The Research of Medical Science Review

Received: 06 October, 2024
Accepted: 06 November, 2024
Published: 19 November, 2024

ISSN: 3007-1208 | 3007-1216
Volume 2, Issue 3, 2024

THE SYNERGISTIC EFFECT OF MORINGA OLEIFERA, MENTHA PIPERITA AND ZINGIBER OFFICINALE ON THE GROWTH OF BROILER CHICKS AND THEIR USE AS AN ALTERNATE SOURCE OF ANTIBIOTICS AGAINST E. COLI BACTERIA.

Muhammad Zeeshan Mubarik*[1], Amara Akhtar[2], Ayesha Nawaz[3], Muhammad Zohaib[4], Hassan Nawaz[5], Qurban Ali[6], Aqeela Qaiser[7]

*[1,2,3,4]*Department of zoology wildlife & Fisheries University of Agriculture Faisalabad, Punjab Pakistan.*
[5]Department of Chemistry, University of Education Lahore, Punjab Pakistan.
[6]College of life Sciences, Nanjing Forestry University, China
[7]College of Marine Sciences, Shanghai Ocean University, China

*[1]chz53153@gmail.com

ABSTRACT

Broiler meat is considered as a rich source of nutrients. Phytobiotics offer immense therapeutic potential and can provide broiler chickens with a natural protein-rich diet. The present study was conducted to analyze the effects of Moringa oleifera, Mentha piperita, and zingiber officinale on the growth performance of broiler chicks and their use as an alternate source of antibiotics. One day old, thirty broilers were purchased from the local hatchery Faisalabad and were divided into five treatments. T0 was fed with basal diet, T1 was fed with 2% Moringa leaf powder (MLP), T2 was fed with 2% peppermint leaf powder (PLP), T3 was fed with ginger root powder (GRP) and T4 was fed with 0.5ml Enrofloxacin per 2L drinking water. The growth performance was calculated by the percentage weight gain and absolute weight gain on the weekly basis. All data was analyzed by using one-way ANOVA. Maximum weight gain was showed in T1 treatment (63.72 ±60.99) fed with 2% Moringa leaf powder and minimum WG was observed in T2 treatment (50.312 ±54.89) fed with 2% Peppermint leaf powder. Maximum average weight gain was showed in T1 treatment (205.25) fed with 2% Moringa leaf powder and minimum Av WG was observed in T2 treatment (143.25) fed with 2% peppermint leaf powder. Maximum absolute weight gain was showed in T1 treatment (260.25) fed with 2% moringa leaf powder and minimum Absolute WG was observed in T2 treatment (240.6) fed with 2% Peppermint leaf powder. Maximum Specific growth rate was showed in T1 2% MLP (19.86) and minimum SGR was observed in T2 2% PLP (18.94). Maximum FCR rate was showed in T4 treatment (1.73) fed with Enrofloxacin and minimum FCR was observed in T1 2% MLP (1.32227). To check the antibacterial activity of these plants powder against E.coli bacteria, ELISA test was performed before and after treating the groups with phytobiotics and Enrofloxacin. All treatments showed positive ELISA test result after affecting them with E.coli bacteria. Phytobiotics and antibiotics treated groups showed negative ELISA test result. Maximum ratio of ELISA negative test result OD (Optical density) was observer in T1 (0.08) 2% MLP and minimum in T4 (0.021) fed with Enrofloxacin. Therefore, it was concluded that these phytobiotics works as natural growth promoters and also alternate of antibiotics.

Key words: *Moringa Oleifera, Mentha piperita, Zingiber officinale, Broiler growth, E.coli, Antibiotics.*

INTRODUCTION

Poultry farming is one of the most important agricultural industries and poultry meat is a crucial source of animal protein for people all over the world (Kearney, 2010). Poultry farming is a sub-sector of livestock that has been developed from ground chicks rising to industrial farming throughout the past four decades. Among all different agricultural industries, it is known as one of the emerging and innovative business (Kumar, 2006). Broiler feed production accounts for 70% of the total production cost of commercial poultry. Poultry is succeeded in obtaining maximum weight gain at low production cost using some selective growth promoters. Some growth promoting agents are applied to enhance feed efficiency, growth, meat quality and minimize production costs (Abdulameer *et al*., 2015).

Phytobiotics enhance the antioxidant status of farm birds by optimizing their diet quality and quantity. This is a crucial and effective way to improve their health and productivity (Kale *et al*., 2003). Use of natural supplements in chicken diets can boost the bird's antioxidant defense mechanisms and mitigate the harmful effects of oxidative processes on the quality of their products (Rafeeq *et al*. 2023). Antioxidant strategies for birds can be amplified by incorporating phytobiotics that contain extracted active compounds (Drannikov *et al*., 2021). *Moringa oleifera*, particularly its dehydrated leaves in broiler chickens has been found to significantly enhance immune response increase Lactobacillus counts in the ileum and reduce the presence of *Escherichia coli* (E. coli). Moringa supporting immune functions and improving the nutritional status of broiler chickens (Yang *et al*., 2006). Moringa Oleifera Leaf Powder (MOLP) is rich in vitamin C, with a content of 22mg/10mg. This high vitamin C concentration may contribute to developing resistance against infectious diseases and enhancing immunity, regardless of whether individuals are exposed to cold or hot climatic conditions (Gowrishankar *et al*., 2010). Moringa oleifera demonstrates antitumor, anti-inflammatory, and antiulcer activities. This is known for being a rich source of natural antioxidants, such as tocopherols, flavonoids, vitamin C, and essential oils (Lewis *et al*., 2010)

Mentha piperita oil as a sedative has gained popularity in tropical regions. It is topically applied to alleviate pain and enhance blood circulation in the affected area (Schuhmacher *et al*. 2003; Jamroz *et al*. 2003). Peppermint oil or peppermint tea is commonly employed as a remedy for relieving gas and indigestion. It is believed to have the potential to enhance the flow of bile from the gallbladder (Mimica Dukic *et al*., 2003). It is also used in alcoholic industry. Production of alcoholic beverages are used in the treatment of conditions such as rheumatism and toothache (Shah and Mello, 2004). *Zingiber officinale* is utilized as a digestive aid, appetite enhancer, and for alleviating arthritic pain (Mishra *et al*., 1994). Ginger rhizome *(Zingiber officinale)* has been employed as both a culinary spice and a medicinal remedy. Use of ginger as an alternative to antibacterial growth-promoting agents offers several advantages for poultry production, including enhanced productivity, improved appetite and feed palatability, increased nutrient absorption, and stimulation of gastric enzyme secretion (Kothari *et al.*, 2019). Altered parameters could enhance bacterial clearance following in vivo challenge with multidrug-resistant *Escherichia coli* (E. coli) O78 (Ahmad, 2019). *Escherichia coli* (E. coli) is a bacterial species belonging to the family Enterobacteriaceae, which is classified within the gamma subgroup of the phylum Proteobacteria (Arif *et al.* 2019). E. coli is widely acknowledged as a prevalent constituent of the normal gut microbiota in humans, reptiles, and other warm-blooded animals (Leimbach *et al*., 2013). Broiler chickens harbor Escherichia coli (E. coli) on their skin, plumage, upper respiratory tract, and intestines. Although the majority of E. coli strains do not pose a risk to the health of broilers, certain strains have the ability to cause disease beyond the intestinal tract Colibacillosis, an infection induced by avian pathogenic Escherichia coli (APEC), can manifest as either a localized or systemic illness (Ahmad *et al*.,2023). This condition is a significant cause of morbidity and mortality, particularly in the global chicken industry (Sargeant *et al*., 2019). Enrofloxacin is commonly considered more effective than oxytetracycline and sulfadimethoxine in the treatment of diseases like colibacillosis, which is caused by *Escherichia coli*

infection, in chickens (Glisson *et al.*, 2004). While there is a wealth of laboratory data on antimicrobial susceptibility that seemingly supports this notion, there is a scarcity of published clinical data to provide poultry veterinarians with the necessary confidence to make such a choice (Allan *et al.*, 1993). Antibiotic alternatives have demonstrated the ability to effectively enhance performance in veterinary medicine while having minimal therapeutic use. These alternatives do not disrupt the normal flora of the gut, are not absorbed into edible tissues from the gut, and do not promote drug resistance at recommended usage levels. Additionally, they do not contribute to environmental pollution and are non-toxic to both birds and humans (Yadav *et al*., 2016).

