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KIRIKKALE UNIVERSITY

INSTITUTE OF HEALTH SCIENCE

EFFECTS OF *ARTEMISIA ANNUA L.* ON GROWTH PERFORMANCE, DIGESTIVE ENZYME ACTIVITIES, DIGESTA pH, INTESTINAL MICROFLORA AND GUT MORPHOLOGY IN BROILER DIET

Hafız Muhammad NOUMAN

DEPARTMENT OF ANIMAL NUTRITION

AND NUTRITIONAL DISEASES

MASTER THESIS

SUPERVISOR

Assistant Professor Dr. Gökhan ŞEN

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THESIS ACCEPTANCE AND APPROVAL

This thesis study titled **EFFECTS OF *ARTEMISIA ANNUA L.* ON GROWTH PERFORMANCE, DIGESTIVE ENZYME ACTIVITIES, DIGESTA PH, INTESTINAL MICROFLORA AND GUT MORPHOLOGY IN BROILER DIET**, prepared by **Hafız Muhammad NOUMAN** was unaniomously accepted as M.S.C. Thesis by the following jury, at Kırıkkale University, Institute of Health Sciences, Department of Animal Nutrition and Nutrıtional Diseaeses.

Examining Committee Members

Supervisor: Assist. Prof. Dr. Gökhan ŞEN

Department of Animal Nutrition and Nutritional Diseases, Kırıkkale University.

I approve/disapprove that this thesis is a Master's Thesis in terms of scope and quality.

Signature……………..

Head of Jury: Assist. Prof. Dr. Hasan Hüseyin ŞENYÜZ

Department of Animal Nutrition and Nutritional Diseases, Necmettin Erbakan University.

I approve/disapprove that this thesis is a Master's Thesis in terms of scope and quality.

Signature……………..

Member: Prof. Dr. Mehmet Akif KARSLI

Department of Animal Nutrition and Nutritional Diseases, Kırıkkale University.
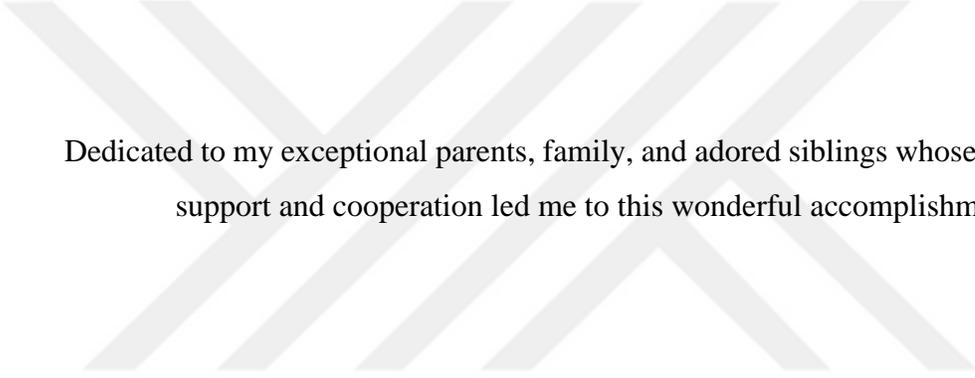
I approve/disapprove that this thesis is a Master's Thesis in terms of scope and quality.

Signature……………..

I certify that this thesis, accepted by the jury, fulfills the requirements for it to be a Master's Thesis.

Prof. Dr. Mehmet Akif KARSLI

Director of Health Sciences Institute

Dedicated to my exceptional parents, family, and adored siblings whose tremendous support and cooperation led me to this wonderful accomplishment.

ETHICS STATEMENT

In this thesis study, which I prepared in accordance with Kırıkkale University, Institute of Health Sciences Thesis Writing Rules;

o I have obtained the data, information and documents I have presented in the thesis within the framework of academic and ethical rules,

o I present all information, documents, evaluations and results in accordance with scientific ethics and morals,

o I have cited all the works I have benefited from in the thesis by making appropriate references,

o I have not made any changes to the data used,

o Thework I have presented in this thesis is original,

Otherwise, I declare that I accept all loss of rights that may arise against me.

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Hafız Muhammad NOUMAN
(Signature)
06/07/2022

</div>

Özet

ARTEMİSİA ANNUA L'NİN ETLİK PİLİÇ RASYONLARINDA BÜYÜME PERFORMANSI, SİNDİRİM ENZİM AKTİVİTELERİ, SINDIRIM pH, BAĞIRSAK MİKROFLORASI VE BAĞIRSAK MORFOLOJİSİ ÜZERİNE ETKİLERİ.

Kırıkkale Üniversitesi
Sağlık Bilimleri Enstitü
Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı, Yüksek Lisans Tezi

Danışman: Dr. Öğr. Üyesi Gökhan ŞEN

Temmuz 2022, 98 sayfa

Kanatlı hayvan endüstrisi, hızlı büyüyen tavukların yetiştirilmesine dayanmaktadır. Son yıllarda, tavuk endüstrisinde büyüme destekleyicileri olarak çeşitli antibiyotikler kullanılmıştır, ancak artan antibiyotik direnci ve insan sağlığı üzerindeki olumsuz etkileri nedeniyle kanatlı endüstrisinde antibiyotik kullanımı yasaklanmıştır. Bilim adamları, antibiyotik direnci arttıkça alternatif antibiyotik ürünleri aramaya başladılar. Probiyotikler, prebiyotikler, organik asitler, bitki özleri, eterik yağlar ve immünolojik uyarıcılar alternatiflerden bazılarıdır. Son yıllarda bu ürünler hayvan performansını iyileştirmiş, hayvan verimliliğini artırmış, enten enfeksiyonlarını önlemiş ve kontrol altına almış ve hayvan tarımında antibiyotik kullanımını azaltmıştır. Bu çalışma *Artemisia annua L*'nin etlik piliçler üzerindeki etkilerini kontrol etmek amacıyla yapılmıştır. *Artemisia annua* ayrıca tatlı pelin, tatlı Annie ve tatlı yıllık pelin (Çince: qugho) olarak da bilinir. Asya'nın ılıman bölgelerine özgü yaygın bir pelin türüdür. *Artemisia* cinsinde bulunan aktif bileşen Artemisinin'dir ve kimyasal olarak hem uçucu hem de uçucu olmayan bileşenler içerir. *Artemisia* cinsinin biyolojik aktiviteleri antibakteriyel, antiinflamatuar, antimalaryal, anti-koksidiyal, antioksidan ve anti-tümör

etkilerini içerir. Sindirim enzim aktiviteleri, bağırsak mikroflorası ve bağırsak morfolojisi kontrol edildi. Mevcut çalışmada 96 günlük Ross 308 etlik piliç kullanıldı. İlk üç hafta boyunca tüm gruplara aynı bazal diyet ad-libitum verildi. Dördüncü hafta itibari ile 4 alt gruplu, bazal rasyonu tüketen grup; Kontrol, bazal yeme 5 mg/kg *linkomisin* ilave edilmiş yemi tüketen grup; Antibiyotik ve bazal yeme 5 g/kg *Artemisia annua L.* ilave edilmiş yemi tüketen grup; *Artemisia* olmak 3 gruba bölündü. Çalışma 42 gün sürmüş ve canlı ağırlık artışı, yem tüketimi ve FCR haftalık olarak belirlenmiştir. Kanatlıların canlı ağırlık artışı, yem tüketimi ve FCR haftalık olarak kontrol edilmiştir. 42 günün sonunda, her tekerrürden rastgele 3 tavuk seçilmiş ve kesim yapılmıştır. Kan ve dışkı örneği ve bağırsaklar toplandı ve daha sonra bağırsaklar morfoloji, bağırsak meroflorası, bağırsak pH'ı ve sindirim enzimi aktivitelerinin kontrol edilmesi için laboratuvarda analizler yapıldı.

