Effect of phytogenic feed additives on hematobiochemical parameters and gut bacterial load in layer hens

M. Yegrem[1], G. Animut[2], Y. Mekuriaw[3], S. Yilkal[4]

[1]Department of Animal science, Debark University, P.O. Box 90, Debark, Ethiopia, [2]Ethiopian Agricultural Transformation Institute, P.O. Box 708, Addis Ababa, Ethiopia, [3]Department of Animal sciences, College of Agriculture and Environmental Sciences; [4]Agricultural Development Center, Bahir Dar University, P.O. Box 5501, Bahir Dar, Ethiopia.

Corresponding author E-mail: minichle18@gmail.com

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Abstract

This study evaluated the effects of neem (*Azadirachta indica*), girawa (*Vernonia amygdalina*), and garlic (*Allium sativum*) powder on the haematological, serum biochemical, and gastrointestinal bacterial counts in layer hens. A total of 128 layer hens were randomly assigned to four dietary treatments: a control diet (T1) with no additives; neem leaf meal (T2); girawa leaf meal (T3); and garlic powder (T4), each at 2.5%. Each treatment had 4 replicates with 32 hens per treatment, and the study lasted for 12 weeks following a Completely Randomized Design (CRD). Blood samples were collected for haematological and serological analysis, and at the end of the study, two chickens from each replicate were slaughtered for bacterial load analysis. The results showed significant effects of dietary treatments on haematological parameters. Packed cell volume (PCV) differed significantly ($P < 0.01$), with the highest value in T2 (35%) and the lowest in T1 (17.43%). Hemoglobin concentration and red blood cell (RBC) count also differed significantly ($P < 0.01$), with the highest values in T2 (9.22 g/dl and 2.61×10^6/mm³, respectively) and the lowest in T1. Lymphocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) showed no significant differences ($P > 0.05$). Serum biochemical indices were not significantly affected ($P > 0.05$). Importantly, bacterial counts in various gut segments were significantly lower in treated groups compared to T1 ($P < 0.01$), with reductions in E. coli and Salmonella counts and increased lactobacillus counts. In conclusion, the inclusion of 2.5% neem, girawa, or garlic powder in the diet did not negatively impact blood profiles and improve haematological health and gut microbiota.

Keywords: Bacterial load; Haematobiochemical; Phytogenic feed additive; Layers

Introduction

Chicken production is popular among small- and medium-scale farmers due to its high turnover and quick return on investment (Afolayan et al., 2014). However, diseases and feed costs have reduced profits (Fasina et al., 2012). Improvements in management practices, such as maintaining chicken health and controlling disease, have boosted productivity (Habte et al., 2017). The health and growth of laying hens, particularly their digestive system, are vital for productivity, emphasizing the need for diets that support gut health (Adedokun and Olojede, 2019).

Historically, antibiotics were used in feeds to promote gut health, but concerns over side effects and public health risks led to their ban in many countries (Gaitov et al., 2021). This has sparked interest in phytogenics as alternatives, driven by concerns about antibiotic resistance and consumer demand for drug-free food (Mahlake et al., 2022; Yonatan et al., 2023).

Studies now focus on plant-based feed additives such as spices and herbs to enhance chicken health (Durmic and Blache, 2012; Kennedy et al., 2019). These additives, rich in bioactive compounds, have antibacterial, antifungal, antioxidant, and functional properties. Girawa (*Vernonia amygdalina*) and garlic (*Allium sativum*) are known for their medicinal and nutritional benefits (Alem, 2024). Neem (*Azadirachta indica*) also promotes growth and feed efficiency in hens, owing to its antibacterial and hepatoprotective properties (Dhana, 2015, Gobezie 2022). Neem leaf meal improves chicken meat and egg pigmentation and stimulates immune responses (Shahrajabian et al., 2022), with no adverse health effects noted when included in layer diets (Pliego et al., 2022).

The haematological system is key to evaluating chicken health (Mishra et al., 2016), highlighting the importance of assessing the safety and nutritional potential of feed. Gut health is crucial to chicken performance (Diaz Carrasco et al., 2019), as medications and toxic compounds can affect the gastrointestinal tract (GIT) and haematological and biochemical parameters (Tavangar et al., 2021). While studies have examined the individual effects of Neem, Girawa, and Garlic on chicken health, limited research exists on their comparative impact on haematobiochemical parameters and gastrointestinal bacterial counts in layer hens. The synergistic effects of these plants in chicken diets remain underexplored, and their influence on blood markers and gut microflora is not well documented. This study aims to evaluate the effects of Neem, Girawa, and Garlic powder supplementation on the health and microbiological balance of layer hens, contributing to more sustainable chicken management.