Material and methods:

The research project was carried out with the blessing of the study's IBBC (Institutional Biosafety and Bioethics Committee). We bought 30 chickens from a hatchery in Faisalabad. After supplementing broiler chickens with antibiotics and phytobiotics for 28 days, we tested their performance. The birds were placed in a sterile, disease-free environment in a building with a powerful ventilation system so that their optimum growth performance could be monitored, as well as any changes in their hematology. Before the chicks arrived, we made sure there was enough food and water available. A few days before the chicks arrive, a 3- to 4-inch layer of litter is laid over a base of clean, dry sand or directly on the clean floor of the brooder room. Diseases including Newcastle and infectious bronchitis (Globivac) were vaccinated against in the birds.

Experimental birds and house management

The experiment was carried out at PARS campus of University of Agriculture, Faisalabad. The duration of the experiment was 28 days. The house was thoroughly cleaned, whitewashed and sanitized 5 days before chick's arrival. Rearing of experimental birds was carried out at the cage system. Sawdust was utilized as litter material a 2-inch layer of sawdust was evenly spread in each of the pens. Caking of bedding material was avoided by racking regularly. Formalin and potassium permanganate were used for fumigation of the house. The door of the house remained close for 48 hours after fumigation. After 48 hours door of the house was opened to provide cross ventilation in order to avoid discomfort to the chick from the remnants of formalin and potassium permanganate**.**

Fig: 1.1 Experimental house and birds' management.

Thirty broiler chicks were obtained from a local hatchery of Faisalabad. Chicks were placed randomly under 5 treatments including control after recording the initial weight of the chicks.

After the placement of chicks in cages they were provided with sugar solution in order to provide flushing of the digestive system of the chicks. Proper managed condition such as space, temperature, light, ventilation and humidity were provided to chicks of all replicates. Throughout the experiment, the birds had unrestricted access to food and water.

Experimental Layout

The experimental setup was purely arbitrary. When the baby birds arrived, they were weighed and then split into five groups of six at random. The first week was brooding time; therefore, the thermostat stayed at 95 degrees Fahrenheit. The experiment was flushed on day one. The chicks were separated into groups and housed in individual enclosures. These replicates were arbitrarily assigned to one of the five treatments. Both food and water were available to the birds at all times. They cleansed the drinkers twice a day. There was following diet plan.

T0: Basal Diets/ Control

T1: 2% of Moringa leaf Powder (260 g MLP/13 kg of the feeds)

T2: 2% of Peppermint Leaf Powder (260 g PLP /13 kg of the feed)

T3: 2 % of Ginger Root Powder (260 g GRP / 13 kg of the feed)

T4: Basal Diets + Enrofloxacin Antibiotics (0.5 ml / 2 liter of the drinking water)

Collection of Moringa leaves, Peppermint leaves, Ginger and feeds.

Feeds was purchased from Jadeed group of companies PVT. Limited Punjab Faisalabad while moringa leaves were harvested from Punjab bioenergy institute of University of Agriculture Faisalabad. The peppermint leaves were harvested from Chaudhary Agricultural farm Bahawalpur. The ginger was purchased from vegetable shop in Faisalabad and sun dried for 10 days and milled into powder form. The moringa and peppermint leaves were picked, air dried for 4 days in the shade, and ground. The leaf meal was then added to the diets of the various treatment groups at a 2% level.

Nutritional composition of *Moringa*, Peppermint and Ginger Powder

Nutrient Component	Moringa	Peppermint	Ginger
Moisture (%)	6	8	3.55
Energy (kcal)	271.54	151	347
Protein (g)	23.78	3.8	10.25
Carbohydrates (g)	28.32	15	74.77
Fat (g)	7.014	0.9	0.82
Fiber (g)	11.8	8	0.4
Vitamin C (mg)	56	31.8	0.9
Beta – carotene (µ)	37800	-	-
Iron (mg)	19	5.08	15.2
Calcium (mg)	3467	234	69.2
Phosphorus (mg)	215	-	-

Growth performance

Weekly gross weight measurements of the chicks from each treatment were used to assess growth performance. By removing uneaten feed from the chicks' cage, feed utilization was measured. Growth performance and feed utilization was evaluated in terms of Feed conversion ratio specific growth rate (SGR) Specific weight gain (WG) and absolute weight gain (AWG) (FCR).

Body weight gain

Body weight of day-old chicks was recorded and after that body weight was recorded on weekly basis. The average weight per chick of each treatment group was calculated from their replicates at the end of every week. The average body weight gain of each replication was calculated by deducting initial body weight from the final body weight of the birds.

Body weight gain = Final weight – Initial weight

Weight gain (%):

The formula below was used to calculate weight

Final weight – Initial weight

Weight gain (%) = ---------- X100 Initial Weight

Absolute weight gain (AWG):

The absolute weight gain was measured by subtracting the final weight from the starting weight of the chicks.

Absolute weight gain (g) = Final weight (g) – Initial weight (g)

Specific growth rate (SGR):

SGR for chicks was determined as follows:

In [Initial weight – Final weight]

SGR= ------- X100 Experimental duration in days

Survival rate (%):

Survival rate of chicks was determined as:

Final number of chicks

Survival rate (%) = --------X100 Initial number of chicks

Weekly feed intake

Weekly feed intake was determined by taking the entire amount of feed supplied over the week and subtracting the amount of rejection. The amount of food each chick consumed was determined using the following formula:

Feed intake = Feed offered – Feed refused

The body mass information was obtained by weighing the birds in each duplicate at the end of each week. The birds and their food were both measured using a computerized scale.

Feed conversion ratio

The formula below was used to calculate the feed conversion ratio, which measures the bird's effectiveness in turning feed weight into increased body weight. Weekly feed conversion ratios (feed: gain) were determined using the following formula:

$$\text{FCR} = \frac{\text{Feed intake in grams}}{\text{Weight gain in grams}}$$

Corrected FCR was calculated by following formula

Corrected FCR = Feed intake ÷ Weight gain + Weight of dead birds

Feces collection

On day 27th and 28th day bird droppings were collected. Excreta were collected twice a day and were separated over polythene sheet. Bird droppings were collected from individual replicate after 24 hours of feeding. Foreign particles and feathers were removed carefully from excreta. Feces were collected in 2 days from each replicate as composite sample and was weighed, mixed, oven dried at 65 °C and grinded for analysis.

Collection of Blood samples

Chicken with good health were taken from the each group T0, T1, T2, T3 and T4. All birds were acclimatized before slaughtering for one day no feed and water were given to them. Birds with maximum weight were selected for slaughtering. Birds were slaughtered after the completion of 28 days of trial. A digital weighing balance were used to measure live body weight.

Fig: 1.2 Blood sampling and slaughtering.

2-3 ml blood was collected from the broilers in EDTA tube for testing. All birds were slaughter and preserve in the EDTA tubes take 2 sample from each T0, T1, T2, T3 and T4. Different test were perfumed to check the hematology of broiler chicken. Birds often only need a 0.2-mL sample for

regular hematologic tests. The size of the bird, the quirks of the species, the collector's preference, the volume of blood needed, and the bird's health all play a role in determining the collection method used to take blood from birds. The jugular vein was the most usual site for blood collection; however, other sites were also employed. The right jugular vein in the featherless part of the neck (apterium) is big and easy to detect and access, making it a convenient site from which to draw blood. One drawback is the need for careful stabilization of the movable jugular vein. The cutaneous ulnar (brachial) vein provides an alternate location for blood collection in medium-sized birds. But we were used to slaughter the birds and blood was taken in EDTA tube to avoid the clotting. 2ml of blood is taken from each bird. This blood was used to perform ELISA test for the detection or presence of antibodies against the E. coli bacteria.

