient digestion (Pourmozaffar et al., 2017), which is linked to trophic effects on the absorptive epithelium, such as increased villi height, which enhances epithelial cell secretions, nutrient digestion, and absorption (Khan and Iqbal, 2016). Organic acids are long recognized as functional supplements which selectively eliminate harmful intestinal bacteria (Yagnik et al., 2018). The

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beneficial organic acids include simple monocarboxylic acids such as acetic, formic, butyric, and propionic acids (Mehdi et al., 2018). Acetic acid is the predominant periobiotic in ACV (Hayajneh, 2019).

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

Currently, we lack a complete understanding of the phytogenic efficacy of ACV and GAE in broilers. Therefore, this study investigated their specific and potential synergistic or complementary phytogenic activity in broilers.

MATERIALS AND METHODS

Experimental Conditions

The study was conducted at the poultry facility in the experimental farm of the School of Agriculture, University of Venda, Thohoyandou, Limpopo Province, South Africa. The study site is characterized by arid to semiarid conditions and temperature ranging from a minimum of 10 °C during winter to a maximum of 40 °C during summer (Odhiambo, 2011). The production setup and broiler management closely simulated local small-scale broiler production. All experimental procedures were approved by the University of Venda Animal Research Ethics Committee (Certificate number: SARDF/19/ANS/20/2011).

Preparation of Additive Plant Extracts

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 the cloves that were then washed with tap water. The cleaned garlic was loaded into a centrifugal juicer (700W Russell Hobbs centrifugal juice maker Model no. RHJM01) and centrifuged at level 2. The extract was packaged in 750-mL glass bottles and stored at 4 °C.

To prepare ACV, Golden Delicious apples were cut into 2 to 3 cm cubic chunks. A 3-liter glass jar was half filled (1,000 g) with the apple chunks followed by the addition of eight tablespoons (80 g) of brown sugar and 1.3 liters 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 to 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 was 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).

Broiler Chicks, Management, and Experimental Design

The study used 390 Ross 308 broiler chicks which had been feather sexed during the second week. The experiment was conducted in a 17.0 m × 9.0 m deep litter, open broiler house with a section divided into 30 experimental unit pen partitions, each of approximately 140 cm × 145 cm (2.03 m^2) floor dimensions, stocked at 13 sexed random birds per pen. Each pen had one tube feeder (height 430 mm, diameter 39 0mm) and one water fountain (height 400 mm, diameter 360 mm, Poltek, Johannesburg, South Africa). The cement floor was covered with wood shavings litter. 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.

A three-phase feeding program was implemented, evenly split into a 0- to 15 days starter, 16–28 days grower, and 29–42 days finisher dietary regimen. During the starter phase, all chicks were on a common 200 g/kg crude protein, coccidiostat, and growth enhancer fortified commercial starter diet manufactured by Meadow Feeds Pty, Ltd (Delmas, South Africa, Budget Range Product V19502), and received Virbac (Pretoria, South Africa) supplementary stress vitamins for the first 6 days following placement. During the grower and finisher phases, broilers were split into five treatment groups in a 2 (sex) × 4 (additive) experiment replicated three times. The five additive treatments comprised antibiotic-free diets with untreated drinking water (negative controls [**NCs**]), with 3mL/L filtered ACV in drinking water (T1), with 2mL/L filtered GAE-treated drinking water (T2), or with mixed (3mL/L ACV + and 2 mL/L GAE) additive drinking water (T3), and antibiotic (15% granular zinc bacitracin and 12% valinomycin sodium, each at 500 g/tonne) diets with untreated drinking water as positive controls (**PCs**). The NC diets were duplicates of the PC diets, respectively, custom and commercial grower (190 g/kg crude protein, 13.0 MJ ME/kg) and finisher (165 g/kg crude protein, 13.2 MJ ME/kg) products manufactured by Brennco Feed Pvt Ltd, Louis Trichardt, South Africa.