Materials and methods

Study Area

The experiment was conducted at Bahir Dar University Chicken Farm, located on the Zenzelma campus. It is situated 565 km west of Addis Ababa. The farm sits at an altitude of 1911 meters above sea level. The experimental site geographical coordinates were latitudes of 11°37'18" N and longitudes of 37°27'47" E that had mean annual rainfall of 1445 mm, and the mean minimum and maximum temperature during the experimental period were 18.5 °C and 30.28 °C, respectively. The mean temperature humidity index (THI) value was 66.1%.

Collection and Preparation of Phytogenic Feed Additives

The fresh garlic was purchased from the local market in Bahir Dar city. To prepare the garlic, the whole piece of garlic was chopped into small pieces, sieved through a 2 mm sieve, and spread on a plate. Plastic shade netting was used to dry the garlic in the sun until it dried completely. Fresh neem (*Vernonia amygdalina*) and girawa (*Azadirachta indica*) leaves were harvested from Woreb and Maraki kebeles in Bahir Dar, respectively. The leaves were cleaned from dirty matter and evenly spread on plastic mats at room temperature. They were allowed to dry for 8 days until they became crispy. The leaves were turned regularly to ensure even drying and keeping it in its green color. The dry, crispy leaves were hammer-milled and sieved through a 2 mm sieve before being stored in an airtight plastic sack to prevent moisture absorption until they were used in the experiment as supplements. The processed leaves and garlic powder were manually mixed with the commercial diet. A small amount (0.625 kg) of each additive was added per 25 kg of feed and then blended properly to prepare homogenous mixtures.

Feeding and Experimental Treatments

All chickens received a commercial diet throughout the experiment. Treatment groups were as follows:

T1 (control): Commercial diet without additives,

T2: Commercial diet + 2.5% of *Azadirachta indica* supplement

T3: Commercial diet + 2.5% of *Vernonia amygdalina* supplement

T4: Commercial diet + 2.5% of *Allium sativum* supplement

Nutrient composition of commercial diet and treatments

The nutrient content of the commercial diet and treatment diets were analyzed at two different laboratories, Bahir Dar and Jima Universities (Table 1). The following parameters were measured in representative samples: Dry matter (DM), crude protein (CP), and total ash were analyzed from Bahir Dar University, and the rest, ether extracts (EE),

crude fiber (CF), and phosphorus (P), were analyzed from Jima University. Nitrogen was determined using the Kjeldahl procedure, and CP was calculated by multiplying nitrogen content by 6.25 AOAC (2000). Metabolizable energy (ME) values were calculated indirectly from the EE, CF, and ash using the following formula, adapted from (Wiseman, 2013):

ME (Kcal/kg DM) = 3951 + 54.4 EE - 88.7 CF - 40.8 Ash

Experimental Chickens and Management

For this study, a total of 128 Bovans brown layers weighing 1.73±0.05 kg/chicken were selected from Bahir Dar University Chicken Farm. Four dietary treatments were used, and each treatment had 32 chickens. Each group, which was repeated 4 times in a completely randomized design (CRD), consisted of 8 chickens per replicate. The layers were acclimated to the experimental diets for seven days and then fed for 90 days. Each replicate was housed in 2x1 m^2 pens with wire-mesh partitions on deep litter bedding covered with 5 cm of *teff* straw. Before the start of the experiment, watering and feeding troughs were thoroughly cleaned and disinfected. The experimental pens were sprayed against external parasites. A measured amount of feed was offered twice daily at 08:00 and 17:00 hours to the layers in each pen, with water available *ad libitum.*

The daily temperature (T°c) and relative humidity (RH) were recorded throughout the experimental period using a digital thermo-hygrometer. This instrument was hung at the center of the pens inside the experimental layer house to record the maximum and minimum values of temperature and RH. The average temperature humidity index (THI) was calculated weekly using the equation below according to Ravagnolo et al. (2000) and was analyzed using: THI = (1.8 × T + 32) - [(0.55 - 0.0055 × RH) × (1.8 × T -26)]. *Where* T = temperature (° c) and RH = relative humidity (%). The average THI is presented in (Figure 1).