Fig: 1.3 Collection of blood in EDTA tubes.

ELISA Testing

All the treatments except the control group were infected twice with E. coli bacteria during the whole trial. After affecting them for the first time, the ELISA test was performed to check the status of the results. Then these affected treatments (T1, T2, T3, and T4) were supplemented with Moringa, peppermint, ginger root powder, and Enrofloxacin antibiotics, respectively, and then again an ELISA test was performed to check whether the results were still positive or not. *E. coli* bacteria were purchased from the Faisal Institute of Health Sciences in Faisalabad.

Fig: 1.4: E.coli bacteria.

Statistical Analysis:

ANOVA was used to statistically analyse the data (One-way analysis of variance). Level of significance was 0.05.

Results and discussion

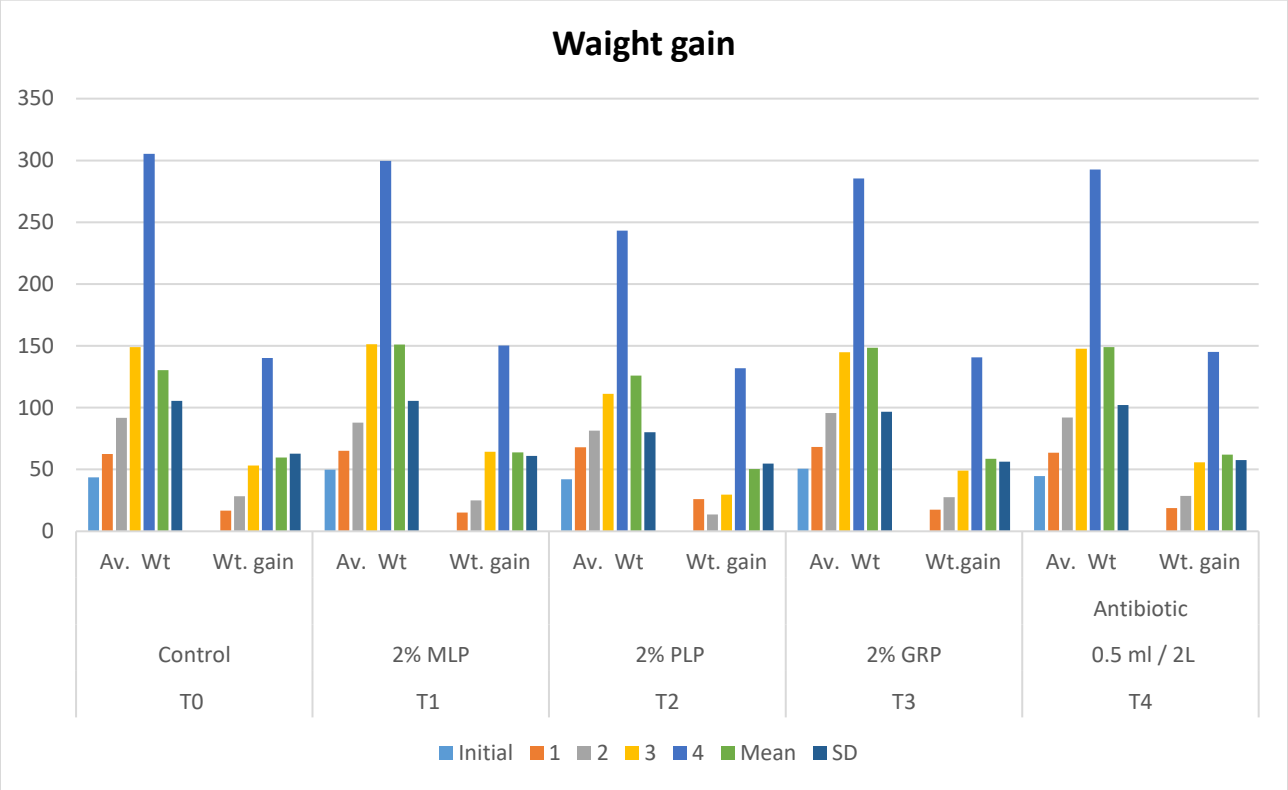
The use of intensive indoor methods to carry out broiler chicken production has gradually led to the maximum production efficiency (Robins and Phillips, 2011). Broiler meat is considered to be highly reliable, flexible and delicious. Consumers demand trends suggest an increase in white meat consumption and a decrease in red meat consumption over the world. Broiler meat and eggs comprise a significant portion of the animal protein consumed by humans (Butcher and Yegani, 2009). The poultry industry has undergone a gradual transition towards an industrialized and fully integrated system of broiler production, resulting in a substantial rise in the utilization of broilers and a notable decrease in market prices for these birds (Lipp *et al*., 2019). At present, commercial broiler chicken strains have been developed through a successful selection process aimed at enhancing two key productive traits: rapid growth and desirable body conformation (Scheuermann *et al*., 2003).

Growth Parameters.

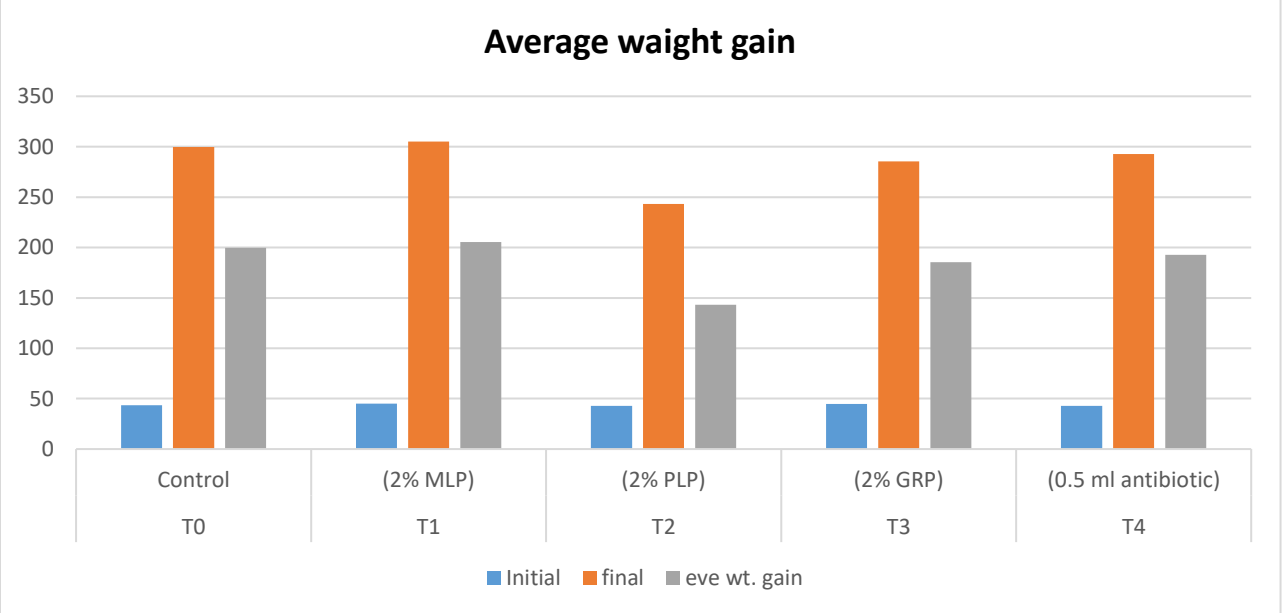
Growth performance of broiler chicks fed with Moringa leaf powder, Peppermint leaf powder, Ginger root powder and Antibiotics was observed during the trial. Weight gain absolute weight gain specific growth rate survival rate and FCR were

determined in terms of growth performance. The density of chicks in each cage was 6.