Data Collection

The pen feed intake and weights of three random birds per pen replicate were measured every 7 d, from which the FCR was estimated. Mortality was monitored daily and recorded whenever it occurred. On day 42, one random bird per replicate was selected for slaughter. The slaughter protocol followed that was described by Benyi et al. (2015). Birds to be slaughtered were subjected to 24 h of feed withdrawal with free access to drinking water after which they were slaughtered and dressed. The viscera (gizzard, heart, liver, small intestine, cecum, 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 h 14 °C postpartum 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 were checked for outliers, and to confirm normal distribution and homogenous variance, and subjected to ANOVA for a 2 × 5 factorial experiment using the General Linear Model 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 GAE on the 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 ($P > 0.05$). The 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 were 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 were not influenced ($P > 0.05$) by sex.

During the finisher phase, 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. Weight gain followed a similar pattern with the exception that the feed intake of male birds on the NC was higher than of male birds ($P < 0.05$) on T3. The mortality of male birds during the finisher phase was not affected by dietary additives ($P > 0.05$).

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 ($P < 0.05$).

Table 1. Effect of †apple cider vinegar and or ‡garlic extract on production performance of male and female broiler chickens

Treatments		Grower (days 15–28)				Finisher (days 29–42)				Overall (15–42 days)			
		Weight gain, g per bird	Feed intake, g per bird	FCR	Mortality, %	Weight gain, g per bird	Feed intake, g per bird	FCR	Mortality, %	Weight gain, g per bird	Feed intake, g per bird	FCR	Mortality, %
Sex	Additives												
Male	NC	903.33^{ab}	1,672.05	1.87	0.00	853.33^{a}	1,923.85^{b}	2.28	0.00	1,757.67^{a}	3,595.90^{ab}	2.06	0.00
	T1	776.67^{a}	1,600.00	2.10	0.00	687.67^{a}	1,711.28^{ab}	2.49	0.00	1,462.33^{a}	3,311.28^{a}	2.28	0.00
	T2	858.67^{ab}	1,565.90	1.84	0.00	741.00^{a}	1,792.56^{ab}	2.48	0.00	1,599.00^{a}	3,358.46^{a}	2.13	0.00
	T3	771.00^{a}	1,547.18	2.01	0.00	569.00^{a}	1,465.64^{a}	2.74	0.00	1,340.67^{a}	3,012.82^{a}	2.26	0.00
	PC	1,051.00^{b}	1,609.74	1.54	0.00	1,398.67^{b}	2,512.48^{c}	1.80	2.67	2,449.00^{b}	4,122.22^{b}	1.68	2.67
	SE	57.509	51.135	0.118	0.000	75.08	96.405	0.272	1.193	1.000	143.739	0.123	1.193
Female	NC	804.33^{ab}	1,457.44	1.81^{b}	0.00	639.00^{a}	1,450.51^{a}	2.35	0.00	1,443.33^{a}	2,907.95^{a}	2.02	0.00
	T1	722.33^{a}	1,474.10	2.05^{b}	0.00	684.33^{a}	1,574.62^{a}	2.35	0.00	1,406.33^{a}	3,048.72^{a}	2.17	0.00
	T2	681.00^{a}	1,403.85	2.06^{b}	0.00	631.00^{a}	1,456.56^{a}	2.31	2.67	1,312.00^{a}	2,860.41^{a}	2.19	2.67
	T3	746.67^{a}	1,464.61	1.98^{b}	0.00	632.33^{a}	1,439.74^{a}	2.31	0.00	1,378.67^{a}	2,904.36^{a}	2.12	0.00
	PC	1,032.00^{b}	1,520.77	1.48^{a}	0.00	1,276.67^{b}	2,234.10^{b}	1.76	0.00	2,308.67^{b}	3,754.87^{b}	2.06	0.00
	SE	54.205	73.505	0.069	0.000	70.239	66.773	0.234	1.193	77.979	134.236	0.087	1.193
Additives													
	NC	853.83^{a}	1,564.74	1.84^{b}	0.00	746.17^{a}	1,687.18^{a}	2.31	0.00	1,600.00^{a}	3,251.92^{a}	2.04^{ab}	0.00
	T1	749.00^{a}	1,537.05	2.08^{b}	0.00	685.50^{a}	1,642.95^{a}	2.42	0.00	1,434.33^{a}	3,180.00^{a}	2.22^{b}	0.00
	T2	769.33^{a}	1,484.87	1.95^{b}	0.00	686.00^{a}	1,624.56^{a}	2.42	1.33	1,455.50^{a}	3,109.43^{a}	2.16^{ab}	1.33
	T3	758.33^{a}	1,505.90	1.99^{b}	0.00	600.67^{a}	1,452.69^{a}	2.53	0.00	1,358.67^{a}	2,958.59^{a}	2.19^{b}	0.00
	PC	1,041.50^{b}	1,565.26	1.51^{a}	0.00	1,336.67^{b}	2,373.29^{b}	1.78	1.33	2,378.33^{b}	3,938.55^{b}	1.87^{a}	1.33
	SE	39.514	44.771	0.068	0.000	51.407	58.636	0.179	0.843	63.308	98.337	0.075	0.843
Sex													
	Male	871.73^{b}	1,598.97^{b}	1.867	0.00	849.53	1,881.16^{b}	2.36	0.53	1721.33	3,480.14^{b}	2.08	0.53
	Female	797.07^{a}	1,464.15^{a}	1.876	0.00	772.47	1,631.11^{a}	2.23	0.53	1,569.40	3,095.26^{a}	2.11	0.53
	SE	24.991	28.316	0.043	0.000	32.512	37.084	0.133	0.533	40.040	62.194	0.048	0.533
Significance													
Additives (A)		*	NS	*	NS	*	*	NS	NS	*	*	*	NS
Sex (G)		*	*	NS	NS	NS	*	NS	NS	NS	*	NS	NS
A × G		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, nonsignificant; SEM, standard error for the mean.
For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different ($P < 0.05$).
NC—Antibiotic-free diet plus untreated drinking water.
T1—Antibiotic-free diet plus 3 mL/L ACV-treated drinking water.
T2—Antibiotic-free diet plus 2 mL/L GAE-treated drinking water.
T3—Antibiotic-free diet plus 3 mL/L ACV + 2 mL/L GAE-treated drinking water.
PC—Antibiotic (15% granular zinc bacitracin and 12% valinomycin sodium, each at 500 g/tonne)-fortified diet plus untreated drinking water.
*$P < 0.05$.
†Pure, filtered garlic extract (GAE), preserved at 4 °C.
‡4-Week fermentation of 1,000 g fresh Golden Delicious apples in 1.3 liters of water with 80 g brown sugar at room temperature, preserved at 4 °C.