Table 1. The nutrient content of experimental diets (%)

Ingredients	Parameters						
	DM	CP	CF	EE	P	Ash	ME(Kcal/kg DM)
Commercial diet (T1)	92.43	18.34	4.07	6.00	0.4400	5.20	3533.04
Azadirachta indica	90.62	16.78	7.54	3.40	0.0095	18.1	2728.68
Vernonia amygdalina	93.28	22.62	8.01	3.60	0.0096	16.3	2771.31
Allium sativum	90.78	11.38	7.89	3.46	0.0079	20.1	2619.30
Treatments							
T2	94.69	18.76	4.26	6.080	0.450	5.65	3552.91
T3	94.76	18.91	4.27	6.090	0.449	5.61	3552.17
T4	94.70	18.62	4.27	6.086	0.451	5.70	3555.83

T1=commercial diet without additives; **T2**=2.5% neem leaf meal+commercial diet; **T3**=2.5% Girawa leaf meal+ commercial diet; **T4**=2.5% garlic+commercial diet; DM: Dry matter, CP: Crude protein, CF: Crude Fiber, EE: Ether extract, P: Phosphorus, Ash: Total ash, ME: Metabolizable energy

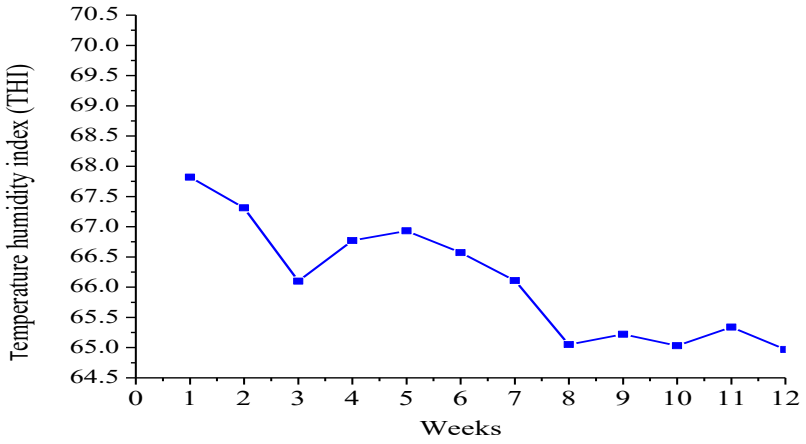

Figure 1. Weekly Temperature Humidity Index throughout the experiment

Blood Sample Collection and Analysis

At the end of the 12-week feeding trial, 8 hens were randomly selected from each treatment group. Blood samples were collected from the overnight-fasted hens via the wing vein using a 5 ml syringe fitted with a 22-gauge sterile hypodermic needle. Blood samples were collected using disposable specimen tubes containing EDTA (ethylene diamine tetraacetic acid) as an anti-coagulant for haematological analysis. The blood sample from each Layer hen was separated into two labeled bottles for haematological and serum biochemical tests. The bottles were then immediately transferred to the laboratory. Red blood cell (RBC) count, hemoglobin (Hb) content, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined by an automatic haematology analyzer, Hemax330. Packed cell volume (PCV) was determined using the microhaematocrit method. Serum biochemical traits, including total protein, albumin, triglyceride, total cholesterol count, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), were analyzed by an automated chemistry analyzer (Weiss and Wardrop, 2011). Globulin values were calculated by subtraction of albumin from serum total protein (Doumas et al., 1985).

Total and Selected Bacterial Counts in Gut of Layer Hens

At the end of the experiment, two hens per replicate (a total of eight hens from each treatment group) were slaughtered. This allowed for the determination of total bacterial counts in the gastrointestinal tract (GIT) and the count of selected bacteria in the cecum. Digesta samples were collected from the crop, gizzard and small intestine (SI), ceca, and feces. The samples were aseptically transferred to sterile sampling tubes and transported immediately for microbial analysis in the laboratory.