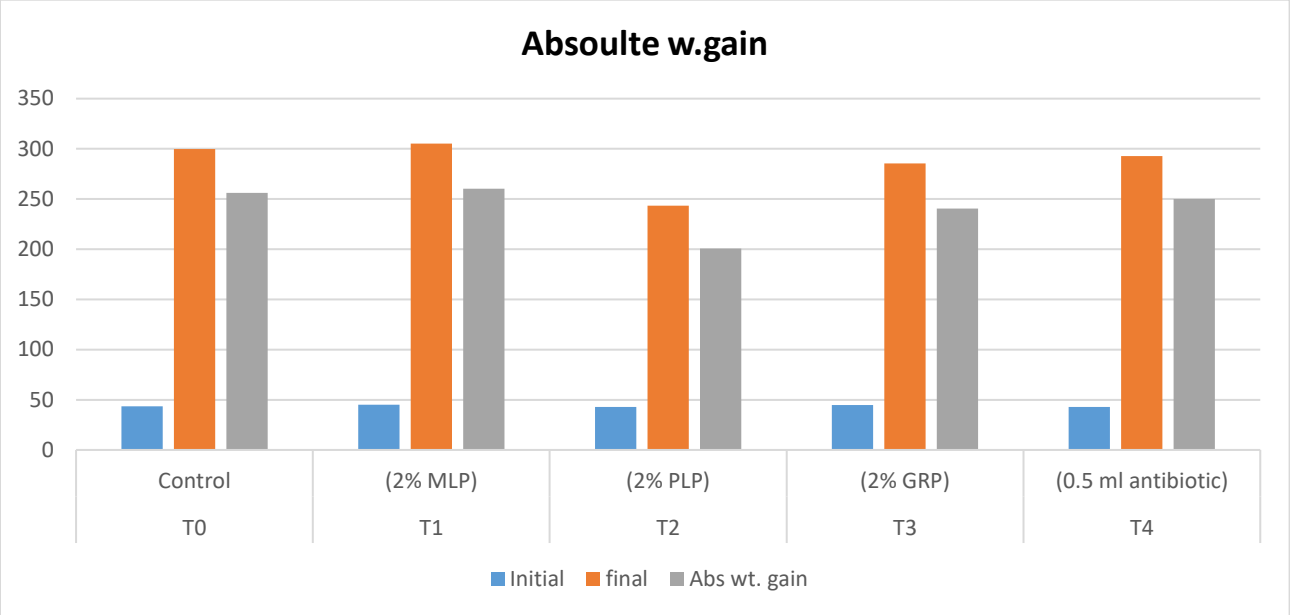
Effect of Supplementation MLP, PLP, GRP and Antibiotics to broiler diets on body weight gain of broiler chickens at different week.



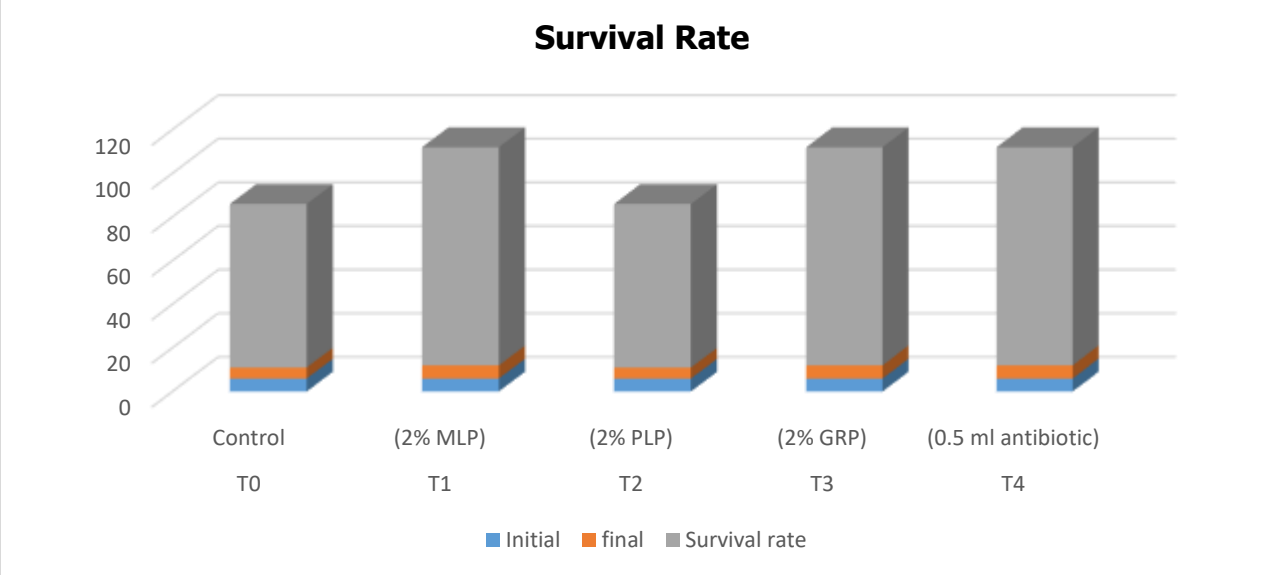
It was observed that in comparison of Moringa leaf powder, Peppermint leaf powder, Ginger root powder and antibiotics, the maximum weight gain was showed in T1 treatment (63.72 ±60.99) fed with 2% MLP and minimum WG was observed in T2 treatment (50.312 ±54.89) fed with 2% PLP. Total weight of chick fed with MLP, PLP, GRP, and antibiotics.



It was observed that the maximum average weight gain was showed in T1 treatment (205.25) fed with 2% MLP and minimum Av WG was observed in T2 treatment (143.25) fed with 2% PLP. Av weight gain of chick fed with MLP, PLP, GRP, and antibiotics.

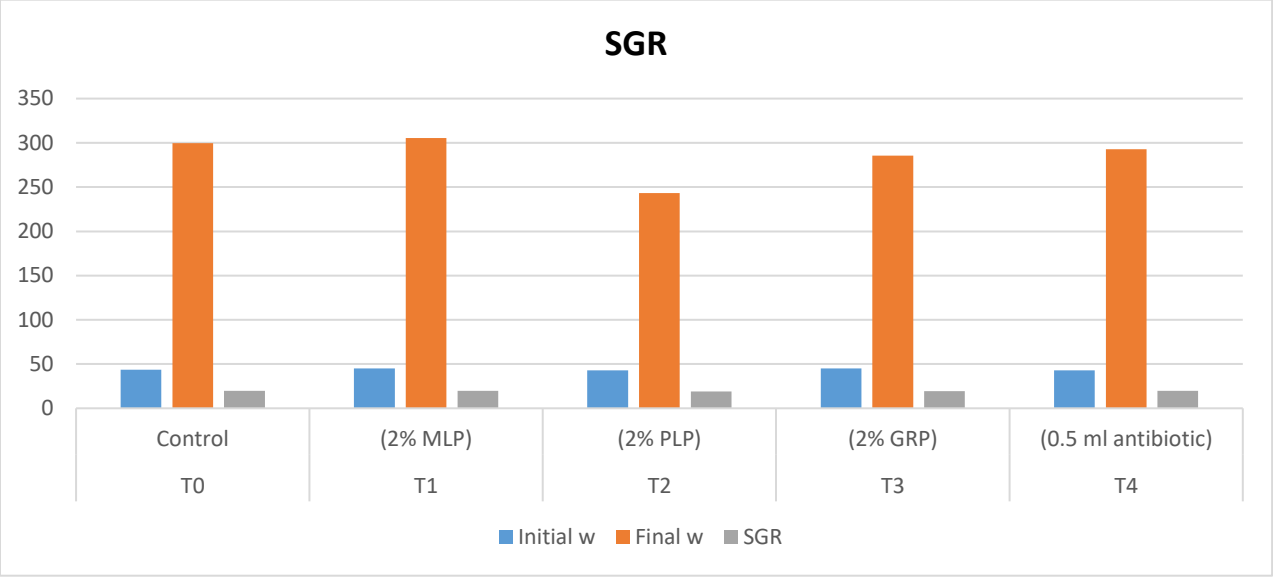


It was observed that the maximum absolute weight gain was showed in T1 treatment (260.25) fed with 2 % MLP and minimum Av WG was observed in T2 treatment (240.6) fed with 2% PLP. Absl weight gain of chick fed with MLP, PLP, GRP, and antibiotics.

