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Effects of Dietary Supplementation of Phytogenic Feed Additives on Broiler Feed Conversion Efficiency and Immune Response against Infectious Bursal Disease Vaccine

Tesfaye Engida D[1,2*], Berhan Tamir[1], Mihretu Ayele[3], Hika Waktole[4], Berhane Wakjira[5], Fekadu Regassa[6], Fikru Regassa[5], and Takele Beyene Tufa[5]

[1]*Department of Animal Production Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia*
[2]*Department of Animal Sciences, School of Agriculture, Guder Mamo Mezemir Campus, Ambo University, Ambo, Ethiopia*
[3]*Alage ATVET College, Ministry of Agriculture, Alage, Ethiopia*
[4]*Department of Veterinary Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia*
[5]*Department of Biomedical Sciences, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia*
[6]*Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia*

*Corresponding author's E-mail: tesfaye.engida@yahoo.com; tesfaye.engida@ambou.edu.et

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ABSTRACT

The ban on antibiotic growth promoters in livestock feeding has encouraged the utilization of phytogenic feed additives. These phytogenics recently attracted much attention and are generally recognized as residue-free ideal feed additives in animal Production. The current study was conducted to investigate the effects of the phytogenic herbs on feed intake, feed conversion ratio, and immune responses associated with the infectious bursal disease (IBD) vaccine in broiler chickens. For this study, 360 day-old broiler chicks were randomly assigned to six feeding trials, each with three replicates containing 20 chicks. The control group (T1) was only fed a basal diet alone, while the treatment groups were given the basal diet supplemented with 1% of basil (T2), lemongrass (T3), peppermint (T4), rosemary (T5), and thyme (T6) leaves powder, respectively. Body weight, feed intake, and feed conversion ratio were recorded. All chicks were vaccinated against IBD on days 7 and 19. A serology test was conducted to check the antibody titer against the IBD vaccine. The findings of this study showed that chickens in group T2 had significantly consumed more feed, followed by T1 and T6. During the overall study period, chickens in group T4 had significantly better feed conversion efficiency, followed by T3 and T6. Chickens in groups T5 and T6 showed a more pronounced antibody titer against the IBD vaccine at days 21 and 42 of the experiment. Therefore, these findings indicated that supplementation of basil leaf powder improved feed intake. Moreover, peppermint and lemongrass leaf powder improved the feed conversion ratio. In addition, supplementation of rosemary and thyme enhances the immune status of broiler chickens and could be considered a natural growth promoter feed additive. Therefore, further studies should be done to discover their beneficial effects to use as alternative feed additives in broiler chickens.

Keywords: Body weight, Broiler chicken, Feed conversion, Feed intake, Immune response, Infectious bursa disease

INTRODUCTION

Poultry production is a fast growing industry in the meat-producing agriculture sector, with the demand for protein sources to meet the growing population worldwide (Bogale and Engida, 2022; El-Sabrout et al., 2022). In order to ensure this demand for animal-origin food, efforts are made by the producers to develop new strategies for animal production (El-Sabrout et al., 2022). Poultry producers widely used antibiotic growth promoters (AGPs) for the last decades because of their useful effect on production performance and the health condition of the chickens (Barreto et al., 2008; Mirzaei et al., 2022). This long-term utilization of antibiotics as a growth promoter in chickens led to reduced beneficial endogenous bacteria, the development drug resistant pathogenic microorganisms, and resulting in the accumulation of antibiotic residues in the environment

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and animal consumable products, which is a threat to consumer health (Barreto et al., 2008; Kurniawati et al., 2021; Mirzaei et al., 2022). These conditions force the world to ban the utilization of AGPs as feed additives on food-producing animals and motivated the search for alternative resources which are safer to use as feed additives (Singh and Yadav, 2020; Petričević et al., 2021).