Denemenin 2. ve 3. haftarda tüm grupların vücut ağırlıkları istatistiksel olarak benzerdi (P>0.05), ancak Antibiyotik ve *Artemisia* gruplarının vücut ağırlıkları, kontrole kıyasla sayısal olarak daha yüksekti (P>0.05). Araştırma denemesi sonunda, kontrol, Antibiyotik ve *Artemisia* gruplarının vücut ağırlıkları sırasıyla 2668.33, 2683.50 ve 2752.25 g idi (P>0.05). 2 ve 3 haftalık çalışmada, tüm grupların vücut ağırlığı artışları arasında istatistiksel olarak benzerdi (P>0.05). Diğer taraftan Antibiyotik ve *Artemisia* gruplarının 2 haftalık canlı ağırlık kazanımları kontrole göre sayısal olarak daha yüksek iken, 3. haftada sadece *Artemisia* grubu kontrole göre sayısal olarak daha yüksekti. *Artemisia* grubunun 1" ve 3 haftalık yem tüketimi diğer iki gruba göre sayısal olarak daha yüksek iken, 2 & 4 haftalık antibiyotik ve *Artemisia* gruplarının yem tüketimi kontrol grubuna göre daha yüksekti. Genel dönemde *Artemisia* grubunun yem tüketimi kontrol grubuna göre daha fazlaydı. ve antibiyotik gruplarında fark önemli değildi (P>0.05) Kontrol, Antibiyotik ve *Artemisia* grupları için araştırma günlerinde genel yem tüketimi sırasıyla 2889.18 g, 2873,23 g ve 2917.87 g idi. İstatistiksel olarak fark yoktu (P >0.05) deney süresi boyunca gruplar arasında FCR, ancak gruplar arasında sayısal farklılıklar gözlendi.Amilaz için gruplar arasında istatistiksel fark bulunmamakla birlikte (P>0.05), *Artemista* grubu kontrol ile karşılaştırıldığında maksimum sayı (405.08 ± 26.41) gösterdi ve antibiyotik grubu (337.75 ± 13.99) Lipaz durumunda antibiyotik

grubu (23.78 ± 1.11) kontrol ve *Artemisia* grubuna göre maksimum sayıya sahipken, *Artemisia* (21.15 ± 0.69)) ve kontrol (23.10 ± 125) kanda hemen hemen benzer miktar gösterdi, Tripsin için yine antibiyotik grubu kontrol (103.43 ± 12.05) ve *Artemisia* grubuna (114.34 ± 9.25) göre daha yüksek bir sayım (139.82 ± 21.16) gösterdi. Benzer şekilde, Antibiyotik grubu tüm gruplar arasında en düşük pH'a sahiptir. Bunu sırasıyla kontrol ve antibiyotik grupları izledi.

Bağırsak mikroflorasında *Koliform, E. coli, Lactobacillus spp.* ve toplam anaerobik bakteri olmak üzere dört bakteri maddesinin kolonileri sayıldı. Gruplar arasında *koliform* bakteri sayısında istatistiksel bir fark (P>0.05) gözlenmedi. Ancak kontrol grubu, araştırma grubuna kıyasla sayısal olarak daha yüksek miktarda *koliform* bakteriye sahiptir. *E.coli* bakterilerinin miktarları gruplar arasında istatistiksel olarak anlamlı farklılık göstermektedir (P<0.05). *E.coli* sayısı antibiyotik grubunda en düşük, kontrol grubunda en yüksekti. *Lactobacillus spp.* durumunda gruplar arasında istatistiksel olarak fark bulunmadı (P>0.05), ancak bakteri sayısı *Artemisia* grubunda sayısal olarak daha yüksekti. Toplam anaerobik bakteri sayısında, deney ve kontrol grupları arasında istatistiksel fark mevcuttu (P<0.05). Antibiyotik grubu en düşük anaerobik bakteri sayısına sahipken, kontrol grubu maksimum anaerobik bakteri sayısını göstermektedir.

Villus yüksekliği ve villus hayır durumunda tüm gruplarda istatistiksel fark yoktu (P>0.05) ancak sayısal fark mevcuttu. *Artemisia* grubu (686.86 ± 21.07) ile kontrol grubu (632.33 ± 19.42) arasında villus yüzey alanı olması durumunda istatistiksel fark bulunurken, villus yokluğunda antibik, *Artemisia* grubunda maksimum villus sayısı (44.49 ± 4.1) olduğu için. ve antibiyotik (40.25 ± 2.00) grupları.

Antibiyotik grubu, villöz yüzey alanı durumunda (P=0,006) diğer iki gruba göre istatistiksel olarak farklılık göstermiştir.

Sonuç olarak *Artemisia annua L.*, kanatlı endüstrisinde hayvanların gelişimini olumsuz etkilemeden bakterilerde gelişebilecek anti-mikrobiyal direnci önlemek için iyi bir alternatif üründür. Bununla birlikte daha yüksek dozlarda ve besleme süresinin

tamamında gelişecek etkilerini de ortaya koymak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: *Artemisia annua*, Antibiyotik, Direnç, Alternatif, *Lincomycin,* Kanatlı Endüstrisi

Abstract

EFFECTS OF *ARTEMISIA ANNUA L,* ON GROWTH PERFORMANCE, DIGESTIVE ENZYME ACTIVITIES, DIGESTA pH, INTESTINAL MICROFLORA AND GUT MORPHOLOGY IN BROILER DIET

Kırıkkale Unıversity
Institute of HealthSciences
Department Of Animal Nutrition and Nutritional Diseases, Master's Thesis

Supervisor: Assistant Prof. Dr. Gökhan ŞEN

July 2022, 98 page

The poultry industry is based on raising fast-growing chickens. In recent decades, several antibiotics have been employed as growth promoters in the chicken industry, but antibiotic use is banned in the poultry industry due to increased antibiotic resistance and negative impacts on human health. Scientists started searching for alternative antibiotic products as antibiotic resistance increased. Probiotics, prebiotics, organic acids, plant extracts, etheric oils, and immunological stimulants are some of the alternatives. In recent years, these products have improved animal performance, increased animal productivity, prevented and controlled enteric infections, and reduced antibiotic use in animal agriculture. *This* study has been done for checking the effects of *Artemisia annua L* on broiler. *Artemisia annua* is also known as sweet wormwood, sweet Annie, and sweet annual wormwood (Chinese: qngho). It's a common wormwood species native to Asia's temperate zones. The active ingredient present in the *Artemisia* genus is Artemisinin, and chemically it contains both volatile and non-volatile constituents. Biological activities of the *Artemisia* genus include antibacterial, anti-inflammatory, anti-malarial, anti-coccidial, anti-oxidant, and anti-tumor effects. In this study effects of plant extract on broiler growth performance, FCR, intestinal pH, digestive enzyme activities, intestinal microflora, and intestinal morphology were checked. In the current study, 96 day old Ross 308 broiler birds were used and divided into three groups