Samples were diluted in phosphate-buffered saline (10^{-1} to 10^{-10}) for bacterial counts. Each 1 g sample was weighed and suspended in 9 mL of buffered peptone water solution. The suspension was then subjected to serial dilution ranging from 10^{-1} to 10^{-10}. 0.1 mL of each diluted sample was spread on plate count agar selective media for counting total bacteria. For gut bacteria quantification, diluted samples were seeded on freshly prepared bacterial growth media for quantitation of particular gut flora. E. coli colonies were enumerated on Eosin Methylene Blue (EMB) Agar incubated aerobically at 37°C for 24 hours. Lactobacilli counts were determined on MRS agar incubated anaerobically at 37°C for 72 h. Salmonella colonies were enumerated using a colorimetric reaction on Xylose Lysine Deoxycholate (XLD) Agar. Samples were incubated on XLD Agar at 37°C for 48 hours.

Statistical Analysis

The data for all measured variables were analyzed using a completely randomized design with the General Linear Model procedure in the Statistical Analysis System (SAS) (version 9.4; SAS Institute Inc., Cary, NC). Tukey test was used to determine mean differences at $P<0.05$.

The model used for statistical analysis was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: Yij = the observed value of each of the response variable;

μ = overall mean; T_i = observed effect of the i^{th} dietary treatment, and, and e_{ij} = random error

Results and Discussion

Haematobiochemical Parameters

Haematological parameters of layer hens

The haematological profile of laying hens fed diets supplemented with neem, girawa, and garlic showed significant effects on certain parameters (Table 2). Red blood cell count (RBC), hemoglobin (Hb), and packed cell volume (PCV) all exhibited significant differences ($p < 0.01$). The incorporation of neem, girawa and Garlic in the layer diet enhanced ($p < 0.01$) the blood concentration of red blood cell count (RBC), hemoglobin (Hb), and packed cell volume (PCV). Similar findings were made by Oluwafemi et al. (2021) who reported that broiler chickens given different inclusion levels of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) oil mixture had significantly higher ($p < 0.05$) RBC, PCV, and Hb levels compared to the control group. But the current result is inconsistent with the finding of Odoh and Bratte (2015), who stated that adding neem leaf meal at different level of inclusions had non-significant effect on RBC, Hb, and PCV counts when compared to the control group.

Hens fed neem, girawa, and garlic supplements had significantly higher PCV values (26–35) compared to the control group (17.43) ($P < 0.01$). This result corroborated with the findings of Handique et al. (2019) who found higher PCV values in garlic treated groups as compared to control. This improvement may be attributed to increased protein intake and better nutrient availability, likely due to active compounds in these supplements that stimulate digestive enzyme secretion, enhancing nutrient digestion and utilization. PCV is a key indicator of anemia and dehydration, with values below 22% and above 42% indicates anemia and dehydration respectively (Joshua et al., 2022).

RBC count was significantly higher ($p < 0.01$) in hens fed neem leaf meal (T2) and girawa leaf meal (T3) compared to the control group (T1). The RBC values in this study were slightly lower than the normal range (2.2–4.0 × 10^6/µl) reported for healthy broiler chickens (Ubua et al., 2018; Joshua et al., 2022) but comparable to those found by Olumide and Odunowo (2019) for broiler chickens fed garlic-supplemented diets (3.06–3.96 × 10^3/µl). Hemoglobin (Hb) concentration was significantly higher ($p < 0.01$) in the supplemented groups compared to the control group. The Hb concentrations in this study were within the range (8.43–9.68 g/dl) reported by Solanki et al. (2020) for healthy hens, indicating that the hens in this study were likely in similar health to those considered healthy. The Hb concentrations were also comparable to those found by Unigwe and Igwe (2022), who observed Hb levels ranging from 7.23 to 10.27 g/dl in hens fed garlic and ginger powder meals. However, contradictory findings were reported by Morshedul et al. (2015) and Nodu et al. (2016), who found that neem supplementation did not affect Hb concentration in broilers. Inconsistent findings were also reported by Zeryehun et al. (2017), who found no significant difference in Hb levels in White Leghorn layer chickens fed 0, 1, 2, and 3% garlic powder for 90 days. Hemoglobin concentrations reflect the efficiency of oxygen and nutrient transport in hens. PCV, RBC, and Hb are crucial indicators of hen health and nutrition, as changes in blood constituents can reveal metabolic status and feed quality.