The international animal feed industry faced the challenge of increased feed costs, the prohibition of the utilization of AGPs, and consumers' awareness of food safety (Osman et al., 2010; Madhupriya et al., 2018). Therefore, poultry nutritionist develops an interest in natural feed additives to use as an alternative additive to replace the AGPs with natural products (Upadhaya and Kim, 2017; El-Saadany et al., 2022; El-Sabrout et al., 2022). Among abundant natural feed additives that are available for chicken feed, phytogenic feed additives (PFAs) are widely advocated and received consumer acceptability (Saeed et al., 2018; Odunowo and Olumide, 2019; Abd El-Ghany, 2020; Kurniawati et al., 2021). PFAs are natural heterogeneous groups, less toxic, residue-free, and ideal non-antibiotic growth promoters derived from plants, herbs, fruit, spices, and their essential oil used as feed additives in meat animal production (Upadhaya and Kim, 2017; Madhupriya et al., 2018; Abd El-Ghany, 2020).

Phytogenics and their extract are supplemented to animal ratio to stimulate their appetite and improve nutrient digestibility, growth enhancer, improve gastrointestinal morphology (by stimulating the development of the gastrointestinal macro-architecture) and physiological functions, have antioxidant properties, help to treat certain diseases and immune modulator activities in chickens have been reported by different researchers (Upadhaya and Kim, 2017; Madhupriya et al., 2018; Babazadeh and Asasi, 2021). Furthermore PFAs active ingredients improve chicken performance by inducing digestive enzyme secretion, leading to enhance feed digestion and nutrient absorption (Odunowo and Olumide, 2019) and improving the feed conversion ratio in poultry (Huang and Lee, 2018). Currently, many scholars have revealed that supplementation of PFAs in animal feed enhances the immune response and can guard the gastrointestinal tract against external stressors. Their phytochemicals are considered the most promising feed additives due to their positive effect on regulating some viral vaccines' immune response in broiler chickens (Upadhaya and Kim, 2017; Huang and Lee, 2018; Abdelli et al., 2021).

One of the means advisable to enhance the productivity of chickens is through improving their feeds for optimum utilization as well as by enhancing their well-being, especially through feed obtained from phytogenics. The PFAs are comparatively young classes of poultry feed additives that have attracted the attention of poultry nutritionists in recent years (Abd El-Hack and Alagawany, 2015; Engida et al., 2023). Phytogenics have a beneficial role in disease prevention and control strategies to enhance the immune response against disease and for superior prevention of disease challenges (Gado et al., 2019). Broiler chickens supplementing with phytogenic essential oil significantly improve *infectious bursal disease (IBD)* antibody titer (Nahed et al., 2020). In this regard, it is necessary to extend the outlook on PFAs to promote their use as an alternative source to improve production performance in chickens. The PFAs evaluated in this research are available in almost all areas of the country, which are affordable or freely available and easy to process and utilize. Therefore, this study aimed to evaluate the utilization of basil, lemongrass, peppermint, rosemary, and thyme leaves powder as PFAs on the performance and immune response of broiler chickens regarding IBD vaccination.

MATERIALS AND METHODS

Ethical approval

All procedures related to animal handling, blood collection, and their routine manipulations were carried out according to animal care guidelines protocols approved by the Institutional Review Board of the College of Veterinary Medicine and Agriculture (CVMA, Ethiopia) animals ethics committee with approval number VM/ERC/01/13/12/2020.

Study areas

The experiment was conducted in the CVMA poultry house of Addis Ababa University, Bishoftu, Ethiopia, from January 1 to February 18, 2020. The area is located 47 km southeast of Addis Ababa at an altitude of 1900 m above sea level in the central highland of the country, a latitude of 8.44` North and a longitude of 38.57` East. The average annual rainfall is 686.9 mm with an average minimum and maximum temperature of 10.9^{o}C and 27^{o}C, respectively, and the average relative humidity is 60.0%.

Preparation of experimental herb powders

The experimental herbs included 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), found in Hawassa, Ethiopia. The herbs were washed, and the leaves were detached from the stem, spread out on a plastic sheet, and allowed to dry at room temperature in the physiology laboratory of CVMA. The dried leaves were prepared in powder form and stored in plastic material until used.