(Antibiotic group received *lincomycin* 5 mg/kg of basal diet, Control group received basal diet, and *Artemisia annua* group received 5 g/kg grinded plant). Each group consists of 32 birds having four replicate. During the first three weeks, same basal diet ad-libitum was provided for all groups. From 4th to 6th week, control group received finisher diet, Antibiotic and *Artemisia* group received experimental diet. Birds body weight gain, feed consumption, and FCR were checked weekly. At the end of 42nd day, 3 birds were selected randomly from each replicate and slaughtered. Blood and fecal sample, and intestine were collected, and later on analysis has been done in laboratory for checking intestinal morphology, intestinal microflora, intestinal pH, and digestive enzyme activities. In 2nd and 3rd weeks of study, body weights of all groups were statistically similar (P>0.05), however, body weights of Antibiotic and *Artemisia* groups were numerically higher as compared to control (P> 0.05). At the end of research trial, body weights of control, Antibiotic, and *Artemisia* groups *were* 2668.33, 2683.50, and 2752.25 g respectively (P>0.05). In 2nd and 3rd weeks of study, among body weight gains of all groups were statistically similar (P>0.05). On the other hand, in 2nd week body weight gains of Antibiotic and *Artemisia* groups were numerically higher as compared to control whereas, in 3rd week only the *Artemisia* group was higher numerically compared to control. In 1st and 3rd week feed consumption of *Artemisia* group was numerically higher compared to other two groups while in 2nd week feed consumption of antibiotic and *Artemisia* groups was higher than control group. During the overall period, feed consumption of *Artemisia* group was greater than control and antibiotic groups but the difference was not significant (P> 0.05). Overall feed consumption during research days for Control, Antibiotic, and *Artemisia* groups were 2889.18 g, 2873.23 g, and 2917.87 g, respectively. There was no statistically difference (P>0.05) among groups FCR during the experimental period, but numerical differences were observed among groups. Although no statistical difference found among groups (P> 0.05) for Amylase, *Artemisia* group showed maximum count (405.08 ± 26.41) as compared to control (364 ± 16.28) and antibiotic group (337.75 ± 13.99). In case of Lipase, antibiotic group had (23.78 ± 1.11) maximum count as compared to control and *Artemisia* group while *Artemisia* (21.15 ± 0.69) and control (23.10 ± 1.25) showed

almost similar amount in blood. For trypsin, again antibiotic group showed a higher count (139.82 ± 21.16) than control (103.43 ± 12.05) and *Artemisia* group (114.34 ± 9.25). Similarly, Antibiotic group has lowest pH among all groups. It was followed by control and antibiotic groups, respectively.

In intestinal microflora, colonies of four bacteria items including *Coliform*, *E. coli*, *Lactobacillus spp*., and total anaerobic bacteria were counted. There was no statistical difference ($P>0.05$) observed in the number of *coliform* bacteria among groups. But control group has a numerically higher amount of *coliform* bacteria as compared to research group. Amounts of *E. coli* bacteria have statistically significant difference among groups ($P<0.05$). *E. coli* count was lowest in antibiotic group whereas it was maximum in control group. In case of *Lactobacillus spp*., there was no difference statistically among groups ($P>0.05$) but bacterial count was numerically higher in *Artemisia* group. In the number of total anaerobic bacteria, statistical difference was present among experimental and control groups ($P<0.05$). Antibiotic group has the lowest anaerobic bacteria count while control group shows maximum anaerobic bacteria counts.

In case of villous height and villous no., there was no statistical difference in all groups ($P> 0.05$) but numerical difference was present. While in case of villous surface area statistical difference present among groups ($P< 0.05$). Villous height was higher in *Artemisia* group (686.86 ± 21.07) as compared to control (632.33 ± 19.42) and antibiotic (632.33 ± 19.42) group. Similar results were found in case of villi no. as *Artemisia* group had maximum villous no (44.49 ± 4.50) as compared to control (42.53 ± 2.87) and antibiotic (40.25 ± 2.00) groups.

Antibiotic group showed statistical difference in case of villous surface area ($P=0.006$) as compared to other two groups. In conclusion, *Artemisia annua* showed only positive effects on bird's health, microflora, pH and morphology of intestine. There were no side effects observed in this study. Increase in dose rate may show better positive result of *Artemisia annua* used in broilers. So, it is the good alternative product for avoiding anti-

microbial resistance in the poultry industry without compromising growth and production.

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS

BW: Body Weight

ECDC: European Centre for Disease Prevention and Control

EC: European Commission

FCR: Feed Conversion Ratio

ADG: Average Daily Gain

VH: Villous Height

VA: Villous Area

AA: *Artemisia annua*

EA: Enzymatically treated *Artemisia annua*

IFA: In Feed Antibiotics

EO: Essential Oils

HS: Heat Stress

ROS: Reactive Oxygen Species

CD: Crypt Dept

1. Introduction

Poultry industry is big global industry that is dealing with chickens, ducks and turkeys for the purpose of meat, egg and feathers. This vast global industry helps for meeting food requirement throughout the world at cheaper cost with rich protein sources. This poultry industry developed a lot in last two decades due to innovative production technologies, improvement in disease controlling system, genetic upgrading, bio security measurements, increase in human population and urbanization. These different changes offered multiple opportunities for poultry farmers specially smallholders for increasing their business.

Factors those threat poultry industry includes immune status of birds, disease outbreaks, production and health status (Cavani et al., 2009). Moreover, quality of meat and eggs, consumer satisfaction level and products verities is challenging factors for optimum poultry production (Hafez, 2005). Further, food borne diseases, eradication and control pose major threat to poultry industry. In last 20 to 30 years, Antibiotic resistance becomes global problem and world poultry industries are really suffering economical issues due to this problem (FAO Statistics, 2020). Antibiotic residual levels in end products such as meat and eggs creating serious problems for public and animals (Mulder, 2011).

Antibiotics were using for two purposes in ancient times, either for treating diseases or as a growth promoter (Compendium of Veterinary Products, 2003). Antibiotics those used as a growth promoter, usually added in feed in small amount and this usage termed as sub therapeutic. Usually same antibiotics were used for management of different diseases and for growth promotion of birds but lower quantity were added for growth factor. These growth promoting antibiotics were added in feed only (Jones & Ricke, 2003).

Basic question is that why do farmers feel need to add antibiotics as a growth promoter in feed? So answer is that, according to some researchers addition of antibiotics in feed can increase feed efficiency and body weight gain of birds. But with some benefits, it was becoming serious problem due to high antibiotic resistance. In United State and other European countries, customers started demand about antibiotics free meat and eggs and this condition urge scientists to look for non antibiotic growth promoting agent in poultry industry throughout the world. The European Centre for Disease Prevention and Control (ECDC) states that antibiotic resistance continues to be a serious public health threat worldwide. The European Commission (EC) decided to ban all commonly used feed antibiotic-growth promoters due to increased concerns about the potential for antibiotic resistant strains of bacteria and residues in tissues in 2006.

There are variety of non antibiotic growth promoting agents invented including Probiotics, Prebiotics, Organic acids, Intestinal acidifiers, Herbal extracts, Antibacterial and Enzymes those decrease the production of harmful bacteria's such as clostridium perfiringes and staphylococcus species (Elwinger et al., 1992; Hofacre et al., 1998). There are many bacterial species responsible for economic loss for poultry industry including *E. coli*, *Salmonella* and *Clostridium* (Barnes et al., 2008).

