



Effects of Phytogenic Feed Additives on Body Weight Gain and Gut Bacterial Load in Broiler Chickens

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ABSTRACT

Phytogenic feed additives (PFAs) have promising importance in chicken production as antibiotic alternatives to balance chicken gut microorganisms and improve productivity. The objectives of this study were to evaluate the body weight gain (BWG) and gut bacterial load of broiler chicks fed on selected herbs. For this experiment, 360 unsexed one-day-old broiler chicks of Cobb 500 with an average weight of 40.74 g were randomly allocated into six treatment groups with three replicates of 20 chicks in each pen. The treatment 1 (T1) group was fed by a basal diet alone. Chickens of T2, T3, T4, T5, and T6 were fed the basal diet containing 1% of basil, lemongrass, peppermint, rosemary, and thyme leaves powder, respectively for 49 days. Body weight (BW), BWG, and average daily weight gain (ADWG) data were recorded every week and at the end of every phase. On days 21 and 42, three chickens from each replicate were slaughtered for microbiological analysis (pathogenic and normal flora) of cecum contents aseptically. The obtained result showed that chickens kept on T3 had significantly higher BW, BWG, and ADWG during the starter and grower phases. Significantly highest final BW was recorded during the finisher phase on T3 and T6. Chickens that consumed T3 and T6 had significantly higher overall BWG and ADWG. The lowest *Escherichia coli* counts were seen in chickens fed on rosemary (T5) on both days 21 and 42 of the experimental time. Similarly, the highest *Lactobacilli* counts were recorded on chicken fed on T5 (day 21) and T3 (day 42). On the other hand, almost all treatment herbs showed a higher *Enterococcal* count, with the highest recorded for T3 (day 21) and T6 (day 42). The present findings suggest that supplementing lemongrass and thyme leaf powder improves BW performance and gut microbial composition. Likewise, rosemary leaf powder enhances the beneficial microbial composition and reduces pathogenic bacteria. However, the underlying detailed biological mechanisms and dose standardization of these herbs for inclusion in the diet of broiler chickens need to be studied further.

Keywords: Antimicrobial, Body weight gain, Broiler chicken, Feed additive, Gut bacteria, Phytogenic

INTRODUCTION

Poultry production has increased recently due to the increase in human population, incomes, and standard of living, which have pressured the poultry industry to expand and produce high-quality products for consumers (Farrell, 2013). Efficient nutrition improvements in the poultry sector have accelerated the utilization of antibiotic-based feed additives, which became widely used to increase eggs and meat production and improve the chickens' health by maintaining a healthy gut environment (Alloui et al., 2014). However, this extensive chemical antibiotic utilization increases the chance of their accumulation in animal products as residues and the environment and increases the resistance of pathogens to antibiotics (Haque et al., 2020; de Mesquita Souza Saraiva et al., 2022). These situations force the world to restrict the utilization of antibiotic growth promoters (AGPs) in animal feed (Alloui et al., 2014; Hady et al., 2016), and the international livestock feed industries have been directed toward non-antibiotic feed additives. Among them, the feed additives of plant origin, called Phytogenic Feed Additives (PFAs), are gained a lot of attention as a suitable alternative to AGP (Alloui et al., 2014; Abd El-Ghany, 2020).

Phytogenics and plant derivative products attract a lot of attention as safe alternatives for AGPs in poultry. Recently research has shifted away from chemical-based feed additives to the use of phytogenics that exists naturally in the environment (Alloui et al., 2014). These plant-origin PFAs are natural, less toxic, residue-free, and ideal feed additives in meat animal production when compared to inorganic antibiotics or organic chemicals (Hady et al., 2016). Evidence also suggests that herbs, spices, and various plant extracts have antimicrobial, appetizing, and digestion-stimulator properties (Diniz et al., 2020). Phytogenic compounds have antibacterial and immunostimulant properties and could be used as alternatives to AGP to enhance chicken production performance. Hence, phytogenics are currently

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considered as feed additives in poultry ration to improve body weight gain (BWG) and their antimicrobial activities (Hady et al., 2016; Abd El-Ghany, 2020).