Experimental design and animals management

A total of 378 day-old broiler chicks of Cobb-500 strain were purchased from Bishoftu Alema farms, (Ethiopia). Blood collection was performed from 18 randomly selected chicks on day one of their age before vaccination by incising the jugular vein and transferring it into the non-heparinized tubes (Legese et al., 2022). The remaining chicks (n = 360) were weighed and randomly assigned into six different treatment groups with three replicates based on a completely random design. Chicks were reared in a wire-meshed wood partitioned deep litter floor housing system (1.20 m × 1.80 m) for 49 days of the experimental period. Electric power was used as a source of heat and light according to the recommendation of the broiler management guide (Anonymous, 2018). All experimental chickens were vaccinated against IBD. The chicks were fed commercial broiler feed (1-10 days for starter, 11-30 days for grower, and 31-49 days for finisher) throughout the study, as per the recommendation by the feed supplier (Alema Koudijs Feed PLC, Ethiopia). Chicks of the control group were fed only broiler commercial feed (basal diet), whereas the other treatment groups were fed a basal diet plus 1% of one of the five herbs prepared in powder form as treatment herbs. The prepared treatment herb was homogeneously mixed with the broiler diet manually (Nielsen, 2010).

All diets were provided in mash form. Water and weighed feed were provided *ad libitium* to the chicks throughout the experiment. The standard bio-security protocol was employed throughout the experiment (USDA, 2014; SAPA, 2022). The study protocol for the starter, grower, and finisher broiler diet during the experiment included T1 as the control group receiving the basal diet, T2 receiving the basal diet + 10g of basil leave powder per kg of basal diet, T3 receiving the basal diet + 10g of lemongrass leave powder per kg of basal diet, T4 receiving the basal diet+ 10g of peppermint leave powder per kg of basal diet, T5 receiving basal diet + 10g of rosemary leave powder per kg of basal diet, and T6 receiving basal diet + 10g of thyme leave powder per kg of basal diet.

Nutrient composition of basal diets and treatment herbs

Nutrient compositions of basal diet and herbal powder of the treatments were analyzed from their representative sample at two different laboratories, namely Animal Product, Veterinary Drug and Feed Quality Assessment Center, and the Ethiopian Institute of Agriculture Laboratory (Table 1). Samples were analyzed for crude protein (CP), dry matter (DM), crude fiber (CF), Ether extract (EE), phosphorus (P) and total ash (Ash). Nitrogen was analyzed using the Kjeldahl method, and CP was calculated by multiplying nitrogen content by 6.25. The metabolizable energy (ME) value of the basal diet and experimental herb samples were calculated indirectly from the EE, CF, and ash using the following formula adopted from the equation proposed by Wiseman (1987).

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

Data collection

Body weight gain

At the beginning of the experiment and then every week and at the end of each phase (starter, grower and finisher) all chicks were weighed to determine the body weight gain (BWG) of chicks respective to treatment groups. BWG was determined by subtracting initial weight from successive body weight (BW) for each replication during the experimental time (Engida et al., 2023).

Feed intakes and feed conversion ratios

Feed intake (FI) was calculated as the difference between feed offered and feed leftover for each replication. Feed conversion ratio (FCR) was calculated as the ratio of total consumed feed (gm) to total BW gain (gm, El-Ghousein and Al-Beitawi, 2009). At the same time, cumulative FI was computed at the end of the experiment.

Immune response measurement

Blood collection was performed from 18 randomly selected chicks on hatching before vaccination to harvest serum samples to have baseline immune data (Legese et al., 2022). All the chickens were vaccinated against IBD (Gumboro) with the recommended dose and as per the schedule of the product on days 7 and 19 (CEVAC® IBD L, France). Then, 3 ml of blood samples were collected into a plain vacutainer tube using a disposable 3 mL syringe with a