There were no significant differences ($p > 0.05$) observed in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) or lymphocyte counts across the groups. This result is consistent with the findings of Prakash et al. (2022), who reported no significant differences in the hematological parameters (MCV, MCH, and MCHC) of Japanese quail provided with neem leaf extract at different levels. The current result is also similar to the findings of Odoh and Bratte (2015), who reported that varying levels of neem leaf meal inclusion in layer diets had no significant ($p > 0.05$) effect on MCV, MCH, MCHC, and plasma proteins.

Serum Biochemistry Parameters of Layer Chickens

The effects of dietary inclusions of neem leaf meal, girawa leaf meal, and garlic on serum chemistry parameters in laying hens are shown (Table 3). The results indicate that there were no significant differences ($p > 0.05$) between the dietary treatments, suggesting that the tested interventions had no significant impact on the blood chemistry profiles. These findings corroborate previous research by Odoh and Bratte (2015)**,** who similarly found no significant effects of neem leaf meal on serum biochemistry in hens. On the contrary Oluwafemi et al. (2021), found that broiler chickens fed different inclusion levels of ginger and garlic oil mixture showed statistically significant ($p < 0.05$) effects on serum biochemical parameters, including albumin, globulin, and total protein among the treatments.

The total cholesterol levels observed in this study ranged from 80.07 to 139.47 mg/dl. These values were slightly lower than those reported by Khawaja et al. (2013), who found a cholesterol range of 130.70–138.80 mg/dl in layer chickens. Cholesterol is a critical component of cell membranes and plays an essential role in various metabolic processes, including hormone production. In this study, the cholesterol levels fall within the expected range, suggesting that the dietary treatments did not cause any abnormal lipid metabolism in the chickens.

Total protein levels ranged from 4.2 to 5.5 g/dl, which aligns well with the range of 4.25–5.53 g/dl reported by Ojambati et al. (2020) for broiler chickens. Total protein concentration reflects the balance of albumin and globulin in the blood, which are crucial for immune function and maintaining osmotic pressure. Interestingly, this value was lower than the range of 6.16–9.37 g/dl observed by Zeryehun et al. (2017) in layer hens fed garlic powder. The difference in these protein levels could be attributed to several factors, including differences in the type of chickens or the specific feed additives used in each study.

The albumin levels in this study ranged from 2.7 to 3.4 g/dl, which plays a critical role in maintaining blood osmotic pressure and serves as a carrier for various molecules in the blood. The globulin levels observed in this study ranged from 1.53 to 2.2 g/dl. Globulin is another important protein in the blood, contributing to immune defense through antibodies and other immune functions.

In this study, triglyceride levels ranged from 86.43 to 152.4 mg/dl, which are within the expected range for healthy chicken. The lack of significant differences in triglyceride levels between groups suggests that the feed treatments did not alter lipid metabolism or energy storage in a way that would be considered harmful to the hens. The HDL levels in this study ranged from 80.7 to 101.1 mg/dl, and the LDL levels ranged from 13 to 17 mg/dl. These values were consistent with previous studies indicating no significant effects of phytogenic feed additives, such as those in this study, on serum cholesterol and lipoprotein levels. Specifically, Saki et al. (2014) found similar results in laying hens, where phytogenic feed additives did not significantly affect serum HDL or LDL levels. This suggests that the use of such additives may not drastically alter lipid profiles in chickens.

Overall, the serum biochemical parameters in this study suggest that there was a balanced chemical composition across all tested treatments (Table 3), indicating no adverse effects from the dietary interventions. Layer hens fed a diet with inclusion of neem, girawa leaf meal, and garlic exhibited comparable levels of total protein, albumin,

Table 2. Haematological indices of laying hens as affected by the supplementation of *Azadirachta indica*, *Vernonia amygdalina leaves meal* and *Allium sativum* powder Meal.

Indices	Treatments				SEM	P-value
	T1	T2	T3	T4		
PCV (%)	17.43^{b}	35^{a}	31.2^{a}	26.68^{ab}	0.51	0.002
RBC (10^6/mm^3)	1.3^{b}	2.61^{a}	2.27^{a}	1.87^{ab}	0.04	0.0059
Hb (g/dl)	4.8^{b}	9.22^{a}	8.68^{a}	7.67^{a}	0.13	0.0013
MCV (fl)	134.3	134.5	138.5	143.95	0.41	0.1208
MCH (pg)	37.3	35.48	39.2	42.2	0.32	0.3009
MCHC (g/dl)	27.33	26.32	28.2	29.13	0.14	0.4143
Lymphocytes (%)	92.8	88.80	75.20	90.76	0.76	0.1520

T1=commercial diet without additives; **T2**=2.5% Azadirachta indica leaf meal+commercial diet; **T3**=2.5% Vernonia amygdalina leaf meal+ commercial diet; **T4**=2.5% Allium sativum + commercial diet; $^{a, b}$ Means within a row with different superscript letters are significantly different, (P<0.05); SEM=Standard error of the mean

Table 3. Serum biochemical indices of laying chickens as affected by the supplementation of *Azadirachta indica*, *Vernonia amygdalina leaves meal* and *Allium sativum* powder Meal.