Plant extract were started using as a replacement by farmers. These are complex compounds having different compositions and multiple active components. Plant extracts contains proteins, peptides, oligosaccharides, fatty acids, vitamins and micro-minerals. Plant extract have diverse activities and their active secondary plant metabolites generally belong to classes of isoprene derivatives and flavonoids (Glisson, 1998). A great number of plant extracts contain chemical compounds exhibiting antioxidant, antimicrobial, anti-inflammatory, anticoccidial and anthelmintic properties (Kähkönen et al., 1999; Tajodini et al., 2015).

There are many benefits for adding plant extract in animal diet including higher body weight gain, appetite stimulation, endogenous enzymes betterment and beneficial effects on gut physiology (Hsieh et al., 2001). On the other side, some researchers described plant extract have no effect on gut health and growth. There use in poultry industry may

be beneficial due to increase in performance of birds and positive effects on digestibility. Many plants extract those are tested on poultry shows positive effects on health and performance of birds while no harmful effects observed for using plant extract as a feed additive in bird diet. Therefore, they can be used as alternative feed additives in poultry production.

The aim of this research includes use of plant extract *Artemisia annua L* as an alternative of antibiotic for avoiding antimicrobial resistance in poultry industry. *Artemisia annua* is an annual herb native in Asia, especially in China. The name of the plant is Qinghao. It has become naturalized in many countries all over the world, like Argentina, Bulgaria, France, Hungary, Italy, Romania, Spain and USA (Pradeep & Kuttan, 2004; Arab et al., 2006). *Artemisia annua* L., also known as sweet wormwood, sweet annie, sweet sagewort and annual wormwood and belongs to the family of the *Asteraceae* with great therapeutic and economic importance. *Artemisia annua* has biological activities such as antibacterial, anti-inflammatory, anti-malarial, anti-coccidial, anti-oxidant, angiotensin converting enzyme inhibitory, cytokinin like and anti-tumor effects (Hoste et al., 2006).

2. Literature Review

2.1. Overview of Poultry Industry

The way commercial poultry is produced has changed dramatically since then 1950s. Most commercial poultry is raised entirely indoors in an enviournmentally controlled or semi-ecologically controlled buildings. They are guided to maximize yields, such as by feeding them a diet that suits their nutritional needs at different stages of the rearing or laying cycle. Furthermore, photoperiod and light intensity stimulate growth or spawning. An important source of variation in growth and spawning has done by strong genetic selection. This has led to the development of two different types of poultry- meat poultry and table hens, managed by two different sectors of poultry industry (Karcher & Mench, 2018).

Due to these factors, as well as better prevention and fighting disease through vaccines and antibiotics, poultry industry has grown significantly over the past few decades, with poultry meat and eggs are increasing globally and are expected to continue to surge, especially in developing countries. Although there are many different types of poultry meat and eggs grown around the world, the three main types include chickens (meat and eggs), ducks and turkey.

Over the past few decades, the poultry industry has adjusted dramatically to meet demand for cheap and safe supplies of meat and eggs. In the last three decades, the poultry industry has grown at more than 5% per year (compared to 3% for pork and 1.5% for beef) and its global share for meat production has increased from 15 % to 30% in last 30 years (Gerber et al., 2007).

The poultry sector playing vital role in Pakistan for limiting the gap of protein demand and supply. In Pakistan commercial poultry production started after 1950 and with decades it showed more growth. Due to beneficial policies from government and commitment from poultry related community, this sector grows a lot in early days (Sadiq, 2004).

Poultry farming is most motivated and well managed sector of Pakistan who contributed 26.8%, 5.76% and 1.26% for total meat production, agriculture sector and GDP respectively. Over the last few years, poultry department showed excellent growth and provide source of income to more than 1.5 million people (Hussain et al., 2015).

2.2. Antibiotics use in poultry production

First of all, in 1940 antibiotic growth promoter effects was observed while chlortetracycline residues originated from *Streptomyces aureofaciens*, improved bird growth. According to different experimental trials, increase in body weight gain was associated to interactions with intestinal microbial populations (Dibner & Buttin, 2005). In 1951, antibiotics used as feed additives were approved by United State Food and Drug administration. In the middle of 1950-1960, different European states made rules and regulations policies for antibiotics used in animals at national level (Jones and Ricke, 2003). Antibiotics including *bacitracin manganese, neomycin, soframycin, hygromycin-B, tylosin, lincomycin* and *erythromycin* were started using as a feed additives. These antibiotics were used according to instructions given by each state at national level (Castanon, 2007).

Risk related to antibiotic resistance from edible tissues that can create allergic reactions in humans or animals was negligible due to use of antibiotics those were not absorbed in digestive tract. But long term use of these antibiotics can create resistant in bacteria. These resistant microbes can create problems in humans if they transferred through food parts. That's why later World Health Organization and Social Committee for Eurpean Union concluded that anti microbials use can create public health issues (Donoghue, 2003).

Antimicrobial agents can be used for two purposes; appropriately for treating infections and inappropriately (self prescription from humans and as a growth promoter in livestock production). Other then faulty use of medicine for public, antibiotic resistance also arising due to excessive and improper use in agriculture practices. First of all, antibiotic resistance cases observed in 1963 in UK in a particular strain of *Salmonella*

typhimurium (Khachatourians,1998). Approximately 90% antibiotics those use for treating infection are using as promoting agent. In early days, recommended level of antibiotics was 5-10 ppm but later on its increased 10-20 fold (Tenover & Mc Gowan Jr, 1996).There is a 3 to 5 percent increase in the rate of weight gain and feed efficiency when high-energy feed for meat and dairy cattle, sheep, and goats is supplemented with low levels of antibiotics (e.g., 35-100 mg of *bacitracin, chlortetracycline*, or *erythromycin* per head per day or 7-140 g of tylosin or neomycin per ton of feed) (conversion of daily feed intake into meat) (Gillespie, 1997).

After the use of penicillin and *tetracyclines* in livestock production as a growth promoter, many other antibiotics were also subjected to trial and start using in poultry farming. One of such antibiotic is *lincomycin* that introduced in poultry industry as feed additives in 1970. Some researchers observed that use of *lincomycin* at lower dosage 2.2-4.4 mg/kg in broiler production was effective for increase in body weight gain and feed utilization rate. For example, Marusich et al. (1973), Marusich et al. (1978), Stutz and Lawton (1984) and Dafwang et al. (1985) observed increase in body weight gain when *lincomycin* used at 4mg/kg. However, most of the researches showed that improvement in body weight was limited to 14-28 days and small sample size.

Since 1910, antibiotics have been used in animal production in America and other countries. For cheaper production of large quantity of meat, scientists started using antibiotics and anti-microbials agents in livestock production (Ogle, 2013). In some countries, non-therapeutic use of antibiotics banned due to antimicrobials resistance leads to treatment failures and economic losses (Cogliani, 2011). Among EU countries, Sweden was first country banned using antibiotics as a growth promoter in 1986 and for treatment of bacterial infections in 1988 (Cogliani, 2011). After Sweden, other EU countries such as Denmark, Netherlands also banned using of antibiotics in food producing animals. These countries have banned all of antibiotics used as a prophylactic agent in 2011(Maron et al., 2013). Many other countries also banned using of antibiotics and designed special structure for using antibiotics in animals (Choct, 2001).