22-gauge needle from the wing vein on the 21 and 42 days of age to measure post-vaccination antibody titer from 3 randomly selected chicks. The collected blood sample was kept undisturbed to clot in the laboratory for 30 minutes, then centrifuged at 1500 rpm for 10 minutes for separation of serum, and the serum sample was harvested into labeled cryovials and kept at -20°C until serology was conducted at the National Veterinary Institute laboratory (Ethiopia). The indirect ELISA test (ID screen® IBD indirect, ID.vet, France) was performed according to the directions provided by the manufacturer with the kit for the detection of antibody titer developed against IBD. It is a quantitative test for the determination of IBD-specific antibodies in chicken serum. The antibody titer was computed to $\log_{10}$ as per the instruction of the manufacturer provided with the kit (Tesfaye et al., 2017). The test was valid if the mean optical density value of the positive control was greater than 0.250 and the ratio of the mean value of the positive and negative controls was greater than 3 (Tesfaye et al., 2017).

Statistical analysis

All data pertaining to measured parameters were analyzed using the R tools (R project, 2020). Treatment means were compared by one-way ANOVA.

Significant differences among the treatments' effects were separately analyzed with Duncan's multiple comparison test. Significance mean differences between treatments were considered at $p < 0.05$. The following model was used to analyze the experiment where PFAs are the main effects (Gomez and Gomez, 1984).

$$Yij = \mu + Ti + Eij.$$

Where, *Yij* is an observation of chicken, μ denotes the overall mean of a response variable, *Ti* determines the effect due to treatment herbs, and *Eij* represents the error term.

RESULTS

Nutrient composition of basal diets and treatment herbs

As can be seen in Table 1, basil had a higher CP content compared to other herbs. Additionally, basil exhibited lower DM and EE content in comparison to Peppermint. On the other hand, lemongrass displayed higher ash and ME content than rosemary and basil. Peppermint had lower EE content but higher P content, with an optimum ME level. Rosemary stood out with the highest DM, EE, and CF composition among all the herbs, while displaying the lowest P and ME content. Thyme showcased the highest ME content and the lowest CF content when compared to the other herbs. Additionally, thyme had higher EE content compared to rosemary.

Table 1. Nutrient composition (%) of basal diets and treatment herbs

Sample \ Composition	DM	CP	EE	CF	P	Ash	ME (kcal/kg DM)
Treatment herbs							
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

Feed intake

The effect of the treatment herbs on FI is presented in Table 2. According to the result of the present study, the inclusion of phytogenic herbs significantly affected FI. The chickens kept on treatment herbs had significantly ($p < 0.05$) higher FI, compared to the control group during the starter phase, and there was no significant difference ($p > 0.05$) in FI between treatment herbs. During the grower phase, there was a significant difference between treatments ($p < 0.05$). Significantly higher FI was seen in chickens in group T2 ($p < 0.05$), while significantly lower FI was seen in chickens in group T4 ($p < 0.05$). No significant difference was seen in chickens fed on T1, T3, and T6 regarding FI. During the finisher phase, the highest FI was recorded in chickens kept on T1, whereas chickens fed on T4 had the lowest FI ($p < 0.05$). During the finisher phase, T1, T2, T3, and T6 did not differ significantly in terms of FI. There was a significant difference in FI ($p < 0.05$) between treatments during the overall study period. Chickens fed on T2 had the highest FI, whereas chickens kept on T4 had the lowest FI than all other treatments. There was no significant difference in FI ($p > 0.05$) among T1, T2, and T6 and among T3, T5, and T6.

Body weight gain

Body weight gain data of broiler chickens fed on treatment herbs is presented in Table 3. BWG was highly significant between treatments during the starter, grower, and finisher phases ($p < 0.05$). Significantly higher BWG was seen in chickens kept on T3 and T6 than in other treatments ($p < 0.05$), whereas chickens kept on T1, T4, and T5 developed a lower body weight during the starter phase. Similarly, chickens kept on T3 showed higher BWG, compared to other treatments during the grower phase ($p < 0.05$). No significant difference was seen in chickens fed on T1, T4, and T5 and had lower BWG than other treatments. During the finisher phase, chickens kept on T6 showed significantly higher BWG than other treatments ($p < 0.05$). On the other hand, chickens that consume T4 had lower BWG, followed by T1. There was no significant difference in BWG between T2 and T3 during the finisher phase ($p > 0.05$). During the overall study period, significantly higher BWG was achieved from chickens that consumed T3 and T6 than in all other treatments. However, lower BWG was seen in chickens fed on T1 and T4.