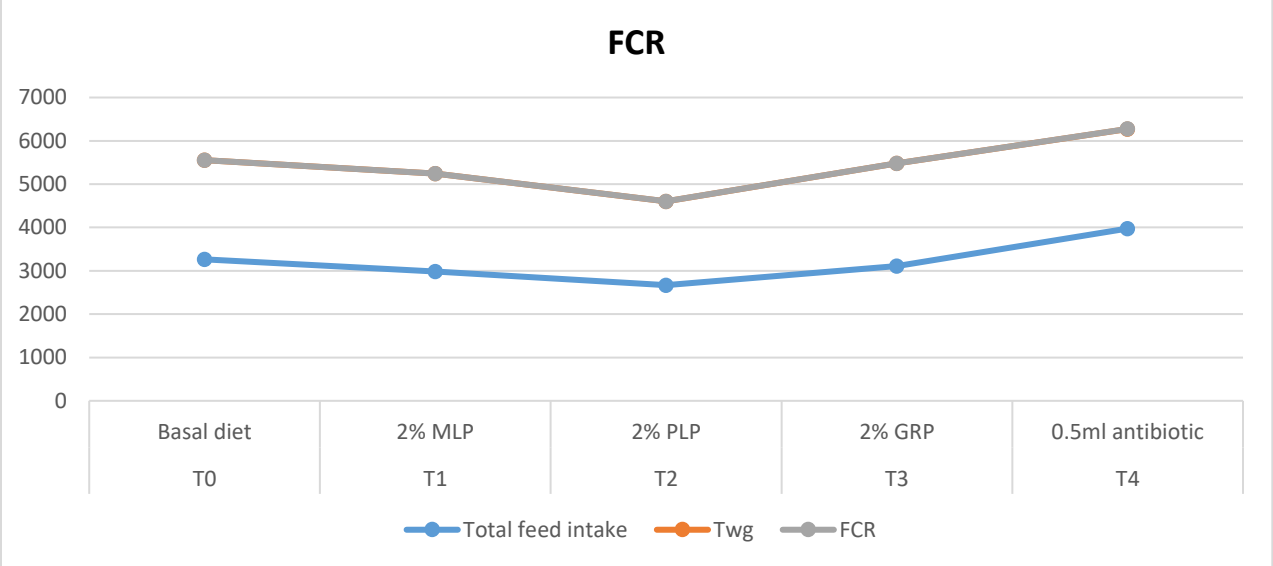


It was observed that the 100 % survival rate was showed in treatment T1, T3, T4 and there was 75% survival rate in treatment T0 and T2. Total survival rate of chick fed with Moringa leaf powder,

Peppermint leaf powder, Ginger root powder and antibiotics.



It was observed that the maximum Specific growth rate was showed in T1 treatment (19.86) fed with 2 % MLP and minimum SGR was observed in T2 treatment (18.94) fed with 2% PLP. SGR of chick fed with MLP, PLP, GRP, and antibiotics.



It was observed that the maximum FC rate was showed in T4 treatment (1.73) fed with 0.5ml Enrofloxacin antibiotic in 2 L drinking water and minimum FCR was observed in T1treatment (1.32227) fed with 2% MLP. FCR of chick fed with MLP, PLP, GRP, and antibiotics.

Table 3.1. ELISA positive testing results after affecting the treatments with E.coli bacteria.

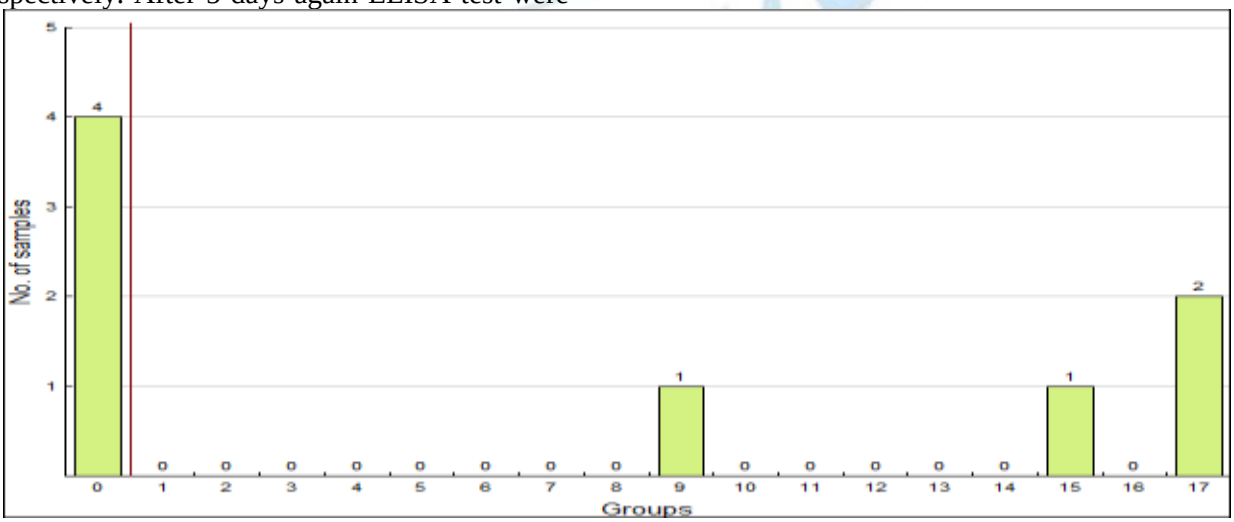
Treatments	Positive Control Dilution (pg/ml)	ELISA OD* Absorbance reading	ELISA Results
T1	20	0.737	Positive +ve
T2	9	0.4	Positive +ve
T3	8	0.22	Positive +ve
T4	5	0.13	Positive +ve

All the treatments except control group were effected with E.coli bacteria by their diet. After affecting them with E.coli bacteria ELISA test was conducted to check the results against E.coli bacteria. All samples were positive. Average OD (Optical Density) An OD > 0.10 is considered a positive result. Maximum ratio of ELISA OD was observer in T1 (0.737) and minimum in T4 (0.1). ELISA results are shown in table. 3.1

Table 3.2. ELISA negative testing results after affecting the treatments with E.coli bacteria.

Treatments	Positive Control Dilution (pg/ml)	ELISA OD* Absorbance reading	ELISA Results
T1	5	0.083	Negative -ve
T2	2.5	0.053	Negative -ve
T3	1.25	0.043	Negative -ve
T4	0.025	0.021	Negative -ve

All the treatments except control group were effected with E.coli bacteria by their diet. After affecting them with E.coli bacteria then the treatments T1, T2, T3 and T4 were supplemented with 2% MLP, 2% PLP, 2% GRP and 0.5 ml Enrofloxacin antibiotic in 2 L drinking water respectively. After 5 days again ELISA test were performed all samples results were negative. Average OD (Optical Density) An OD < 0.10 is considered a negative result. Maximum ratio of ELISA OD was observer in T1 (0.08) and minimum in T4 (0.021). ELISA results are shown in table 3.2.



Graphical representation of ELISA Positive and Negative Results.

Discussion:

Poultry sector has succeeded in obtaining maximum weight gain at low production cost using some selective growth promoters. Some growth promoting agents are applied to enhance feed efficiency, growth, meat quality, and minimize production costs. Usage of growth boosters is very effective for increasing poultry production with low production cost. The main objective of the current study was to determine the effect of *Moringa oleifera*, *Mentha piperita*, *zingiber officinale* and Enrofloxacin on the growth of broiler and their use as an alternate source of antibiotics. Findings showed that usage of these phytobiotics had positive effect on the growth performance of broiler and are good alternatives of antibiotics.