2.3.   Antibiotic resistance in poultry industry

In September 1928, Sir Alexander Fleming made the discovery of penicillin after examining how a contaminant mold prevented the growth of *Staphylococcus aureus. Penicillin* was given as the name for the chemical that caused inhibition after mold, which was later termed *Penicillium notatum*. In 1940, researchers at Oxford University utilized penicillin for treatment after it had been produced and purified (particularly Ernst Boris Chain and Howard Walter Florey). These three scientists received the Nobel Prize for Physiology and Medicine in 1945 (Alanis, 2005).

Antibiotic resistance was described soon after discovery of penicillin. In 1945, Sir Fleming warned about antibiotic resistance in his interview with New York Times. He said improper use of penicillin responsible for selection of resistant "mutant forms" of *Staphylococcus aureus* that can become reason of more acute infection in animals or humans. After one year of his statement, widespread use of this drug caused resistant in many strains for penicillin. Only a few years later, over 50% were no longer susceptible to this new drug (Mellon et al., 2001).

Abraham and Chain described the first antibiotic resistance mechanism in 1940 (Abraham and Chain, 1940). Penicillinase, an enzyme that inactivates penicillin, was discovered in *E.coli*. In *Staphylococcus aureus*, another scientist, Kirby, described a similar sort of enzyme. As a result, gram positive and gram negative bacteria developed resistance to penicillin before it was widely used (Kirby, 1944). Later in 1970, reports of organisms resistant to *penicillin, aminoglycosides, chloramphenicol,* and *tetracycline* were published.

Availability of antibiotics is one of the great successes of science and miracles of modern life. Diseases responsible for death of our ancestors are now routinely diagnosed controlled. A recent analysis by the Union of Concerned Scientists estimates that over 84% of the antibiotics produced in the U.S. are given to animals in animal production, and most of this is for non-therapeutic purposes (Greko, 1999).

But usually using antibiotics for this purpose cause death of non-pathogen -beneficial-bacteria in body. Bacteria normally present in food animals, so when the antibiotics given to animals, they become reason for death of this beneficial bacteria because beneficial bacteria are sensitive to these antibiotics. Inappropriate use of antibiotics in domestic animals caused the development of resistant variants. These resistant variants will now populate the food animals, and when these animals are slaughtered, these antibiotic resistant bacteria will contaminate the meat.

Bacteria have ability to rapidly transferring antibiotic resistance from one bacterium to another. Bacteria can even transfer it between different species of bacteria. So, if bacteria not present in food supply can got antibiotic resistance (Swartz, 2002). If these non-food bacteria cause disease in animals or humans, it will not be treatable by antibiotics as well.

Despite the predictability of results, some corporate sectors are not ready to admit that excessive use of antibiotics in food animals leads to antibiotic resistant food-borne pathogen. Due to this reason, some researchers in last few years started research about proving that same antibiotic resistant pathogen in a food animal present as an antibiotic-resistant pathogen in animal-based human foods and in humans (White et al., 2002; Hamer & Gill, 2002).

Due to emergence of multidrug resistant organisms, arising of new infections, and the potential use of multidrug resistant agent included in biological weapons, the need of new antibiotics becomes greater (Spellberg et al., 2004). Since the 1970s, few new antibiotics have been discovered, and experts judge that the chance for discovery of new antibiotics is less. This is partially because little research into new antibiotics is being conducted and unfortunately there may be few remaining effective antibiotics to be discovered (Daikos et al., 2008).

"Selective pressure" developed by antibiotic usage in poultry industry is the main factor for emergence of antibiotic resistance in both humans and animals. Inappropriate use of antibiotics i.e., such as overuse or misuse enhances the chances of resistance

(Rosenblatt-Farrell, 2009). Further, role of food industry for creating antibiotic resistance is under observation and there is high concern about transmission of resistant bacteria via food chain.

Two conditions are needed for antibiotic resistance to develop in bacteria. First, the organism must come into contact with the antibiotic. Then, resistance against the agent must develop, along with a mechanism to transfer the resistance to daughter organisms or directly to other members of the same species (Khachatourians, 1998). Recently Alliance report about antibiotics in animals and their impact on resistance published. According to that report we should describe new controlled way of using antibiotics in animals as like we have done for humans (Singer & Hofacre, 2006).

2.4. Antimicrobial Agents Mechanism of Action

Antimicrobial drugs are classified into distinct categories based on their overall mode of action. Antimicrobial drugs commonly have four modes of action: (a) interfering with cell wall production, (b) blocking protein synthesis, (c) interfering with nucleic acid, and (d) interfering with metabolic pathways (McManus, 1997).

Beta-lactams, such as penicillin *cephalosporins, carbapenems*, and *monobactams*, and glycopeptides are antibacterial drugs that impair bacterial cell wall formation. Beta lactams inhibit bacterial cell wall synthesis by interacting with an enzyme required for the development of the peptidoglycan layer (Drlica & Zhao, 1997). Antibacterial effects are achieved by stopping protein synthesis with *macrolides, aminoglycosides, tetracyclines, streptogramins,* and *oxazolidinones* (Drlica & Zhao, 1997). The structure of bacterial and eukaryotic ribosomes differs, which aids antibacterial agents in inhibiting bacterial growth. *Chloramphenicol* binds to the 50S subunit of the ribosome, whereas *macrolides, aminoglycosides, and tetracyclines* bind to the 30S subunit.

Sulphonamides and *TMP* impede the production of folic acid, whilst *fluroquinolones* stop DNA replication by breaking DNA double strands (Yao & Moellering, 2003). TMP, folic acid analogue, and *sulphamethoxazole* were used in conjunction to limit

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bacterial folate production (Petri, 2006). Disruption of bacterial membrane structure, which is less well understood, is another putative mode of action. Polymyxins are thought to work by increasing the permeability of bacterial membranes, causing bacterial contents to leak out (Storm et al., 1977). The cyclic lipopeptide *daptomycin* appears to embed its lipid tail into the bacterial cell membrane, inducing membrane depolarization and ultimately bacterial death (Carpenter & Chambers, 2004).

2.5. Mechanism of action of Bacterial resistance

Bacteria have developed a variety of methods to combat antibiotics, a development that is weakening our ability to control bacterial infections (Rosenblatt-Farrell, 2009). Antibiotic resistance can be inherent, resulting from a microorganism's general physiology or architecture (absence of the target of the antimicrobial agent, poor permeability of cell envelope, production of enzymes that inactivate the antimicrobial, or presence of efflux systems that decrease intracellular antibiotic concentration). Intrinsic resistance is a naturally occurring feature in certain bacterial species (also known as "insensitive" or "unsusceptible") that is unaffected by antibiotic use (or misuse).

Gram-negative bacteria, for example, have an outer layer that makes hydrophobic chemicals like *macrolide* antibiotics relatively impenetrable. Stress-response systems (FVE, 2002) are temporary techniques in which different genes are expressed or silenced to allow bacteria to survive in the presence of antibiotics. Inherent resistance, on the other hand, is not the primary source of concern for human and animal health. Instead, the majority of antimicrobial-resistant bacteria have arisen as a result of genetic alterations gained through mutation (vertical evolution) or horizontal transfer of genetic material from other bacterial strains (Alanis, 2005).