Chicken producers currently face challenges in meeting the consumers' needs with zero antibiotic residues and improved production with optimum quality (Haque et al., 2020). To meet consumer needs, the producers are interested in producing chickens without antibiotic residues by utilizing feed scientifically through sustainable poultry farming principles (Madhupriya et al., 2018). Besides, feed costs share about 70% of the total variable costs of poultry production (Fathi et al., 2019). Hence, PFAs are assuming an apposition of prime importance in poultry nutrition for promoting growth and production with optimum cost (Alloui et al., 2014; Singh and Yadav, 2020)

Supplementation of basil, peppermint, lemongrass, thyme, and rosemary in broiler chicken feed significantly affected chicken health and production. For instance, the supplementation of basil leaf powder significantly improves BWG, nutrient absorption, and the immune system, and maintains normal intestinal microflora in broiler chicken due to its antibacterial activities (ELnaggar and El-Tahawy, 2018). Similarly, feeding peppermint improves growth performance (Gurbuz and Ismael, 2016) and increases the beneficial-to-harmful bacteria ratio (Petričević et al., 2021). Likewise, studies have also revealed that the inclusion of lemongrass in broiler feed improved BWG (Parade et al., 2019), increased the beneficial bacteria, and reduced pathogenic bacterial load in chickens (Weckesser et al., 2007; Alagawany et al., 2021). Utilization of thyme herb in a broiler diet improves BWG (Toghyani et al., 2010), increases nutrient utilization, and has antimicrobial activity against microbiota found in the gastrointestinal tract (El-Ghousein and Al-Beitawi, 2009; Wade et al., 2018). Rosemary powder supplementation in poultry diets resulted in higher BWG (Ghazalah and Ali, 2008), reduced pathogenic bacteria load, and had significant antimicrobial activities (Weckesser et al., 2007).

Several investigators elsewhere reported that phytochemicals in broiler diets improved body weight (BW) and BWG (Alloui et al., 2014; Hady et al., 2016). However, there is limited published work conducted on phytochemicals herbs from Ethiopia. Hence, evaluating the effects of locally available herbs on chicken performance and gut microbiota has paramount importance as PFAs enhance the productivity of chickens by improving their feeds for optimum utilization as well as by enhancing their well-being. Therefore, this study was conducted to determine the effect of supplementation of dried basil, lemongrass, peppermint, rosemary, and thyme leaves powder on the BWG and gut bacterial load of broiler chicken.

MATERIALS AND METHODS

Ethical approval

All procedures related to animal handling and their routine manipulations were carried out according to animal care guidelines and protocols approved by the institutional animal ethics committee of the College of Veterinary Medicine and Agriculture (VM/ERC/01/13/12/2020).

Study areas

The experiments were conducted in the College of Veterinary Medicine and Agriculture poultry house of Addis Ababa University, Bishoftu campus, Ethiopia. The area is situated 47 km East of Addis Ababa at an altitude of 1900 m above sea level, a latitude of 8.44°N, and a longitude of 38.57°E. The average annual rainfall is 686.9 mm with an average minimum and maximum temperature of 10.9°C and 27°C, respectively, and the average relative humidity is 60.0%.

Experimental herbal powders

The treatment herbs, namely basil (*Ocimum basilicum*), lemongrass (*Cymbopogon schoenanthus*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), and thyme (*Thymus vulgaris*) green leaves, were purchased from Green mark herbs private limited company (PLC), horticulture farm found in Hawassa, Ethiopia, incorporate in the diets of broiler chickens as PFAs. The herbs were washed, and the leaves were detached from the stem and dried at room temperature. The dried leaves were prepared in powder form and were homogeneously mixed with the broiler diet manually (Nielsen, 2010).

Experimental design

In this study, 360 unsexed one-day-old broiler chicks of Cobb 500 with an average BW of 40.74 g were purchased from Bishoftu Alema poultry farms in Ethiopia. On arrival, all chicks were checked for any abnormalities and weighed. They were then randomly assigned to one of the 6 different feeding groups, each with three replicates with 18 pens containing 20 chicks each (Table 1) based on a completely randomized design. The chicks were reared in a wire-meshed wood partitioned (1.2 m × 1.8 m) deep litter floor housing system for 49 days of the experimental period. Before the arrival of the chicks, the watering equipment and feeding troughs were thoroughly cleaned, disinfected, and sprayed

against pathogen and external parasites, and the room was fumigated by mixing formalin 10% solution with potassium permanganate (KMnO₄) powder.