The present study showed that the relative weight gain (g) of broiler chickens in the dietary group T0, T1, T2, T3, and T4 were 59.625±62.67, 63.72±60.99, 50.312±54.89, 58.68±56.26, 62±57.5 respectively. The highest relative weight gain was found in T1 (63.72±60.99) with 2% Moringa leaf powder and lowest relative weight gain was in T2 (50.312±54.89) with 2%

peppermint leaf powder. The highest weight gain in T1 might be due to presence of essential amino acids such as Methionine, Lysine and Tryptophan that are the building blocks of proteins and are crucial for growth and development. Moringa leaves and seeds contain healthy fats, including omega-3 fatty acids which are important for overall health and might be contribute to weight gain when part of a balanced diet. Moringa is believed to have immune-stimulating properties, which promote overall health and reduce the risk of diseases in broilers. A healthy bird is more likely to grow and weight gain efficiently. Moringa is rich in vitamins and minerals, including vitamin A, vitamin E, vitamin K, calcium, iron, and zinc. These nutrients play essential roles in maintaining a healthy immune system. These findings are in lined with those of Banjo, O.S. (2012), who discovered that meals containing 2% M. oleifera leaf meal resulted in noticeably greater body weights because its leaves contain a good amount of protein, vitamins, calcium, magnesium and iron. Minimum weight gain in T2 2% peppermint as compare to T1 might be due to less nutritional value of peppermint as compare to moringa which has high nutritional values as proteins vitamins and minerals. Peppermint has been studied for its potential to reduce appetite. It is believed that the aroma of peppermint may help suppress appetite, leading to reduced food intake. Other reason of minimum weight gain might be due to infecting T2 by E.coli bacteria. E. coli infection lead to diarrhea in broilers. Diarrhea can result in nutrient loss and dehydration both of which contribute to slower growth and weight loss. Smith *et al.*, (1985) showed in their study E. coli colonize and invade the intestinal lining causing inflammation and compromising the absorptive capacity of the gut in broiler. This can result in malabsorption of nutrients reducing the efficiency of feed utilization and reduce the bird's growth.

The present study showed that maximum average weight gain was observed in T1 treatment (205.25) fed with 2 % Moringa leaf powder and minimum Average weight gain was observed in T2 treatment (143.25) fed with 2% Peppermint leaf powder. The reason for the improved average weight gain might be attributed to high amino acids, a highly potent anti-inflammatory and hepatoprotective properties. The HPLC analysis indicated the presence of phenolic acids (gallic, chlorogenic, ellagic and ferulic acid) and flavonoids (kaempferol, quercetin and rutin) in moringa. Natural antioxidants for broiler meat might be found in Moringa oleifera leaf meal. Pari and Kumar, (2002) showed in their research that moringa oleifera have high amount of amino acid and protein which increased the average weight gain of broiler chicks. Their research lined with the present results. Minimum Average weight gain in T2 might be due to Peppermint's cooling properties which affect broiler thermoregulation, especially in hot environments. If the birds' body temperature drops significantly due to peppermint consumption, it could impact their metabolic rate and growth. Yarmohammadi Barbarestani *et al.,* (2017) used peppermint in their research and found that peppermint has low nutritional values which decreased the total hematology and weight of the broiler.

The present study showed that maximum absolute weight gain was showed in T1 treatment (260.25) fed with 2 % Moringa leaf powder and minimum absolute weight gain was observed in T2 treatment (240.6) fed with 2% Peppermint leaf powder. Maximum absolute weight gain in T1 might be due to the presence of minerals present in Moringa such as calcium, magnesium, phosphorus, potassium, and iron. These minerals are necessary for bone development, nerve function, enzyme activity and other physiological processes in broilers. The improvement in live body protein content of Moringa leaf powder as claimed by (Kakengi *et al* 2003) and (Olugbemi *et al*., 2010) in which they demonstrated M. Oleifera plant which was reported to contain various amino acids Histidine, Phenylalanine and serine that play an important role for building muscles in broiler. Minimum absolute weight gain in T2 might be due to introducing peppermint to broiler chickens' diets cause gastrointestinal disturbances due to its potential to relax the lower esophageal sphincter and interfere with the normal digestion process. Demonstrated that peppermint mix diet causes the lower digestibility in broilers and enhanced FCR. Their finding lined with present result.

In present study maximum Specific growth rate was showed in T1 treatment (19.86) fed with 2 % MLP and minimum SGR was observed in T2 treatment (18.94) fed with 2% PLP. The maximum

SGR in T1 might be due to zeatin and kinetin present in moringa which potentially play a role in promoting growth and development in broiler chickens. Zeatin promotes cell division which is essential for growth and development. It helps in the formation of new tissues contributing to overall growth. The substantial levels of vitamins (A, B, and C), calcium, iron, and protein in M. oleifera leaf powder may potentially account for the observed weight gain in broilers as a result of supplementation. These findings are in line with (Nkukwana *et al*. 2012) which discovered that when M. oleifera leaf meal was added to the meals of birds, those birds gained more weight compared to the control group. Minimum specific growth rate in T2 might be due to Peppermint's strong aroma and taste deter broiler chickens from consuming their regular feed, leading to reduced feed intake and slower growth rates. Waheed *et al.,* (2021) reported that the peppermint contains low neutrois values which alter the function of growth. Reported the moringa contains high vitamins and mineras as compares to peppermint supplemented diet. Their research matched with present result.

In present study 100 % survival rate was showed in treatment T1, T3, T4 and there was 75% survival rate in treatment T0 and T2. Total survival rate of chick fed with Moringa leaf powder, Peppermint leaf powder, Ginger root powder and antibiotics. This may be due to the presence of bioactive compounds such as alkaloids, flavonoids, phenolics, and glucosinolates in moringa which have been shown to possess antimicrobial properties. Present study is supported by (Ullah *et al.* 2022) who demonstrated that moringa is believed to have immune-stimulating properties, which could enhance the birds' resistance to diseases, improve their overall health and high survival rate. In T3 ginger treated group, maximum survival rate was observed because, it may help to improve gut health and digestion in broilers, leading to better nutrient absorption and utilization. These findings are in line with (Shakya *et al*., 2015) they determined when birds can efficiently extract nutrients from their feed, they are more likely to grow and thrive, which could result in higher survival rates. In T4 antibiotic treated group maximum survival rate might be due to the effectiveness of antibiotics against different diseases. Enrofloxacin is very effective to kill E.coli bacteria. These findings are in line with (Glisson *et al.,* 2004) who investigated that enrofloxacin is superior to oxytetracycline and sulfadimethoxine for the control of morbidity and mortality caused by E. coli in broiler chickens

The present study showed that maximum FCR rate was showed in T4 treatment (1.73) fed with 0.5ml Enrofloxacin antibiotic in 2 L drinking water and Minimum FCR was observed in T1 treatment (1.32227) fed with 2% Moringa leaf powder. Maximum FCR in T4 because Antibiotics not only target harmful bacteria but can also affect beneficial gut microbiota in broiler chickens. This disruption can lead to digestive problems, reduced nutrient absorption, and compromised immune function. When birds are affected by diseases, their health and appetite can be negatively impacted, leading to reduced feed intake and, consequently, a higher FCR. Dumonceaux *et al*., (2006) demonstrated some antibiotics can disrupt the balance of the gut microbiota in broiler chickens, potentially leading to digestive issues and decreased nutrient absorption. Minimum FCR in T1 might be due to prebiotic properties of moringa promoting the growth of beneficial gut microorganisms. A balanced gut microbiota is essential for efficient digestion and nutrient absorption, thus contributing to better FCR. Moringa leaves and pods are rich in essential nutrients such as protein, vitamins (e.g., Vitamin A, B, C, and E), minerals (e.g., calcium, magnesium, and potassium), and amino acids. These nutrients can help meet the broilers' nutritional requirements and support better growth and development, leading to improved feed efficiency. Moringa leaves can serve as an alternative protein source in broiler diets, which can reduce the reliance on traditional protein sources like soybean meal. This substitution can lead to cost savings and potentially positive effects on FCR. The present study is sported by David *et al*. (2012), Safa & El-Tazi (2012) and (Aderinola *et al*., 2013) they demonstrated Broiler chicks fed a control diet exhibited a considerably greater FCR than those fed diets with 0.5, 1, or 2% MOLM. Birds fed diets containing M. Oleifera had a lower feed conversion ratio than those fed control diets.