Mutational resistance occurs when a region on the microbial chromosome that determines susceptibility to a certain antibiotic undergoes spontaneous mutation. Spontaneous mutations frequently result in antimicrobial target modifications (e.g., chromosomal changes that result in quinolone resistance) and are vertically transmissible. Antimicrobial resistance can be exacerbated by mutations in regulators or

regulatory areas that cause the overproduction of intrinsic resistance determinants, such as efflux pumps (Flensburg & Sköld, 1984), or the target itself (Mulvey & Simor, 2009).

Bio film formation, changes in surface permeability, efflux or enzymatic inactivation of the compound before it reaches its target site, modification or overproduction of the target site, and acquisition of alternative metabolic pathways to those inhibited by the drugs are all microbial strategies for overcoming the effects of antimicrobials (Wilson et al., 2020).

Figure 1: Mechanism of action of antimicrobial resistance.
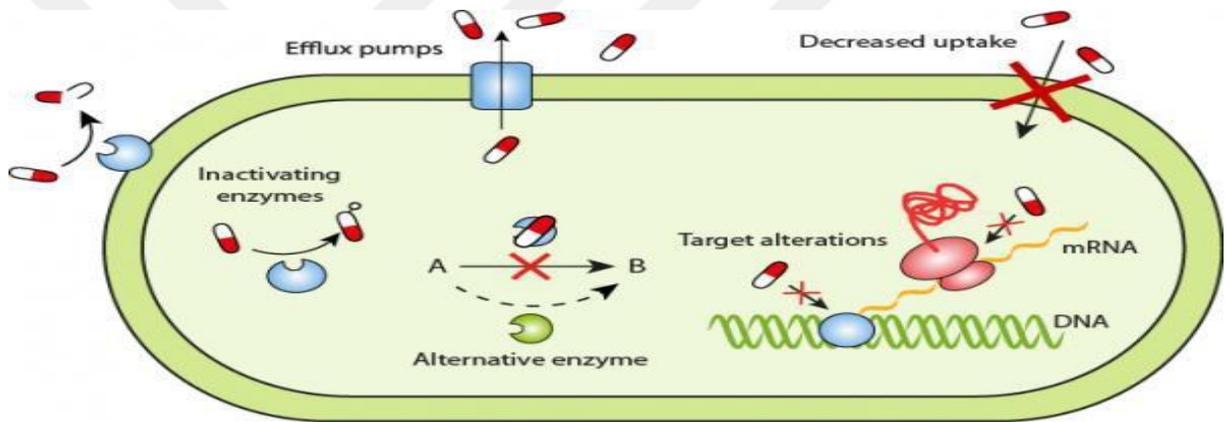
2.6. Consequences of Antibiotic Resistance

Treatment fails to respond in infections caused by resistant bacteria and these caused increase in economics. Also, some issues associated with morbidity and mortality (Neu, 1992). Infections caused by resistant bacteria are responsible for financial burden on health care systems and farmers. Majorly antibiotic resistance creating problems for human beings as same antibiotics are using in food animals and humans. Due to resistance problems, human pathogen is becoming more active and diseases are becoming more severe and tough to cure in humans. If this problem persists for long time, it may possible in future that no antibiotic will work against infectious diseases and it will lead to economic burden and human loss due to untreatable infections.

Antibiotic resistance is making it difficult to treat food-borne bacterial illnesses. For example, *Staphylococcus aureus*, a prominent cause of disorders like septicemia, pneumonia, and wound infections, has developed resistance to all antibiotics except vancomycin, however there have been reports of *vancomycin* resistance as well (Sieradzki et al. 1999; Smith TL et al. 1999). If this resistant virus spreads to someone's blood, that person will succumb quickly. Other infections-causing bacteria species are getting increasingly resistant and difficult to treat as time goes on. Multiple drug-resistant infections *Neisseria gonorrhoeae* is still the most common cause of venereal illness. Many experts in the area are concerned about what may happen if/when antibiotic resistance emerges in particularly dangerous species. Is it possible that we may face major epidemics that medicine will be unable to manage in the future? (Goldman, 2004)

2.7. Antibiotics Alternative

Because in feed antibiotics (IFAs) are no longer allowed in poultry feed, the industry must look for other ways to preserve or improve the health and performance of the birds. Although the efficacy of antibiotic substitutes must be determined through the establishment of standards and multi-factorial models (Rosen, 2003), it is encouraging to know that the majority of the alternatives examined in this context can promote growth; in fact, some of the effects are comparable to those of IFAs. The flip side of the coin is that these growth-promoting effects are (very) variable; in certain cases, the alternatives can even have a negative impact on performance.

Poultry development and feed efficiency are tightly linked to the quantity of intestinal bacteria, wall structure, and immune system activity. Several antibiotics were employed as growth promoters in chicken diets to boost growth, feed efficiency, and minimize mortality in the poultry business (Denli & Demirel, 2018). Their effects on gut microbiota, on the other hand, interact with digestive physiology and consequently growth in a variety of ways, which can be impacted or even determined by a variety of other factors such as diet compatibility, hygiene standards, and animal husbandry techniques (Yang et al., 2009).

There are many alternatives currently using for poultry production including Bacteriocins, Bacteriophages, Probiotics, Prebiotics, Phytobiotics, Symbiotics, Organic acids, Enzymes, Mannan Oligosaccharides, Essentail oils and Plant extract. These alternatives same beneficial effects as like antibiotics but they don't harm beneficial bacteria and most of them have no harmful effects on health of birds and for humans and enviournment.

2.7.1. Bacteriocins

Many bacteriocins have characteristics that suggest they might be useful in clinical situations. However, the primary focus of these bacteriocins' utilization has been on animal health rather than human health to date. Thiostrepton is currently used in combination therapy ointments for treating dermatological indications in domestic animals, and nisin is used as the active ingredient in the mastitis prevention medication Wipe Out (Immu Cell Corporation) (Cotter et al., 2013).

2.7.2. Bacteriophages

Bacteriophages could be utilized to treat bacterial infections. Although phage therapy has various benefits and few adverse effects have been observed, it is impossible to rule out underreporting. However, more well-designed trials are needed to determine the role and safety of phage therapy in treating patients with diverse infections in daily clinical practice. Furthermore, direct usage of phage-encoded proteins such as endolysins, exopoly saccharidases, and holins has demonstrated their potential as a viable antibacterial option (Wittebole et al., 2014).

2.7.3. Fibre-degrading enzymes

Enzymes are naturally occurring proteins that all living organisms create to catalyze chemical processes. Bedford (2000) divided the effects of enzymes on gut microbiota into two phases: an ileal phase and a caecal phase. Enzyme supplementation can result in a 2-5 percent increase in feed/gain ratio and a 2-3 percent increase in growth rate (Broz and Beardsworth, 2002).

2.7.4. Prebiotics

A prebiotic, according to Gibson and Roberfroid (1995), is a non-digestible food element that improves the host's microbial balance by selectively encouraging the growth of and/or activating the metabolism of one or a small number of health-promoting bacteria in the digestive tract. Endogenous microbial population types such as *bifidobacteria* and *lactobacilli* are activated in a certain way, and these bacteria are thought to be helpful to animal health.