The chicks were brooded with gradual height adjustment using 200-watt bulbs suspended as heat and light sources for each pen to ensure adequate and uniform distribution of heat and light. The lighting program was 23 hours of light and 1 hour of darkness for the first week, then gradually decreased and kept constant at 18 hours at the age of the second week. The room was well heated at a constant temperature of 32°C two days before and on the arrival of the chicks, then was gradually reduced after a week by 3°C per week from 32°C to a final temperature of 20°C on day 28 and then kept constant. Chicks of the control group were fed broiler commercial concentrate feed as a basal diet purchased from Alema Koudijs Feed PLC, Bishoftu, Ethiopia. In contrast, each treatment group was fed on a concentrate basal diet containing 1% of one of the five herbs prepared in powder form as treatment (Table 1). Chicks were fed their respective prepared diet at their respective ages (starter feed from day 1-10, grower feed from day 11-30, and finisher feed from day 31-49) throughout the study time, as per the recommendation by the feed supplier by Alema Koudijs Feed PLC. All diets were provided in mash form. The chicks freely accessed tap water using manual drinkers and weighed feed throughout the experimental time (for 49 days). The standard bio-security protocol was employed throughout the entire experimental period (SAPA, 2022; USDA 2014). All chicks were vaccinated against New Castle Disease (HB1 on day 1, NEW Lasota on days 12 and 24) and Infectious Bursal disease on their days 7 and 19 of age as per the recommended local vaccination schedule/program of the Alema farms hatchery.

Table 1. The starter, grower, and finisher broiler diets for treatment groups during the experiment

Treatments group	Experimental diet	Number of chicks per	
		Replication	Treatment
T1	Only concentrate feed (Basal diet)	20	60
T2	Basal diet + Basil leaf powder	20	60
T3	Basal diet + lemongrass leaf powder	20	60
T4	Basal diet + Peppermint leaf powder	20	60
T5	Basal diet + Rosemary leaf powder	20	60
T6	Basal diet + Thymus leaf powder	20	60

Nutrient composition of basal diets and treatment herbs

Nutrient compositions of basal diet and treatment herbs powder of the representative samples were analyzed at two different laboratories; 1) the Animal product, Veterinary Drug and Feed Quality Assessment Center, and 2) the Ethiopian Institute of Agriculture Laboratory (Table 2). Samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), phosphorus (P), and total ash (Ash). Nitrogen was determined by the Kjeldahl procedure, and CP was calculated by multiplying nitrogen content by 6.25 (AOAC, 2000). The metabolizable energy (ME) values of the sample were calculated indirectly from the EE, CF, and ash using Formula 1, adopting the equation proposed by Wiseman (1987).

$$\text{ME (Kcal/kg DM)} = 3951 + 54.4 \text{ EE} - 88.7 \text{ CF} - 40.8 \text{ Ash} \quad (\text{Formula 1})$$

Table 2. Nutrient composition of basal diets and different herbs in broiler chicken diets in during the experiment

Sample	DM	CP	EE	CF	P	Ash	ME (kcal/kg DM)
Treatment herb							
Basil	85.7	31.56	2.14	38.52	0.36	6.72	376.516
Lemongrass	88.17	14.9	4.6	19.52	0.45	14.21	1890.05
Peppermint	88.81	26.92	1.88	21.72	0.52	11.58	1654.24
Rosemary	90.22	12.45	7.62	42.95	0.17	6.76	280.055
Thyme	88.88	11.96	6.12	18.09	0.27	11.03	2229.32
Basal diet							
Starter diet	90.9	21.95	4.02	7.86	0.48	9.88	3069.4
Grower diet	90.79	20.51	4.85	7.62	0.39	8.95	3173.79
Finisher diet	91.08	19.0	5.63	7.25	0.32	9.42	3230.07

DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude Fiber, P: Phosphorus, Ash: Total ash, ME: Metabolizable energy

Data collection

Body weight and average body weight gain measurement

At the beginning of the experiment, chickens were weighed using a sensitive balance with a sensitivity of 0.01g in a group per pen, and the average weight of the pen was calculated and recorded as initial BW. To measure the chicken's BWG, the chicks' BW was weighed weekly and at the end of every phase (starter, grower, and finisher) to determine

weight change. An average BWG for each phase was calculated for each treatment as the difference between the two successive weights divided by the number of chickens. The average daily body weight gain (ADWG) was determined by dividing the average body weight change by the number of experimental days (Formula 2, [Kidane et al., 2017](#)) for each phase.