Table 2. The effect of inclusion of treatment herbs on feed intake of broiler chickens during the starter, grower, and finisher phases

Parameter	Treatment	T1	T 2	T3	T4	T5	T6	SEM	P-value
FI (g/bird)	**Age (days)**								
Starter	1-10	288.21^{b}	308.13^{a}	308.37^{a}	309.46^{a}	312.71^{a}	308.79^{a}	2.26	0.003
Grower	11-30	1959.94^{b}	2059.58^{a}	1929.47^{bc}	1871.13^{d}	1899.41^{cd}	1984.31^{b}	16.07	0.000
Finisher	30-49	3593.12^{a}	3520.68^{ab}	3358.29^{ab}	3004.87^{c}	3297.82^{b}	3363.26^{ab}	53.34	0.004
Overall	1-49	5841.27^{ab}	5888.39^{a}	5596.14^{bc}	5185.47^{d}	5509.94^{c}	5656.37^{abc}	63.42	0.001

$^{a-e}$ Means within the same row bearing different superscripts are significantly different at $p < 0.05$, FI: Feed intake, SEM: Standard Error of Mean, g: Gram, 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% thyme leaf powder

Table 3. The effect of treatment herbs on body weight gain of broiler chickens during the starter, grower and finisher phases

Parameter	Treatment	T1	T 2	T3	T4	T5	T6	SEM	P-value
BWG (g)	**Age (days)**								
Initial BW	1	40.85	40.79	40.7	40.75	40.67	40.68	0.04	0.810
Starter	1-10	171.29^{c}	179.54^{b}	194.45^{a}	168.91^{c}	168.32^{c}	190.24^{a}	0.85	0.000
Grower	11-30	644.38^{c}	680.77^{b}	716.49^{a}	654.80^{c}	647.36^{c}	688.20^{b}	2.10	0.000
Finisher	30-49	1098.75^{d}	1131.02^{bc}	1137.91^{b}	1085.80^{e}	1120.87^{c}	1156.04^{a}	2.10	0.000
Overall	1-49	1922.84^{d}	1991.34^{b}	2033.97^{a}	1920.18^{d}	1947.65^{c}	2023.48^{a}	3.14	0.000

$^{a-e}$ Means within the same row bearing different superscripts are significantly different at $p < 0.05$, BWG: Body weight gain, BW: Body weight, g: gram, SEM: Standard Error of 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% thyme leaf powder

Feed conversion ratio

The mean FCR data are presented in Table 4. The obtained results indicated that the inclusion of phytogenic herbs significantly affected FCR during the starter, grower, finisher, and overall experimental period ($p < 0.05$). During the starter phase, FCR was found to be significantly better in chickens fed on T3 followed by T6 compared to other treatments ($p < 0.05$), whereas chickens kept in T4 and T5 had lower FCR. Significant FCR was seen between treatments during the grower phase ($p < 0.05$). Chickens kept on T3 had better FCR than all other treatments, and significantly lower FCR was recorded for chickens fed on T1 and T2 among the treatments ($p < 0.05$). During the finisher phase, significantly better FCR values were seen in chickens kept on T4 than in all other treatments, and significantly lower FCR was recorded in T1 and T2 chickens ($p < 0.05$). However, there was no significant difference among T2, T3, T5, and T6 in FCR regarding the finisher phase ($p < 0.05$). As to the current study finding, FCR was significantly affected by PFAs during the overall study periods ($p > 0.05$). Chickens fed T4 had significantly better FCR followed by T3 ($p < 0.05$). On the other hand, chickens kept on T1 had significantly low FCR.