Indices	Treatments				SEM	P-value
	T1	T2	T3	T4		
Total protein (g/dl)	5.3	5.5	4.2	4.5	0.07	0.388
Albumin (g/dl)	3.1	3.4	2.7	3.0	0.08	0.895
Globulin (g/dl)	2.20	2.11	1.54	1.53	0.07	0.815
Cholesterol (mg/dl)	135.5	137.0	80.1	139.5	3.06	0.262
Triglyceride (mg/dl)	96.65	86.43	115.43	152.4	3.31	0.330
High density Lipo-protein (mg/dl)	91.2	101.1	80.7	97.7	0.82	0.123
Low density lipo-protein (mg/dl)	17.25	15.00	16.75	12.75	0.96	0.367

***T1**=commercial diet without additives; **T2**=2.5% Azadirachta indica leaf meal+commercial diet; **T3**=2.5% Vernonia amygdalina leaf meal+ commercial diet; **T4**=2.5% Allium sativum + commercial diet, SEM=Standard error of the mean*

Table 4. The influence of *Azadirachta indica*, *Vernonia amygdalina* leaves meal and *Allium sativum* powder on bacterial populations (log cfu/g) in the GIT digesta

Parameters	Dietary Treatments				SEM	P-value
	T1	T2	T3	T4		
Crop	6.00^{a}	4.03^{b}	4.34^{b}	4.23^{b}	0.06	0.002
Gizzard	5.00^{a}	3.54^{b}	4.19^{ab}	3.5^{b}	0.04	0.002
Small Intestine	7.00^{a}	3.50^{b}	4.71^{b}	3.98^{b}	0.10	0.000
Ceca	10.5^{a}	7.32^{b}	7.17^{b}	6.85^{b}	0.11	0.000
Feces	6.53^{a}	4.12^{b}	3.66^{b}	3.73^{b}	0.08	0.000

T1=commercial diet without additives; **T2**=2.5% Azadirachta indica leaf meal+commercial diet; **T3**=2.5% Vernonia amygdalina leaf meal+ commercial diet; **T4**=2.5% Allium sativum + commercial diet; $^{a, b}$ row means with different superscripts are significantly different at (p< 0.05), SEM = Standard error of mean

Table 5. Cecal bacterial counts of layer hens supplemented with *Azadirachta indica, Vernonia amygdalina* leaves meal and *Allium sativum powder*.

Bacterial counts (log cfu/g)	Dietary Treatments				SEM	P-value
	T1	T2	T3	T4		
Total bacterial count	10.5^{a}	7.32^{b}	7.17^{b}	6.85^{b}	0.11	0.000
Lactobacillus	3.51^{b}	4.90^{a}	4.70^{ab}	4.91^{a}	0.20	0.018
Escherichia coli	2.91^{a}	1.78^{b}	2.21^{ab}	1.79^{b}	0.16	0.014
Salmonella	2.01^{a}	0.86^{ab}	0.64^{b}	0.65^{b}	0.20	0.025

***T1**=commercial diet without additives; **T2**=2.5% Azadirachta indica leaf meal+commercial diet; **T3**=2.5% Vernonia amygdalina leaf meal+ commercial diet; **T4**=2.5% Allium sativum + commercial diet; ^{a,b} row means with different superscripts are significantly different at (p< 0.05), SEM = Standard error of mean*

globulin, triglycerides, cholesterol, HDL, and LDL to those fed the control diet. These comparable biochemical profiles demonstrate that both the control diet and the diets incorporating supplemented diets provide similar nutritional quality for laying hens.