$$\text{Average daily body weight gain (g)} = \frac{\text{Body weight gain (g)}}{\text{No. of experimental days}} \quad (\text{Formula 2})$$

Caecal bacterial load determination

On days 21 and 42 of the experiment, three representative chicks from each pen (nine chicks per treatment) were randomly selected ([Petričević et al., 2021](#)) for bacterial load determination. The selected chickens were humanely slaughtered by severing the jugular vein, exsanguination in a clean slaughter room, their viscera were exposed, and fresh caecal contents from representative chicks were collected immediately under aseptic conditions, pooled in a group on sterilized 25-ml screw capped tubes respective to the treatment, ligated and carefully placed on sterile plastic bags and put on ice and transported to a laboratory for bacteriological assays. For enumeration of the *Enterococci*, *E. coli*, and *Lactobacilli* colonizing the intestinal tract of chickens among different treatment groups, Bile Esculin Azide agar, Violate Red Bile agar and de Man, Rogosa and Sharpe (MRS) agar (all from HiMedia, India) were used, respectively ([Upadhaya and Kim, 2017](#)). Culture plates of Bile Esculin Azide agar and Violate Red Bile agar were incubated at 37°C for 24 hours, whereas MRS agar plates were incubated at 37 °C for 72 hours in an anaerobic environment ([Upadhaya and Kim, 2017](#)). The bacterial colony counts were calculated as colony-forming units per milliliters (CFU/ml) of caecal digesta (Formula 3). The counted data were transformed into logarithmic form \log_{10} CFU/ml ([Oyeagu et al., 2019](#)).

$$\text{Colony Forming} \left(\frac{\text{CFU}}{\text{ml}} \right) = \frac{\text{Average number of colonies}}{\text{Dilution factor} \times \text{Volume plated}} \quad (\text{Formula 3})$$

Data analysis

The results of the treatment means were analyzed by one-way ANOVA using R tools (R project, 2020). When differences among the treatment effect were found significant, means were separately analyzed using Duncan's multiple comparison tests. Significance differences were considered at ($p < 0.05$). The following model was used to analyze the experiment where PFAs are the main effects (Formula 4, [Gomez and Gomez, 1984](#)).

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \quad (\text{Formula 4})$$

Where, Y_{ij} represents an observation of chicken, μ is the overall mean of a response variable, α_i denotes the effect due to treatments herb, and ε_{ij} is the error term.

RESULTS

Body weight gain

The difference in BWG and ADWG between the treatment herbs is presented in Table 3. At the beginning of the experiment, BW of broiler chicks was similar in all treatments ($p > 0.05$, Table 3). BWG and ADWG were highly significant ($p < 0.05$) between treatments during the starter, grower, and finisher phases. Chickens kept on T3 and T6 had the highest BW, BWG, and ADWG, compared to others during the starter phase ($p < 0.05$). In contrast, chickens fed on T1, T4, and T5 had significantly ($p < 0.05$) lower mean BW, BWG, and ADWG, compared to T2 during the starter phase. During the grower phase, chickens kept on T3 had significantly highest BW, BWG, and ADWG than all other chickens. No significant difference ($p > 0.05$) between chickens consumed on T1, T4, and T5 and had the lowest BW, BWG, and ADWG than T2, T3, and T6.

Similarly, no significant difference was observed between T2 and T6 during the grower phase ($p > 0.05$). Significantly highest final body weight was recorded during the finisher phase on chickens that consumed T3 and T6. While chickens kept on T1 and T4 had the lowest final body weight compared to other treatments. Chickens kept on T6 showed significantly highest BWG and ADWG than all other treatments during the finisher phase, while chickens kept on T4 had the lowest BWG and ADWG among treatments. There was no significant difference in BWG and ADWG between T2 and T5 as well as T2 and T3, during the finisher phase ($p > 0.05$). At the end of the experiment, there was a significant difference between all treatments on overall BWG and ADWG. Chickens that consumed T3 and T6 had significantly higher overall BWG and ADWG than all other treatments, and chickens that consumed T4 and T1 had the lowest overall BWG and ADWG. A Similar trend was exhibited for BWG and ADWG gain records at the end of the experiment as previously obtained weights in T3 and T6.