Table 4. The effect of treatment herbs on a feed conversion ratio of broiler chickens during the starter, grower, and finisher phases

Parameter \ Treatment		T1	T 2	T3	T4	T5	T6	SEM	P-value
FCR	**Age (days)**								
Starter	1-10	1.68^{bc}	1.71^{b}	1.58^{d}	1.83^{a}	1.85^{a}	1.62^{dc}	0.025	0.000
Grower	10-30	3.04^{a}	3.02^{a}	2.69^{c}	2.85^{b}	2.93^{b}	2.88^{b}	0.029	0.000
Finisher	30-49	3.27^{a}	3.11^{ab}	2.95^{bc}	2.76^{c}	2.94^{bc}	2.91^{bc}	0.046	0.006
Overall	1-49	3.03^{a}	2.95^{ab}	2.75^{c}	2.70^{c}	2.83^{bc}	2.79^{c}	0.032	0.001

[a-e] Means within the same row bearing different superscripts are significantly different at $p < 0.05$, FCR: Feed conversion ratio, SEM: Standard Error of 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% thyme leaf powder

Immune response

The current study determined the effects of the experimental phytogenic herbs on broiler chickens' immunity, with a recommended vaccination program against IBD/Gumboro disease. During the study, the experimental herb that had the response of immune against the IBD vaccine was conducted via antibody titer from representative chickens' serum samples by a serological test. The effect of the experimental herbs on IBD (log_{10}) immune responses is illustrated in Figure 1. The mean IBD antibody titer from the serum samples of day-old chicks was (log_{10}) 3.60. The statistically significant difference means value antibody titers were observed in chickens fed different treatments ($p < 0.05$). On day 21, chicks fed on T5 and T6 as a supplement in their diet had developed the highest mean antibody titer of 3.853 and 3.567, respectively, and chickens fed on T2 and T3 had developed almost similar mean antibody titer values. In contrast, the lowest antibody titer was seen on chickens fed on T4 with 1.271 antibodies. Similarly, the mean antibody tillers value at the age of 42 days further increases in chickens fed the same treatment herb. Their mean antibody titer was 4.463 for T5 and 3.850 for T6, followed by chickens fed on T3 with a titer of 2.990. The lowest titer was recorded in chickens fed on T4, with a titer of 1.562 (Figure 1).

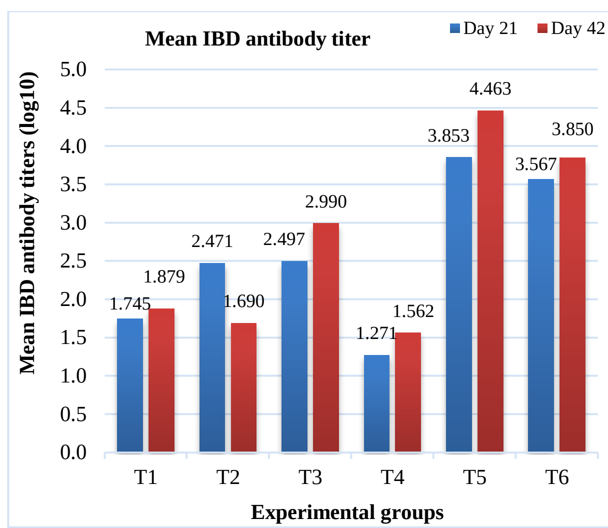

Figure 1. Mean antibody titer of broiler chickens treated with different herbs against IBD vaccine during days 21 and 42. T1: Control (basal diet), T2: Basal diet + 1% basil leaf powder, T3: Basal diet +1% lemongrass leaf powder, T4: Basal diet + 1% peppermint leaf powder, T5: Basal diet + 1% rosemary leaf powder, T6: Basal diet + 1% thyme leaf powder

DISCUSSION

Feed intake

The present study result showed that mean total feed consumption was lower in chickens that consumed peppermint and rosemary during the entire period. Considering the treatment herbs, the highest feed consumption data were recorded in chickens supplemented with basil and a control diet and as well as in a thyme leaf followed by lemongrass leaf powder at the end of the experiment. This result is supported by different scholars' findings that the utilization of PFAs in poultry feed improves feed intake and can stimulate digestion (Pournazari et al., 2017; Madhupriya et al., 2018). Similarly, the result also coincides with the finding of Gurbuz and Ismael (2016), who stated that the biological properties of basil leaf have an appetizing activity, improved feed intake, enhanced digestion process, and intestinal motility (Al-Kelabi et al., 2013). As a natural feed additive, thyme is known to improve feed palatability and stimulate appetite (Hassan and Awad, 2017). Similarly, Witkowska et al. (2019) also noted the highest feed intake in broiler chickens fed on thyme essential oil. Contrary to the present study, Parade et al. (2019) reported that broiler chickens supplemented with lemongrass leaf powder consume lower feed than the control group.