FCR and mortality of female birds during the finisher phase were not affected by dietary feed additives ($P > 0.05$). Across sex, the plant additive treatments had similar feed consumption and weight gain ($P > 0.05$), with higher ($P>0.05$) gain and feed consumption on the PC. The FCR and mortality of birds across sex were not affected by additives ($P > 0.05$). Male birds consumed more feed ($P < 0.05$) than female birds. However, weight gain, FCR, and mortality were not influenced ($P > 0.05$) by sex during the finisher phase.

Overall within the 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 was 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 the 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 ($P > 0.05$).

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 that 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 to birds on the T3. Dressing percentage of female birds on the PC was higher than that 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. Significant interaction ($P < 0.05$) between sex and diet occurred for dressing percentage, whereby female birds on the negative control exhibited a higher dressing percentage than male birds. Digesta pH, relative weight of the liver, spleen, gizzard, proventriculus, gastrointestinal tract length, and dressing percentage of Ross 308 broiler chickens were not influenced ($P > 0.05$) by sex. Male broilers exhibited higher ($P < 0.05$) breast meat pH compared to females. Meat pH was not affected by the additives ($P > 0.05$).

DISCUSSION

In the present study, the phytogenic effects of GAE and ACV were evaluated in sexed Ross 308 broilers. In previous studies, male broiler chickens typically outperform the females (Abdullah et al., 2010; Osei-Amponsah et al., 2011; Benyi et al., 2015). The sex influence on broiler performance derives from genetic metabolic factors that determine growth, and confer male competitive food advantage in mixed rearing systems (Madilindi et al., 2018). In this study, males consumed more feed with similar FCR in the grower–finisher phases, and only surpassed female growth during the grower phase. Under the same experimental conditions as the present study, Madilindi et al. (2018) reported similar FCR and mortality in Cobb Avian48 broilers. Previously, better female FCR was recorded in Ross 308 broilers, and in broiler strains distinguished by contrasting breast size (Trocino et al., 2015). Conversely, Ikusika et al. (2020) reported heavier females with higher feed intake in Ross, Aboaca, and Anak broiler strains.