Table 3. Effect of inclusion of different herbs on body weight gain and average body weight gain of broiler chickens during starter, grower, and finisher phases

Treatment		T1	T2	T3	T4	T5	T6	SEM	P-value
Parameter									
Initial BW (g)		40.85	40.79	40.7	40.75	40.67	40.68	0.04	0.810
Starter phase	BW	211.96 ^c	220.38 ^b	235.26 ^a	209.71 ^c	209.65 ^c	231.23 ^a	18.60	0.000
	BWG	171.29 ^c	179.54 ^b	194.45 ^a	168.91 ^c	168.32 ^c	190.24 ^a	0.85	0.000
	ADWG	17.14 ^c	17.97 ^b	19.46 ^a	16.90 ^c	16.84 ^c	19.04 ^a	0.85	0.000
Grower phase	BW	864.76 ^c	901.15 ^b	936.87 ^a	875.18 ^c	867.74 ^c	908.58 ^b	2.10	0.000
	BWG	644.38 ^c	680.77 ^b	716.49 ^a	654.80 ^c	647.36 ^c	688.20 ^b	2.10	0.000
	ADWG	32.21 ^c	34.03 ^b	35.82 ^a	32.74 ^c	32.36 ^c	34.41 ^b	0.10	0.000
Finisher phase	BW	1963.51 ^d	2032.17 ^b	2074.78 ^a	1960.98 ^d	1988.61 ^c	2064.62 ^a	23.87	0.000
	BWG	1098.75 ^d	1131.02 ^{bc}	1137.91 ^b	1085.80 ^e	1120.87 ^c	1156.04 ^a	2.10	0.000
	ADWG	57.82 ^d	59.52 ^{bc}	59.89 ^b	57.14 ^e	58.99 ^c	60.84 ^a	0.11	0.000
Over-all	BWG	1922.84 ^d	1991.34 ^b	2033.97 ^a	1920.18 ^d	1947.65 ^c	2023.48 ^a	3.14	0.000
	ADWG	39.24 ^d	40.63 ^b	41.5 ^a	39.18 ^d	39.74 ^c	41.29 ^a	0.06	0.000

^{a-c} Means within a row with different superscripts differ significantly ($p < 0.05$), SEM: Standard error of the mean, BW: Body weight, BWG: Body weight gain in, ADWG: Average daily weight gain, T1: Control (basal diet), T2: Basal diet + 1% basil leaf powder, T3: Basal diet +1% lemongrass leaf powder, T4: Basal diet + 1% peppermint leaf powder, T5: Basal diet + 1% rosemary leaf powder, T6: Basal diet + 1% thymus leaf powder

Caecum bacterial count

The results for caecal bacterial load for *Enterococci*, *Lactobacilli*, and *E. coli* are shown in Table 4. During this study, statistically significant differences were recorded in the mean logarithmic bacterial colony count between treatments during both days 21 and 42 of experimental time ($p < 0.05$). A statistically significant difference was noted in the *E. coli* colony count between treatments on day 21 ($p < 0.05$); the highest *E. coli* colony count was seen on chickens fed T1 and T6. However, the lowest *E. coli* counts were seen on chickens fed on T5 followed by T3. Chickens fed on T2 and T4 also reduced *E. coli* bacterial colony count. There was no significant difference in *E. coli* bacteria colony count between T1 and T6. There was a significant difference in *Enterococcal* count between treatment herbs and control during day 21 of bacterial colony count ($p < 0.05$). Almost all treatment herbs had shown higher caecal *Enterococcal* bacterial colony count, compared to the control. The highest *Enterococcal* count was recorded on chickens fed on T3 followed by T6. There was no significant difference in *Enterococcal* colony count between the treatment herbs ($p > 0.05$). Similarly, there was also a highly significant difference in *Lactobacilli* count between treatments during day 21 of experimental time ($p < 0.05$). The highest *Lactobacilli* colony counts were recorded from chickens fed T5 followed by T3; whereas the lowest *Lactobacilli* count was recorded from chickens fed T1. There was no significant difference in *Lactobacilli* bacterial colony count between chickens fed on T4 and T6 during 21 days of the experiment.