Body weight gain

Body weight gain of broiler chickens was improved by supplementing phytogenic herbs with their diet. The highest BWG observed in chickens fed on the treatment herbs may be due to the beneficial effect and properties of the treatment herbs. In the current study, chickens fed on peppermint had the lowest BWG, compared to the other treatment herb. Similarly, Khodadust et al. (2015) found that peppermint leaf extract did not improve body weight in broiler chickens. During the finisher phase, chickens fed on thyme leaf were recorded with the highest BWG, followed by lemongrass. This result coincides with El-Ghousein and Al-Beitawi (2009), who reported that supplementation of thyme in the basal diet of broiler chickens improved BW, BWG, and FCR. Considering the overall period, chickens fed on lemongrass recorded the highest BWG. The result of the current study agrees with Parade et al. (2019) and Mmereole (2010), who stated that the utilization of a lemongrass leaf powder improved BW and BWG in broiler chicken. Alagawany et al. (2021) and Shaheed (2021) also clarified that supplementation of lemongrass essential oil in the quail diet improved growth performance and boosts health status. A similar trend was also observed by Khattak et al. (2014) and Tiwari et al. (2018), where their experiment revealed that the inclusion of lemongrass essential oil in the broiler diet improved BWG and had positive effects on FCR. The retained heaviest BWG in chickens fed on lemongrass started during the starter and grower phase and continued up to the end of the experiment, which enabled the broilers to utilize their diets more efficiently and grow more. This growth trend was similar to Mmereole (2010), who found continued growth from the first week to the end of the experiment in broiler chickens that consumed lemongrass leaf powder. The higher BWG recorded in chickens fed on lemongrass leaf powder could be due to the active compounds found in the lemongrass improving feed digestion and digestion enzyme secretion in broiler chickens. These digestion physiological activities improved feed intake which was reflected in the weight gain of experimental chickens (Alagawany et al., 2021; Shaheed, 2021). Indeed, natural feed additives could improve the digestibility and absorption of dietary protein in the small intestine, which interns attributed to body weight gain, and chickens grew faster than those fed in the control group (Gurbuz and Ismael, 2016).

Feed conversion ratio

The current experiment indicated that the FCR value was significantly lower in chickens consumed on feed supplemented with lemongrass during the starter phase. Similarly, chickens fed on lemongrass, peppermint, and thyme had better feed conversion efficiency during the grower phase, and this trend continued up to the end of the experiment. This is an indication that the amount of feed consumed by chickens supplemented with these herbs was significantly lower to gain a unit of body weight than other treatments. Consistent with this result, Sadek et al. (2014) and El-Ghousein and Al-Beitawi (2009) reported that supplementing thyme leaf powder significantly improved mean FCR throughout the entire period. Similarly, Al-Kassie (2010) noted that supplementing peppermint in the diet stimulates growth performance and improved FCR in broiler chickens. Supplementing peppermint to broiler chicken improved average BWG with better FCR than control over the whole experimental period (Petričević et al., 2021). Other scholars have also argued that the supplementation of lemongrass to broiler chick improved FCR (Tiwari et al., 2018; Parade et al., 2019). Similarly, Alagawany et al. (2021) also reported that supplementation of lemongrass essential oil in the quail diet for up to five weeks improved FCR. Contrary to the present study, Sariözkan et al. (2016) stated that supplementation of lemongrass to the quail diet did not affect FCR.