In the present study, sex influences on slaughter performance and meat quality were minimal. Dressing percentages were comparable between the sexes, similar to previous studies (Novele et al., 2008; Olawumi and Fagbuaro, 2011) though contrasting to others (Hussein et al., 2019). The length of the gastrointestinal tract was not influenced by the sex of broilers, consistent with findings by Schneider et al. (2012). However, in the same strain, Novel et al. (2008, 2009) reported longer intestinal length in males, which, similar to this study, could not be attributed to the feed intake, suggesting a genetic effect. Breast meat pH was below the range of 5.5 to 6.0, which is considered optimum (Schneider et al., 2012; Mir et al. 2017). In the present study, meat pH was higher in males (5.0) compared to the females (4.87), similar to findings on the same strain by Schneider et al. (2012). Low pH than 5.8 (Schneider et al., 2012) is associated with reduced water-holding capacity due to drip loss, resulting in dark, firm, and dry meat. These effects are linked to preslaughte, and postslaughter handling stressors in relation to glycogen metabolism (Mir et al., 2017). Glycogen is broken down into lactic acid during the postmortem conversion of muscle to meat, with the cumulative lactic acid contributing to the postmortem pH drop in meat (Mir et al., 2017). Therefore, in the present study, it is possible the males were more active or restless due to stress during the preslaughter feed withdrawal and at slaughter, stress which induced rapid glycogen depletion (Mir et al., 2017). This likely led to a slower postmortem pH decline, resulting in a higher meat pH. Overall, sex differences in broiler growth, slaughter, and meat parameters among studies suggest genotype and sex-dependent interaction with environmental factors that influence genetic expression.

In the present study, the PC birds outperformed birds on the NC, and on the plant extracts, with broilers on the plant additives largely attaining overall similar growth performance to birds on the NC. In this study, plant extract test dosages relied on the pharmacological and phytogenic thresholds

Table 2. Effect of †apple cider vinegar and or ‡garlic extract on digestive organs, digesta pH, dressing percentage, and meat pH of male and female Ross 308 broiler chickens

Treatments		Gut digesta, pH	Liver weight, %	Spleen,%	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
	SEM	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
	SEM	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
	SEM	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]
	SEM	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 × G		NS	NS	NS	NS	NS	NS	*	NS

NS, nonsignificant; SEM, standard error for the mean.
For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different ($P < 0.05$).
NC—Antibiotic-free diet plus untreated drinking water.
T1—Antibiotic-free diet plus 3 mL/L ACV-treated drinking water.
T2—Antibiotic-free diet plus 2 mL/L GAE-treated drinking water.
T3—Antibiotic-free diet plus 3 mL/L ACV + 2 mL/L GAE-treated drinking water.
PC—Antibiotic (15% granular zinc bacitracin and 12% valinomycin sodium, each at 500 g/tonne)-fortified diet plus untreated drinking water
*$P < 0.05$.
†Pure, filtered garlic extract (GAE), preserved at 4° C.
‡4-Week fermentation of 1,000 g fresh Golden Delicious apples in 1.3 liters of water with 80 g brown sugar at room temperature, preserved at 4 °C.