Table 4. Caecal bacterial count (CFU/ml) of *Enterococci*, *Lactobacilli*, and *Escherichia coli* on days 21 and 42 in broiler chickens

Treatments		Bacterial count	T1	T2	T3	T4	T5	T6	SEM	P-value
Experimental days										
Day 21	<i>Enterococci</i>		6.804 ^b	7.694 ^a	7.819 ^a	7.689 ^a	7.686 ^a	7.700 ^a	0.104	0.000
	<i>Lactobacilli</i>		7.238 ^e	8.234 ^d	9.977 ^b	9.504 ^c	10.375 ^a	9.554 ^c	0.325	0.000
	<i>E. coli</i>		8.168 ^a	7.987 ^b	6.127 ^d	7.203 ^c	5.329 ^e	8.145 ^a	0.327	0.000
Day 42	<i>Enterococci</i>		7.371 ^b	8.250 ^b	8.997 ^{ab}	9.123 ^{ab}	8.395 ^b	9.612 ^a	0.229	0.007
	<i>Lactobacilli</i>		9.796 ^b	10.025 ^b	12.695 ^a	10.246 ^b	12.462 ^a	11.842 ^a	0.360	0.000
	<i>E. coli</i>		8.08 ^b	7.661 ^{bc}	7.747 ^{bc}	8.421 ^b	7.034 ^c	9.225 ^a	0.201	0.004

^{a-c} Means within a row with different superscripts differ significantly ($p < 0.05$), CFU/ml: Colony forming units/ml, SEM: Standard Error of the Mean, T1: Control (basal diet), T2: Basal diet + 1% basil leaf powder, T3: Basal diet +1% lemongrass leaf powder, T4: Basal diet + 1% peppermint leaf powder, T5: Basal diet + 1% rosemary leaf powder, T6: Basal diet + 1% thymus leaf powder

Similarly, on day 42 of the experiment, there was a significant difference in bacterial count between treatments ($p < 0.05$). The highest *E. coli* colony count was recorded in chickens fed on T6, compared to all other treatments. On the contrary, the lowest *E. coli* count was seen in chickens fed on T5. There was no significant difference in mean logarithmic bacterial colonies count between chickens fed on T1, T2, T3, and T4 ($p > 0.05$).

There was also a significant mean difference in *Enterococci* count between treatments on day 42 of the experiment ($p < 0.05$). The highest *Enterococci* count was recorded on chickens fed T6, followed by T4 and T3, but there was no significant difference between chickens fed on T3, T4, and T6 ($p > 0.05$). On the other hand, the lowest *Enterococci* count was recorded on chickens fed on T1. The highest *Lactobacilli* counts during day 42 were recorded in chickens fed on T3 and T5, followed by T6. However, the lowest *Lactobacilli* counts were recorded in chickens fed on T1. No significant difference was recorded in chickens fed on T1, T2, and T4 in *Lactobacilli* count during day 42 of the experiment ($p > 0.05$).

DISCUSSION

Phytogenic feed additives considerably impact the gut environment, either directly or indirectly (Khan et al., 2022). In this study, the effect of the studied phytogenic herbs was found to be very effective in improving the performances of broiler chickens and exhibiting antimicrobial activities. Likewise, the findings of the present study and other reports suggest that phytogenics inclusion in the broiler diet improves the total number of beneficial bacteria, such as *Lactobacilli*, and reduces pathogenic bacteria growth, such as *E. coli* in the cecum (Riyazi et al., 2015a; Ahmed et al., 2016; Alagawany et al., 2021). These could lead to improved resistance to gut disease, enhance chickens' immunity, improve digestion, improve nutrient absorption, and in turn significantly improve the BWG of chickens.

Body weight gain

In this experiment, chickens fed on diets mixed with different phytogenic herbs showed higher BW, BWG, and ADWG. Chickens fed on lemongrass had higher BW, BWG, and ADWG values during the starter, grower, and overall period, compared to the control. This result was in line with Parade et al. (2019), who reported that the inclusion of 1.5% lemongrass leaf powder in the diet improved BWG and can reduce the time of harvesting broiler with proper slaughter weight. Similarly, another study also revealed that feeding a broiler with the inclusion of 2% lemongrass leaf powder improves their BWG compared to the standard diet (Shaheed, 2021). The difference in BW and BWG recorded in this study continues up to the end of the experiment. Higher BW improvement could be due to the active compounds found in lemongrass having antimicrobial and antioxidant activities. These compounds improve feed digestion and increase the secretion of digestive enzymes (Alagawany et al., 2021; Shaheed, 2021). These beneficial activities are reflected in the growth and BWG of the experimental chicks.