Total bacterial Counts in Gut of Layer Hens

There was a significant difference (P < 0.01) in total bacterial populations across the GIT segments as shown in (Table 4). The bacterial load in the crop was reduced with neem, girawa, and garlic supplementation. The phytogenic additives, which significantly lowered bacterial populations compared to the control diet, also improved gut health in

the supplemented groups due to the active compounds in the additives, indicating their antimicrobial properties. This finding aligns with similar results reported for other phytogenic feed additives (Murugesan et al., 2015).

The total bacterial count in the gizzard was significantly lower ($P < 0.05$) in treatments T2 and T4 compared to the control group, indicating a possible inhibitory effect of neem and garlic supplementation, respectively (Table 4). However, no significant difference ($P > 0.05$) was observed with T3 supplementation. The total bacterial population in the small intestine was significantly ($P < 0.05$) reduced by treatments T2, T3, and T4 compared to T1. It is hypothesized that phytogenic additives may inhibit microbial growth by interfering with electrostatic forces or transcriptional mechanisms (Murugesan et al., 2015).

Layers in the supplemental treatment groups had a significantly lower total bacterial count in the cecum ($P < 0.05$) compared to the control group. The cecum plays a crucial role in the fermentation process and nutrient absorption. The decrease in bacterial counts is likely due to the antimicrobial properties of the phytochemicals present in the phytogenic plants. The overall reduction in gut bacterial load from these phytogenic feed additives (PFAs) may lead to immune system-sparing effects, allowing more energy to be directed toward improved performance.

Dietary phytogenic supplements also significantly ($P < 0.05$) reduced bacterial populations in hens' feces compared to the control diet. This finding is consistent with a study by Sakthi et al. (2017), who revealed that layers fed tulsi, garlic, ginger, fenugreek, and curcumin, exhibited significantly lower fecal colony-forming units. Similarly, Odoh and Bratte (2015) demonstrated the antibacterial effect of neem leaf meal, which reduced fecal bacterial counts in Shikka Brown layers compared to the control group. Additionally, Al-Obaidi et al. (2019) reported a decrease in the total fecal bacterial count, including coliforms, among layer hens supplemented with parsley (*Petroselium sativum L*). So this suggests that phytogenic products have the potential to reduce pathogenic populations, thereby improving gut health.

Selected Bacterial Counts in Ceca

Lactobacillus counts were consistently higher in all supplemented groups compared to the control (Table 5). The highest levels of *E. coli* (2.91 log cfu/g) and Salmonella (2.01 log cfu/g) were found in the control group (T1), indicating lower immunity in layers. The lowest *E. coli* colony count was observed in T2 (1.79 log cfu/g) and T4 (1.78 log cfu/g), while the lowest Salmonella count was found in T3 (0.64 log cfu/g) and T4 (0.65 log cfu/g). This result suggests that the addition of those additives to the diet reduces the number of pathogenic bacteria in the ceca and improves gut health. This finding is consistent with a study by Sakthi et al. (2017), which reported that tulsi, garlic, ginger, fenugreek, and curcumin effectively reduce pathogenic bacterial loads and possess significant antimicrobial activity.

The lowest Lactobacillus colony count was found in the control group (T1) (3.5 log cfu/g), while the highest counts were observed in T2 (4.9 log cfu/g) and T4 (4.91 log cfu/g). This result aligns with the findings of Tesfaye et al. (2023), who reported a higher number of Lactobacilli and a lower *E. coli* count in the intestinal content of chickens fed lemongrass, peppermint, and rosemary leaf powder as a supplement. Similarly, Chowdhury et al. (2023) found that incorporating phytogenic feed additives into chicken diets increased Lactobacillus counts and decreased coliform, *E. coli*, and Salmonella counts compared to the control group. Moreover, the addition of 2.0% mint leaf in broiler diets significantly reduced Salmonella and *E. coli* counts in the ceca compared to the control group and improved the overall health status of the broilers (Akter and Asaduzzaman, 2023).

Conclusions

The study on the haematological profile and gut health of laying hens supplemented with neem, bitter-leaf, and garlic improved key haematological parameters, including RBC, Hb, and PCV counts with no significant effect of other parameters and with reduced bacterial load in the gastrointestinal tract. Thus, this finding suggests that phytogenic feed additives are a natural alternative to enhance the health and productivity of laying hens.

Ethics approval

This experiment was reviewed and approved by the Institutional Ethical Committee of Bahir Dar University (Ref. No: 005-2025). The animal experiment was conducted in full compliance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

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