2.7.5. Probiotics

Probiotics are live microorganisms that, when ingested in sufficient concentrations, are said to have health advantages. Many experiments with various strains of fowl showed that probiotic supplementation in the feed resulted in a beneficial reaction. The performance of broilers is aided by probiotic microorganisms. Broiler chicks administered probiotics showed significant increases in daily growth and feed intake. Probiotics have been demonstrated to increase feed efficiency in chicken, lower serum cholesterol and triglyceride levels, modulate intestinal microbiota and pathogen inhibition and immune modulation, and reduce oocyst shedding in *Eimeria acervulina* (Denli & Demirel, 2018).

2.7.6. Plant Extracts

In addition to their beneficial benefits on human and animal health, medicinal herbs, spices, and aromatic plants serve an essential role in improving the flavor, scent, and color of human and animal food and feed (Abd El-Hack and Alagawany 2015). Plant extracts are complex molecules with a wide range of compositions and active ingredients. Proteins, peptides, oligosaccharides, fatty acids, vitamins, and micro-minerals make up the majority of plant extracts. Plant extracts contain a variety of actions, and their active secondary plant metabolites are typically isoprene derivatives and flavonoids (Tajodini et. al., 2015). They offer a diverse set of activities. Plant extracts as feed additives in poultry could be beneficial since they allow for better overall performance and nutrient digestibility in chicken.

In the chicken business, a variety of alternatives have been utilized as antibiotics. Many studies found that probiotics and plant extracts, in particular, have antibiotic-like effects in chickens. In order for these alternatives to antibiotics to be more effective in chickens, adequate dosing and application methods are also important (Hsieh et al., 2001).

2.8. **General Introduction of** *Artemisia annua L.*

Artemisia is one of the most populous and extensively spread genera in the *Asteraceae* family. It is a diverse genus with approximately 500 species found primarily in Europe, Asia, and North America's temperate zones. Annual, biennial and perennial herbs are represented in this collection (Iranshahi et al., 2007). The medicinal plant Artemisia annua L is well-known (Bhakuni et al. 2001). The only planta medica authorized by the WHO in China for study and development as the standards of western medicine research is *Artemisia annua*. It is a well-known herb that is well-known for its efficacy and minimal toxicity in the treatment of ague (Wang et al. 2011).

2.8.1. Origin

This plant is native to Asia, and it is most likely to have originated in China, namely in the provinces of Suiyuan and Chahar. China has a long history of cultivating *Artemisia annua* and is skilled in extracting artemisinin using a unique process; as a result, it is the first country to isolate artemisinin from plant extracts. Furthermore, China has surpassed the United States as the leading provider of *Artemisia annua* raw material on the global market (Sharma et al., 2011).

Scientific names: Artemisia annua L.

Vernacular names Chinese: Caohao, Cao Qinghao, Cao Haozi, Chouhao, Chou Qinghao, Haozi, Jiu Bingcao, Kuhao, San Gengcao, Xianghao, Xiang Qinghao, Xiang Sicao, Xiyehao

 English: annual wormwood, sweet wormwood, sweet annie

French: armoiseannuelle

Japanese: Kusoninjin

Korean: Chui-ho, Hwang-hwa-ho, Gae-tong-sook

Vietnamese: Thanh caohoavàng.

2.8.2. General morphology

Small capitula, inflorescence, racemose, capitate or paniculate, alternating leaves, seldom solitary, receptacle flat to hemispherical, sometimes hirsute and without scales, pappus absent, or occasionally a small scarious ring are listed as general morphological traits of the Artemisia genus (Heywood et al., 1977). It's an Asian annual herb, most likely from China. It grows naturally between 1000 and 1500 meters above sea level in northern Chahar and Suiyuan provinces in China. Many countries, including the United States, have naturalized it (Ajah & Eteng, 2010).

The violet or violet-brown stem is upright. *Artemisia annua* leaves are 3–5 cm long and feature two or three tiny leaflets that are split by severe incisions. The leaves have a strong aromatic smell. In dried leaves, artemisinin content ranges from 0% to 1.5 percent (Organization, 2006). *Artemisia annua* hybrids developed in Switzerland can contain up to 2% artemisinin in their leaves (Simonnet et al., 2006). The little flowers are grouped in loose panicles and have a diameter of 2–2.5 mm. Their color is a mix of green and yellow. Brown achenes with a diameter of only 0.6–0.8 mm are the seeds. Their thousand-kernel weight (TKW) is typically around 0.03 g (wheat has a TKW of about 45 g) (Organization, 2006). *Artemisia annua* is a tall shrub with alternate branches that can reach more than 2.0 meters in height and is normally single stemmed. Aromatic leaves with a length of 2.5 to 5 cm are extensively dissected. Both 10-celled biseriate trichomes and five-celled filamentous trichomes can be found on leaves and flowers (Patočka & Plucar, 2003).

2.8.3. Chemical constituents

Table 1: The chemical composition of *Artemisia annua* leaves (Iqbal et al., 2012)

Contents	Contents Amount (% dry weight basis)
Ash	7.5

Carbohydrate	8.3
Fat	6.07
Protein	18
Fiber	14.2
Moisture	11.4
Phytate	140.4
Tocopherol	2.74
Total Tannins	0.61

There are volatile and non-volatile elements in *Artemisia annua*'s chemical composition. Essential oils make up the majority of the volatile components, which range from 0.2–0.25%. Camphene, β-camphene, isoartemisia ketone, 1-camphor, β-caryophyllene, and β-pinene are the major constituents, accounting for roughly 70% of the essential oils. Other minor compounds discovered in the volatile portions of *Artemisia annua* include Artemisia ketone, 1, 8-cineole, camphene hydrate, and cuminal(Malik et al., 2009). Sesquiterpenoids, flavonoids, and coumarins, as well as proteins (such as β-galactosidase and β-glucosidase) and steroids (such as β-sitosterol and stigmasterol), make up the majority of the non-volatile constituents. Sesquiterpenoids such as artemisinin, artemisinin I, artemisinin II, artemisinin III, artemisinin IV, artemisinin V, and artemisinic acid are key chemical elements of *Artemisia annua* (Nofal et al., 2009).

In one study, nutritional composition and biological activities of leaves and stems of *Artemisia annua* were investigated. Moisture, crude lipid, and protein were higher in leaves, while crude fiber, ash, and mineral were more in stems. The proportion of phenols and flavonoids were two times more in leaves than stems (Turner & Ferreira, 2005). Leaves of *Artemisia annua* plant have 90.3% organic matter, neutral detergent fiber 23.3%, acid detergent fiber 12.8%, and artemisinin 1.4g/100g (Turner & Ferreira, 2005). Since its discovery, *Artemisia annua* L. has been used to treat a variety of illnesses in both animals and humans

2.8.4. Pharmacological activities of *Artemisia annua L*

Artemisia annua L is generally used for curing in different ailments in animals and humans since its discovery. Here discussed, some important pharmacological functions of this herbal plant.

2.8.4.1. Anti-hypertensive effects

According to research, feeding diabetic rats and rabbits an aqueous extract of various Artemisia species for a short period of time can cause blood levels to drop. This activity results in an increase in glycosylated hemoglobin levels. In addition, it protects animals from losing their body weight (Das, 2012).