In this study, there was a statistically significant difference in BW and BWG in the rosemary-fed broiler chickens compared to the control during the finisher phase. The results obtained in this study were comparable with the investigation result obtained by Petričević et al. (2018), who reported that the inclusion of 0.6% rosemary in the broiler feed had no significant difference in ADWG during the starter and grower phase; however, significantly improve feed conversion ratio during the finishing phase. Contrary to the present findings, the inclusion of rosemary essential leaf powder in broiler feed had no significant difference in BWG compared to the control (El-Ghousein and Al-Beitawi, 2009). Rosemary herb has a strong flavor, and high crude fiber content, especially cellulose, tannin, and other potentially interfering substances, which may limit nutrient digestion and absorption in chicken (Loetscher et al., 2013).

The inclusion of basil leaf in the broiler diet in the current study significantly improves BW and ABWG more than the control group. This finding was in line with Onwurah et al. (2011), who reported that the utilization of basil leaf at 5g/kg in the diet of broilers had a beneficial effect on the BWG of broiler chicken. Feeding broiler with phytogenics herb can stimulate the digestive system of the animal by stimulating the secretion of endogenous digestive enzymes, improving the utilization of digestive products through enhanced liver function, and reducing bacterial load in the gastrointestinal tract, leading to the development of muscle in broiler chicken (Gurbuz and Ismael, 2016).

Supplementing peppermint in the broiler feed in the present study had no significant effects on BW and BWG compared to chickens fed on a control diet during the entire experimental period. This study is in line with the findings of Amasaib et al. (2013), reporting that supplementing chickens with 1% spearmint in a diet had no statistically significant effect on BWG. In contrast to this result, Witkowska et al. (2019) reported that peppermint oil mist could improve BW and BWG in broilers as the herb has a bactericidal effect and reduces infections.

The current study findings showed that the inclusion of thyme in the diet of broilers leads to the highest BW and BWG during the starter phase next to lemongrass. This result agrees with the previous findings of Toghyani et al. (2010), who reported higher BW in the broilers fed with a mixture of 0.5% thyme powder. Similarly, other studies also reported that higher BW was observed on broilers fed on a thyme essential oil-containing diet (Fotea et al., 2009). Contrary to the current study, thyme leaf powder supplementation had no significant difference in broiler performance compared to the control (Abdel-Ghaney et al., 2017). The BW and BWG performance seen in the broiler chicken supplemented with thyme in this study could be associated with the active principles of thymol and carvacrol found in the thyme herb. These active ingredients increase the secretion of digestive enzymes (Alagawany et al., 2021), have digestion stimulating (Al-

Kassie, 2009) and antimicrobial activity against gut microbiota effects, and improve nutrient utilization through enhancing liver function (El-Ghousein and Al-Beitawi, 2009; Wade et al., 2018).

Caecum bacterial count

The caecum bacterial load analysis results of day 21 of the experiment showed that there was a higher mean logarithmic value of *Lactobacillus* in chickens that consume basil than in control. However, the *Lactobacillus* count was higher in all treatments compared to the control. This finding coincides with Riyazi et al. (2015a), who reported that the inclusion of 600 ppm basil essential oil reduces *E. coli* and increases *Lactobacillus* bacterial colonies count in broiler chicken. The presence of flavonoids in the *Ocimum* spp. improves the immune system, enhances BWG in the broiler, and maintains normal intestinal microflora due to its antibacterial activities (ELnagar and El-Tahawy, 2018).

Lemongrass and rosemary inclusion significantly increased the *Lactobacilli* and *Enterococci* and reduced *E. coli* mean logarithmic bacterial count both during the days 21 and 42 of experimental time. This result aligns with (Weckesser et al., 2007), who reported that rosemary extract could reduce pathogenic bacteria load and had significant antimicrobial activities. The study also reported that the inclusion of lemongrass essential oil (450 mg/kg) in the quail's diet increased the *Lactobacillus* bacterial count and decreased the coliform, *E. coli*, and *Salmonella* bacterial count compared to the control group (Alagawany et al., 2021). Different literature exhibited that the reduction of such pathogenic bacteria in lemongrass-fed broilers could be due to the leaves and their essential oil rich in phytochemical compounds like tannins, flavonoids, and phenolic acid which have antimicrobial properties that can impair the integrity and function of the bacterial cell membranes and inhibit the growth of pathogenic bacteria (Parade et al., 2019; Alagawany et al., 2021; Shaheed, 2021).