The present study showed that maximum ratio of ELISA OD was observed in T1 (0.737) and the minimum in T4 (0.1). After that, T1, T2, and T3

were fed with 2% phytobiotics, Moringa, Peppermint, and Ginger powder, respectively, and T4 was fed with 0.5 ml of enrofloxacin per 2 L of drinking water. Then again, an ELISA test was performed to check the status of the result against E. coli or titers of antibodies against E. coli or Colibacillosis. Results were negative in all treatments that were fed with phtytobiotics and antibiotics. The maximum ratio of ELISA OD was observed in T1 (0.08) and the minimum in T4 (0.021). The mechanism behind the negative ELISA result because phytobiotics prevent excessive production of leukocyte recruitment. They could eliminate the production of monocytes derived macrophage factors like TNF α intlevinin. The decrease in these factors significantly affect antibodies production so ELISA test is negative.

T1 after effecting with e.coli fed with 2% Moringa leaf powder showed negative ELISA result against E.coli bacteria. This might be due to the presence of bioactive compounds such as alkaloids, flavonoids, phenolics and glucosinolates in moringa which have been shown to possess antimicrobial properties. These compounds interfered with bacterial growth by disrupting the cell membrane of bacteria like E.coli. Some organic acids also present in moringa like gallic acid and oleic acid. These organic acids have been investigated as one of the efficient antibiotic alternatives recently due to their antimicrobial activity towards a wide range of pathogenic bacteria. These acids also have the ability to cause a pH decline in the gut, which may enhance the absorption of nutrients in poultry diets. Both single and multiple acid combinations have seen application. Pterygospermin present in moringa is antibacterial. Pterygospermin interact with the bacterial cell membrane which is composed of lipids (fats). It disrupts the membrane's integrity by inserting itself into the lipid layer and causing leakage of essential cellular components. This disruption weakens the bacterial cell and eventually leads to cell death. Present study is in line with (Eidelsburger *et al*., 1992) and (Boling *et al*., 2000) demonstrated the European Union has approved the use of organic acids and their salts in chicken as safe against bacteria. T2 after effecting with e.coli fed with Peppermint leaf powder also showed negative Elisa result against E.coli. This might be due to active compound Menthol and limonnene present in peppermint have antibacterial effects against both gram positive and gram negative bacteria. These active compounds inhibit the growth of E.coli bacteria by disrupting the cell membrane. It interfere with essential metabolic processes, and ultimately lead to the death of bacteria. Menthol inhibit the growth of some bacterial species by interfering with the bacteria's ability to reproduce and multiply, slowing down their growth and reducing their population. Present study supported by (Zandi *et al*., 2016) showed antibacterial activity of M. piperita extracts in their research to control pathogenic bacteria. Mehri *et al.*, (2015) demonstrated Peppermint at the rate of 20–30 g/kg of diet is a promising alternative to antibiotics in order to improve intestinal bacterial populations and absorption surface area broiler. T3 treatment after feeding with 2% Ginger root powder also showed negative ELISA result against E.coli bacteria. Ginger showed antibacterial properties due to the presence of gingerol, paradol and shogaols that have potential to kill the pathogenic bacteria by inhibiting the replication of colon bacteria like E.coli. Bacterial isolates were significantly inhibited by ginger extract, indicating its potent antibacterial properties. Present study was supported by (Shakya *et al*., 2015), which demonstrated that inhibitory zones for both pathogenic S. aureus and E. coli isolates were 24 and 22 mm wide at doses of 16 and 32%, respectively, of ginger extract. In addition, MICs values up to 20 mg/mL indicated that ginger extract has sufficient antibacterial activity. Sensitivity of both isolates was further confirmed using ciprofloxacin disc and/or solution as a control.

T4 fed with 0.5ml Enrofloxacin antibiotic with basal di*et al*so showed negative ELISA test result after effecting with E.coli bacteria. Present study supported by (Da Costa *et al*., 2011) In general 3 day of treatment with enrofloxacin resulted in increased ($P \leq 0.05$) numbers of E. coli resistant to ciprofloxacin, sulfamethoxazole and tetracycline the treatment decreased ($P \leq 0.05$) the number of ESBL-producing E. coli in the AB group.

REFERENCES

Abdulameer .(2015). The prevalence of bacterial vaginosis among women with preterm labour. *Mustansiriya Medical Journal*, *14*(2), 49-53.

Aderinola, O. A., Rafiu, T. A., Akinwumi, A. O., Alabi, T. A., & Adeagbo, O. A. (2013). Utilization of Moringa oleifera leaf as feed supplement in broiler diet. *International Journal of Food, Agriculture and Veterinary Science*, *3*(3), 94-102.

Ahmad, A.A.M. 2019. Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University. "Comparative Immunol. *Microbiol. Infect. Dis.* 101334.

Ahmad, W., S. Noreen, N. Jabeen and A. Gul. 2023. Efficacy of mentha piperita l. *Extract against E. coli in broiler chicken.* 17–27.

Allan, B.J., J.V. Van Den Hurk and A.A. Potter. 1993. Characterization of Escherichia coli Isolated from Cases of Avian Colibacillosis. 146–151.

Arif, M., Z. Hayat, M.E. Abd El-Hack, M. Saeed, H.M. Imran, A.N. Alowaimer, I.M. Saadeldin, A.E. Taha and A.A. Swelum. 2019. Impacts of supplementing broiler diets with a powder mixture of black cumin, Moringa and chicory seeds. *S. Afr. J. Anim. Sci.* 49:564–572.

Banjo, O.S. (2012). Growth and Performance as affected by inclusion of Moringa oleifera leaf meal in Broiler chicks diet. *J. Bio.*5: 2224-3208.

Boling, S. D., Douglas, M. W., Shirley, R. B., Parsons, C. M., & Koelkebeck, K. W. (2000). The effects of various dietary levels of phytase and available phosphorus on performance of laying hens. *Poultry Science*, *79*(4), 535-538.

Butcher, G. D., & Yegani, M. (2009). Investigating Vaccination Failure in Poultry Flocks: VM174/VM136, 11/2008. *EDIS*, *2009*(1).

Da Costa, S., van der Zwaag, W., Marques, J. P., Frackowiak, R. S., Clarke, S., & Saenz, M. (2011). Human primary auditory cortex follows the shape of Heschl's gyrus. *Journal of Neuroscience*, *31*(40), 14067-14075.

David, L. A., Materna, A. C., Friedman, J., Campos-Baptista, M. I., Blackburn, M. C., Perrotta, A., & Alm, E. J. (2014). Host lifestyle affects human microbiota on daily timescales. *Genome biology*, *15*, 1-15.

Drannikov, A., A. Derkanosova, A. Korotaeva, A. Orinicheva, A.Y. Iskusnykh and E. Litvinov. 2021. Phytobiotics as an alternative to antibiotics in feeding farm birds. In: IOP Conference Series: *Earth and Envir. Sci. p* 032061.

Dumonceaux, T. J., Hill, J. E., Hemmingsen, S. M., & Van Kessel, A. G. (2006). Characterization of intestinal microbiota and response to dietary virginiamycin supplementation in the broiler chicken. *Applied and environmental microbiology*, *72*(4), 2815-2823.

Eidelsburger, U., Kirchgessner, M., & Roth, F. X. (1992). Influence of formic acid, calcium formate and sodium hydrogen carbonate on dry matter content, pH value, concentration of carboxylic acids and ammonia in different segments of the gastrointestinal tract. 8. Investigations about the nutritive efficacy of organic acids in the rearing of piglets.

El-Tazi, S. M. (2014). Effect of feeding different levels of Moringa oleifera leaf meal on the performance and carcass quality of broiler chicks. *Int. J. Sci. Res*, *3*(5), 147-151.