Antibody titer

Currently, improving production performance, boosting health status, and enhancing the immune system in the absence of antibiotics are the interest of livestock nutritionists. The PFAs improve and promote the immune response, reduce pathogenic microorganisms, balance intestinal microbial flora, improve disease resistance, promote feed digestibility, absorption, and nutrient availability, and strengthen the immune function (Zhu et al., 2014; Abdelli et al., 2021; Wakjira et al., 2021). Feeding PFAs improves nutrient availability for absorption and boosts the immune of the chicks during critical situation, when there is stress, and support the animal for better growth (Jameel et al., 2014). In the current experiment, the chickens fed on diets supplemented with thyme and rosemary showed a more pronounced antibody titer. This result is in line with the finding of Nahed et al. (2020), who found significantly increased IBD virus antibody titer at 28 days in broiler chicken supplemented with a mixture containing thyme essential oil in drinking water. Similarly, Jameel et al. (2014) indicated that the highest Newcastle antibody titers were for broiler chickens fed on PFAs, and they confirmed that the supplementing broiler fed with 1% thyme powder and also its mixture significantly improved and stimulated the immune system of broiler against infectious organisms. Huang and Lee (2018) also reported that chicken supplemented with PFAs has improved immune response and protective capacity. Similarly, two studies (Genena et al., 2008; Abo Ghanima et al., 2020) also confirmed that rosemary extracts have antioxidant, antiviral, antibacterial, and antifungal activities. Alhajj et al. (2015) and Mandey and Sompie (2021) also reported that broiler chickens supplemented with Chinese star anise as feed additives had a higher antibody titer against the IBD virus and the herb improved performance and could be used as a feed additive to boost the immune responsiveness. The improvement of antibody titer could be due to active compounds in the experimental herb having an immune-modulating effect (Jameel et al., 2014). The highest antibody titer on chickens supplemented with thyme and rosemary in the current study could possibly be due to the immunostimulant properties of essential oil in these plants. Thymol and carvacrol are active ingredients found in thyme and cineol, rosemarinic acid and rosmarole are in rosemary, which has antioxidative properties, antimicrobial activity against intestinal bacteria, promote growth, enhance the health status and improved the immune system of broiler chickens (Jameel et al., 2014; Yildirim et al., 2018). Generally, when antibody production was improved againist the disease producing pathogens, the chickens expended less energy on non specific immune system and the growth and production were improved as a result of the more energy that is available (Jameel et al., 2014; Huang and Lee, 2018).

CONCLUSION

It is concluded all phytogenics tested can be used in Cobb 500 broiler chicks at 1 % of the diet. The findings of the present study indicated that supplementation of lemongrass as well as thyme leaf powder as PFAs improved the growth of the chickens. Feeding peppermint, lemongrass, and thyme as PFAs in the broilers' diet improved FCR, while supplementation of rosemary and thyme developed the highest antibody titer against the IBD vaccine. Supplementation of broilers with PFAs had an appetizing action which was attributed to growth and weight gain performance and boosted immune responsiveness, possibly due to its immunostimulant properties to reduce the risk of vaccine failures. These herbs possess potential beneficial effects and could serve as effective materials for the preparation of pure substances. They have the potential to be developed as alternative feed additives for broiler chickens. However, their effects have not been extensively studied in Ethiopia. Therefore, further research is necessary to explore their potential side effects on the body of broiler chickens.

DECLARATION

Competing interests

The authors have declared that no competing interest exists.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

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Authors' contributions

Tesfaye Engida D. conceptualized the idea and methodology, performed the experiments, collected all samples, analyzed, generated data, compiled information, and prepared the original and final manuscript. Berhan Tamir contributed to the conceptualization, methodology, validation, supervision, and review of the manuscript. Mihretu Ayele contributed to the methodology, performed the experiments, collected the samples, and laboratory analysis of bacteriological data, and reviewing the manuscript. Hika Waktole contributed to the conceptualization and methodology of the study and reviewed the manuscript. Berhane Wakjira contributed to the methodology and laboratory analysis. Fekadu Regassa contributed to the conceptualization and review of the manuscript. 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.

Ethical consideration

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

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