indicated by previous research. Ideally, the logistical, financial, and ecological implications of mass production and processing of the product should also be considered, in relation to the adoption of alternative additives. Overall, establishing threshold plant extract dosages is complicated by the potential interaction of plant genetics, additive extraction, and administration techniques, as well as by animal and environmental variables, factors that explain the inconsistent findings across published literature. Previously, dietary garlic powder at 500 g and 1,000 g/tonne improved Cobb broiler weight gain and the FCR and increased the dressing percentage, with better performance at the lower rate of garlic inclusion (Fayed et al., 2011). Improved weight gain was reported in Hubbard broilers in 0.5% and 1.0% dietary garlic powder (Mahmood et al., 2009), also with greater improvement at the lower dietary inclusion level. Dietary organic garlic extract at 40 and 60 ppm improved the growth performance of Hubbard broilers (Dieumou et al., 2009; Dieumou et al., 2012). Diets supplemented with 1, 1.5, to 2.25 mL/kg garlic extract improved the growth performance and feed efficiency in Ross 308 broiler (Brzóska et al., 2015). In Ross broilers, 0.5% to 1.5% dietary garlic powder improved broiler growth performance, with greater improvement at the highest dietary inclusion (AL-Hijazeen et al., 2022). In contrast, despite increased antioxidant activity, 1.0% and 1.5% garlic powder did not improve Ross 308 broiler growth (Jakubcova et al., 2014). In Cobb 400 broilers, 0.2% dietary garlic powder improved body weight gain and the financial return per unit feed cost (Umatiya et al., 2018). Supplementation of Shaver Starbo broilers with 0.5 and 5 g/kg dietary garlic powder marginally improved weight gain and reduced mortality, with greater improvement at high levels of supplementation (Onibi et al., 2009). In Cobb 500 broilers, though growth parameters were not affected, 0.2% and 0.4% dietary garlic powder increased the blood cholesterol, triglyceride, low- and high-density lipoprotein, and improved the dry matter, crude protein, and fat digestibility (Issa and Omar, 2012). Dietary garlic powder at 100, 150, and 200 g/tonne improved the blood hematology, live body weight, and the FCR of Hubbard broilers, with less feed intake and mortality rate, with most effect at the highest dietary inclusion.

In the present study, the ACV and GAE or their combination did not affect the weight of any of the measured visceral organs. Similar findings were previously reported for GAE supplementation (Mahmood et al., 2009; Dieumou et al., 2012; Elkatcha et al., 2017). The ACV and GAE did not affect gastrointestinal tract length, similar to previous studies (Tatara et al., 2008; Daneshmand et al., 2012; Patel et al., 2017). In the present study, the stable vital organ morphometry at the tested dosages was considered indirectly indicative of low risk of toxicity.

Compared to GAE, there is less research on the phytogenic action of ACV. Reported functionalities include prebiotic, antibiotic, and probiotic effects, as well as enterotrophic and immunogenic properties (Pourmozaffar et al., 2017), with the latter attributing fish growth enhancement to additive vitamins, organic acids (primarily acetic acid) and minerals, and improved nutrient digestibility. Some previous studies tested organic acid supplements within dosages comparable to the present study. Inclusion of acetic acid (3%, at 125 ppm) in drinking water reduced the pH of the intestine in Hubbard broiler chickens, which mitigated the negative effects on the performance of pathogenic *Eimeria tenella* (Abbas et al., 2011). These effects were similarly observed in Abo acre broilers supplemented with 0.25% acetic, butyric, citric, and formic acids (Ndelekwute et al., 2019). Other studies confirmed the broader and greater phytogenic benefits of supplementary ACV at greater dosages compared to the present study. In Ross 308 broilers, 1%, 2%, and 3% dietary ACV improved the overall broiler performance, associated with improved immunity and intestinal histomorphology (Jahantigh et al., 2021). Supplementary 1% and 2% of 5% acetic acid ACV in drinking water improved 1 to 10 d of Ross 308 broiler intake and FCR, immune and intestine health, with increased small intestinal villus height, crypt depth, and decreased muscular thickness and abdominal fat (Allahdo et al., 2018).

In light of the inconsistent outcomes observed across studies, the current findings on the potential of GAE and ACV as potential phytogenic substitutes for conventional growth-promoting additives underscore the need for further research to standardize the plant extract preparation methods, to identify the optimal forms (powder, organic, aqueous), administration routes (dietary, water), and critical dosages of specific functional compounds, in relation to the influence of variables such as heat stress, the disease burden, and farm biosecurity on broiler health and overall performance.

CONCLUSION

In the present study, dietary antibiotics improved growth performance. However, except for increased dressing percentage, GAE (2 mL/L) and/or ACV (3 mL/L) administered through drinking water did not express any phytogenic benefit to justify the substitution for growth-promoting dietary antibiotics. To address inconsistencies across studies, further research is recommended to standardize preparation methods, to identify optimal forms (powder, organic, aqueous), administration routes (dietary, water), and critical dosages of the functional compounds, in relation to effects of critical production variables such as heat stress, the disease burden, and farm biosecurity on broiler gut health.

Supplementary Data

Supplementary data are available at *Translational Animal Science* online.

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Conflict of interest

Authors all declare no conflict of interest.

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