In the current study, lower *E. coli* and higher values of *Enterococci* and *Lactobacilli* bacterial count were also recorded in chickens fed on thymes herb compared to the control. The findings of the current study are in line with the study (Saki, 2014) that reported that the inclusion of 0.20 ml/l thyme essential oil in drinking water significantly prevents the growth of *E. coli* in broiler chickens. Supplementation of thyme in the broiler diet affects the pathogenic bacteria by altering the cell wall permeability, and normal osmotic nature of the cell wall, which results in cytoplasmic membrane damage and protrusion of the content is a lethal condition to cell (Lee et al., 2004).

In addition, the results of the present study showed a significant reduction of *E. coli* and a higher number of *Enterococci* and *Lactobacillus* count in chickens fed peppermint leaf powder during day 21 of the experiment. This result coincides with the finding of Ahmed et al. (2016), who reported that a higher number of *Lactobacilli* and a lower value of some pathogenic bacteria count in the intestinal content of chickens fed on peppermint extract as a supplement. Likewise, the highest coliform bacteria count was reported in the intestine content of chickens fed on the control feed. Similarly, Petričević et al. (2021) found that the inclusion of 0.6% peppermint leaf powder in the broiler diet improves the beneficial-to-harmful bacteria ratio in the cecum. Contrary to our present findings, the study found no statistically significant difference in *Lactobacillus* and total numbers of aerobic bacteria count between control and peppermint powder-supplemented groups. Peppermint essential oil is rich in menthol content which is the main phenolic component and has antimicrobial activities (Schuhmacher et al., 2003), and it has the potential to be used as phyto-genic feed additive in combating bacterial diseases in poultry (Hady et al., 2016; Abd El-Ghany, 2020). In general, phyto-genic herbs have active compounds with selective antimicrobial activities. The active constituents of the phyto-genic herbs diffuse through the cell wall and affect the microbial cell's normal physiology and reduce their growth. This inhibition of bacterial growth by the herb could be due to interference with the microbial enzymes, or by damaging the protein, affecting the DNA and RNA synthesis, and preventing nutrient uptake, transport systems, and energy production of the bacteria (Shan et al., 2007).

CONCLUSION

The finding of the present study indicated that lemongrass and thyme leaf powder supplementation in the broiler diet as PFAs improves growth performance and results in higher final body weight with beneficial cecal microbial composition. Likewise, supplementation of rosemary leaf powder enhances the broiler's gut-beneficial microbial composition and reduces pathogenic bacteria. Therefore, these herbs can have a promising potential to be effectively used as safe and natural growth promoter phyto-genics in broiler chickens and might be replacing growth-promoting antibiotics. Furthermore, detailed studies should be conducted to determine other beneficiary effects and better understand the mechanism of action of these herbs on the growth performance and health of broilers.

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Authors' contributions

Tesfaye Engida D. conceptualized the idea and methodology, performed the experiments, collected all samples, and analyzed, generated data, compiled information, and prepared the original and final manuscript. Mihretu Ayele contributed to the conceptualization and methodology of the study, performed the experiments and, collected the samples of bacteriological data, laboratory analysis, and drafted the manuscript. Hika Waktole contributed to the conceptualization and methodology of the study and reviewed the manuscript. Berhan Tamir and Fikru Regassa contributed to the conceptualization, methodology, validation, supervision, and review of the manuscript. Takele Beyene Tufa contributed to the conceptualization, methodology, validation, and supervision of the study, editing and reviewing the manuscript, project administration and funding acquisition. All authors approved the results of the study and the final version of the manuscript.

Competing interests

The authors have declared that no competing interest exists.

Ethical consideration

All Authors have checked the ethical issues, including plagiarism, consent to publish, misconduct, double submission, and redundancy.

Availability of data and materials

The authors declare that they prepared all necessary data for this study and are ready to send further documents related to this study upon reasonable request.

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