Glisson, B., Colevas, A. D., Haddad, R., Krane, J., El-Naggar, A., Kies, M., & Posner, M. (2004). HER2 expression in salivary gland carcinomas: dependence on histological subtype. *Clinical Cancer Research*, *10*(3), 944-946.

Glisson, J.R., C.L. Hofacre, G.F. Mathis, J.R. Glisson, A.C.L. Hofacre and G.F.M. B. 2004. Comparative Efficacy of Enrofloxacin , Oxytetracycline , and Sulfadimethoxine for the Control of Morbidity and Mortality Caused by Escherichia coli in Broiler Chickens Research Note Comparative Efficacy of Enrofloxacin , Oxytetracycline , and Sulfadimet. 48:658–662.

Gowrishankar R, Kumar M, Menon V, Divi SM, Saravanan M, Magudapathy P, Venkataramaniah K (2010). Trace element studies on Tinospora cordifolia (Menispermaceae), Ocimum sanctum (Lamiaceae), Moringa oleifera

(Moringaceae), and Phyllanthus niruri (Euphorbiaceae) using PIXE. Biolog. *Trace Element Res.* 133(3): 357-363.

Jamroz D., Wertlecki J., Orda A., Wiliczkiewicz A. and Skorupińska J. (2003). Influence of phatogenic extracts on gut microbial status in chickens. 110-112 in Proc. 14th European Symp. Poult. Nutr. Lillehammer, Norway.

Kale, B.P., Kothekar, M.A., Tayade, H.P., Jaju, J.B. and Mateeddin, M. (2003). *Indian J.*

Kearney, J. (2010). Food consumption trends and drivers. *Philosophical transactions of the royal society B: biological sciences*, *365*(1554), 2793-2807.

Kothari, D., W. Do Lee, K.M. Niu and S.K. Kim. 2019. The genus Allium as poultry feed additive: A review. Animals 9:9–11.

Kumar, A., Roberts, D., Wood, K. E., Light, B., Parrillo, J. E., Sharma, S., & Cheang, M. (2006). Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Critical care medicine*, *34*(6), 1589-1596.

Leimbach, A., Hacker, J., & Dobrindt, U. (2013). E. coli as an all-rounder: the thin line between commensalism and pathogenicity. Between pathogenicity and commensalism, 3-32.

Lewis, M.R., S.P. Rose, A.M. Mackenzie, L.A. Tucker, S.P. Rose, A.M. Mackenzie and L.A.T. Effects. 2010. Effects of dietary inclusion of plant extracts on the growth performance of male broiler chickens. 1668.

Lipp, I., Jones, D. K., Bells, S., Sgarlata, E., Foster, C., Stickland, R., & Tomassini, V. (2019). Comparing MRI metrics to quantify white matter microstructural damage in multiple sclerosis. *Human brain mapping*, *40*(10), 2917-2932.

Mehri, S., Abnous, K., Khooei, A., Mousavi, S. H., Shariaty, V. M., & Hosseinzadeh, H. (2015). Crocin reduced acrylamide-induced neurotoxicity in Wistar rat through inhibition of oxidative stress. *Iranian journal of basic medical sciences*, *18*(9), 902.

Mimica Dukic N., Bozin B., Sokovic M., Mihailovic B. and Matavulj M. (2003). Antimicrobial and antioxidant activities of three Mentha species essential oils. *Plan. Med.* 69, 413-419.

Mishra, S., H. Kumar and D. Sharma. 1994. How do mothers recognize and treat pneumonia at home? *Indian Pediatr.* 31:15–18.

Mlozi, M. R. S., Kakengi, A. V. M., Minga, U. M., Mtambo, A. M., & Olsen, J. E. (2003). Marketing of free range local chickens in Morogoro and Kilosa urban markets, Tanzania. *Livestock Research for Rural Development*, *15*(2), 2003.

Nkukwana, T. T. (2012). *The Effect of Moringa Oleifera Leaf Meal on Growth Performance, Get Integrity, Bone Strength, Quality and Oxidative Stability of Meat from Broiler Chickens* (Doctoral dissertation, University of Fort Hare).

Olugbemi, T. S., Mutayoba, S. K., & Lekule, F. P. (2010). Effect of Moringa (Moringa oleifera) inclusion in cassava based diets fed to broiler chickens. *International Journal of Poultry Science*, *9*(4), 363-367.

Pari, L., & Kumar, N. A. (2002). Hepatoprotective activity of Moringa oleifera on antitubercular drug-induced liver damage in rats. *Journal of Medicinal Food*, *5*(3), 171-177.

Rafeeq, M., R.M. Bilal, F. Batool, K. Yameen, M.R. Farag, M. Madkour, S.S. Elnesr, N.A. El-Shall, K. Dhama and M. Alagawany. 2023. Application of herbs and their derivatives in broiler chickens: a review. *Worlds. Poult. Sci. J.* 79:95–118.

Robins, A., & Phillips, C. J. C. (2011). International approaches to the welfare of meat chickens. *World's Poultry Science Journal*, *67*(2), 351-369.

Sargeant, J. M., Bergevin, M. D., Churchill, K., Dawkins, K., Deb, B., Dunn, J., & Winder, C. B. (2019). The efficacy of antibiotics to control colibacillosis in broiler poultry: a systematic review. Animal health research reviews, 20(2), 263-273.

Scheuermann, G. N., Bilgili, S. F., Hess, J. B., & Mulvaney, D. R. (2003). Breast muscle development in commercial broiler

chickens. *Poultry Science*, *82*(10), 1648-1658.

Schuhmacher A., Reichling J. and Schnitzler P. (2003). Virucidal effect of peppermint oil on the enveloped herpes simplex virus type 1 and type 2 in vitro. *Phytomed.*. 10, 504-510.

Shah P. and Mello P.M.D. (2004). A review of medicinal uses and pharmacohogical effects of *Mentha piperita. J. Nat. Prod. Rad.* 3, 214-221.

Shakya, A., Chatterjee, S. S., & Kumar, V. (2015). Role of fumarates in adaptogenics like efficacies of traditionally used Fumaria indica extracts. *CellMed*, *5*(1), 6-1.

Shakya, R., Adhikari, S., Mahadevan, R., Shanmugam, S. R., Nam, H., & Dempster, T. A. (2017). Influence of biochemical composition during hydrothermal liquefaction of algae on product yields and fuel properties. *Bioresource technology*, *243*, 1112-1120.

Ullah, H., Wang, Z., Mohsin, M., Jiang, W., & Abbas, H. (2022). Multidimensional perspective of green financial innovation between green intellectual capital on sustainable business: the case of Pakistan. *Environmental Science and Pollution Research*, *29*(4), 5552-5568.

Waheed, A., Baig, N., Ullah, N., & Falath, W. (2021). Removal of hazardous dyes, toxic metal ions and organic pollutants from wastewater by using porous hyper-cross-linked polymeric materials: A review of recent advances. *Journal of Environmental Management*, *287*, 112360.

Yadav, A.S., Kolluri, G., Gopi, M., Karthik, K., Singh, Y., 2016. Exploring alternatives to antibiotics as health promoting agents in poultry-A review. *J. Exp. Biol. Agric. Sci.* 4, 36.

Yang RY, Chang LC, Hsu JC, Weng BB, Palada MC, Chadha M, Levasseur V (2006). Nutritional and functional properties of Moringa leaves from germplasm, to plant, to food, to health. Moringa leaves: Strategies, standards and markets for a better impact on nutrition in Africa. Moringanews, CDE, CTA, GFU. Paris.

Yarmohammadi Barbarestani, S., F. Samadi, S. Hassani and G. Asadi. 2017. Effects of encapsulated nano- and microparticles of peppermint (Mentha piperita) alcoholic extract on the growth performance, blood parameters and immune function of broilers under heat stress condition. *Iran. J. Appl. Anim. Sci.* 7:669–677.

Zandi, O., & Hamann, T. W. (2016). Determination of photoelectrochemical water oxidation intermediates on haematite electrode surfaces using operando infrared spectroscopy. *Nature chemistry*, *8*(8), 778-783.