2.8.4.2. Anti-microbial activity

Many investigations have shown that essential oils derived from Artemisia annua have significant action. With the exception of *Pseudomonas aeruginosa*, these essential oils have antibacterial activity against all microorganisms. According to certain studies, these oils exhibit the strongest antifungal action against *Saccharomyces cerevisiae* (MIC = 2 mg/ml) and *Candida albicans* (MIC = 2 mg/ml). The oil had a moderate inhibitory effect on Staphylococcus aureus and *Escherichia coli,* with MIC values of 32 mg/ml and 64 mg/ml, respectively. There was no action against *Pseudomonas aeruginosa* (MR et al., 2009).

2.8.4.3. Anti-oxidant Activity

According to the researchers, the leaves and inflorescences had the highest percentages of protein, crude fat, and in vitro digestible fractions, but the lowest levels of detergent fibers, based on experimental models on antioxidant capacity. It contains approximately 14% crude fiber, 6% lipids, and 8% protein. These tissues also have the most major elements, as well as manganese and copper, in their composition (Nofal et al., 2009). Their high antioxidant capabilities are complemented by their comparatively high amino acid and vitamin profiles, which indicate a favorable nutritional balance. *Artemisia annua* has a high concentration of minerals and antioxidants, making it a strong candidate for usage as a human herbal tonic or as a supplementary feed addition in cattle production systems (Saif, 2003).

2.8.4.4. Immunosuppressive activities

In traditional Chinese medicine, Artemisia annua has been used to treat autoimmune illnesses such systemic lupus erythematosus and rheumatoid arthritis. *Artemisia annua* has the ability to inhibit both cellular and humoral responses. *Artemisia annua* possesses immunosuppressive properties that can be used to treat autoimmune disorders (Sadiq, 2014).

Anti-malarial, anti-parasitic, and anti-inflammatory actions are just a few of the pharmacological properties of *Artemisia annua*.

2.9. Effects of *Artemisia annua L* in broiler diets

2.9.1. Growth Performance

The effects of *Artemisia annua* leaves on broiler growth appear to vary between trials. In a dose-dependent way, dried *Artemisia annua* leaves (0–20 g/kg diet) reduced feed intake and body weight, and 10–20 g/kg diet tended to enhance the feed conversion ratio. *Artemisia annua* leaves powder added at 5, 10, and 15 g/kg of meals resulted in enhanced daily body weight gain and a lower feed conversion ratio in chicks (Engberg et al., 2012). The variation could be explained by differences in *Artemisia annua* origin and dosage, feeding management, and animal species.

In other research, the decreased ADG (average daily gain) in 1–21 days of broilers fed a meal supplemented with 5 g/kg AL (*Artemisia* leaves) was linked to non-soluble fiber and lignin, both of which negatively affect broiler performance. Broilers at the beginning stage of development may have an incomplete digestive system, making them vulnerable to diet components (Cherian et al., 2013). Broilers fed a diet containing 1 g/kg EA gained more ADG in 1–21 and 1–42 days than control and 5 g/kg AL groups. This suggests that enzymatically treated *Artemisia annua* could be advantageous for improving broiler growth performance. Corn distiller's dry grains with with xylanase, improve broiler growth performance and nutrient digestibility.

The reason for this could be that enzymatic treatment breaks down plant cell walls, increasing intracellular contents' interaction with digesting medium, improving plant

materials' digestibility, and thus increasing nutrient and bioactive component absorption after ingestion.

2.9.2. Digestive Enzyme Activities

Medical plants may have stimulatory effects on pancreatic secretions such as digestive enzymes, which aid in the digestion and absorption of additional amino acids, monosaccharides, and fatty acids from the digestive tract, according to certain research (Demir et al., 2008; Mansoub, 2011). Protease digests protein into amino acids, whereas amylase digests carbohydrates by breaking them down into smaller units called disaccharides, which are then transformed into monosaccharides like glucose and fructose. Finally, lipase degrades triglycerides into glycerol and fatty acids, which are both key sources of energy in the body and precursors to vital chemicals.

The production of digestive enzymes from the pancreas in broiler chickens was enhanced by dietary feeding Essential oils derived from plants (Jang et al., 2004). In a study with hens, Jang et al. found that a blend of commercial EO components increased the activity and production of digestive enzymes such amylase when compared to a control group (William and Losa, 2001).

Polysaccharides from *Astragalus membranaceus* were found to increase the activity of intestinal digestive enzymes (amylase, lipase, and protease) in broilers in another investigation (Wu, 2018). However, more research is required to fully understand this mechanism.

Amylase, lipase, and protease are all key enzymes in the digestion of food. The effectiveness of probiotics on digestive enzymes has been reported in a variety of ways. *Saccharomyces boulardii* supplementation boosted lipase activity but had no effect on amylase or trypsin activity in the jejunum of broiler hens, according to Rajput et al. Probiotic *Bacillus coagulans* NJ0516 boosted protease and amylase activities but had no effect on lipase activities in broilers (Rajput et al., 2013). However, de Lima et al. found that adding the probiotic *Bacillus subtilis* to the feed had no effect on the activity of digestive enzymes in broiler chicks (Lima et al., 2003).

Thymol and carvacrol are also thought to have a variety of physiological benefits. Thymol has been shown to stimulate digestive secretions in humans, including salivary amylase, bile acids, gastric, pancreatic enzymes (lipase, amylase, and proteases), and intestinal mucosa, as well as intestinal mucosa in rats (Platel and Srinivasan, 2004). Broilers fed different blends of commercial essential oils showed a considerable increase in pancreatic trypsin, amylase, and maltase activity (Jang et al., 2007). Lee et al. (2003), on the other hand, found no discernible impact on enzyme activity in chickens fed dietary thymol and carvacrol after 21 or 40 days.

Heat stress also influenced intestinal absorption by changing the activity of digestive enzymes such lipase, trypsin, and amylase (Yi et al., 2016). The activities of jejunal lipase and trypsin were reduced after high temperature treatment in the current investigation, which was consistent with Yi et al. (2016), and Ruan and Niu's findings (2001). In addition, the HS group had lower jejunal amylase activity quantitatively than the control group, but the difference was not statistically significant. This difference in response could be linked to the degree of heat treatment as well as the species. Enzymatically treated *Artemisia* (EA) supplementation increased the digestive enzyme activities of the jejunum, as expected, showing that EA could help broilers under heat stress digest their food. There is currently no information on the impact of EA on the digestive enzyme activity of heat-stressed broilers. The enhancement of intestinal morphology and integrity by dietary supplementation of EA could be one explanation for the higher digestive enzyme activity.

2.9.3. Intestinal pH

Phytobiotics, also known as phytogenic feed additives, are plant-derived compounds that are added to animal feed to improve performance (Windisch et al., 2008). Acidification of the diet decreases the pH of the feed and digesta, which limits the multiplication of acid-intolerant entero pathogenic bacteria (Islam, 2012).

Acids' antibacterial function is linked to a drop in pH in the environment, which inhibits the growth of bacteria that are less resistant of acid pH. Furthermore, undissociated organic acids can easily enter bacteria and moulds' lipid membranes. Organic acids in

the cell release protons into the alkaline cytoplasm, resulting in a drop in intracellular pH. This disrupts enzymatic activities and the nutrition transport system, causing the bacterial cell to expend energy to release protons, resulting in an accumulation of intracellular acid anion (Jhones & Ricke, 2003). Improvements in digestive enzyme and microbial phytase activity (Jongbloed et al., 2000), increased pancreatic output, and promotion of gastrointestinal cell proliferation are also connected with acidification (Dibner and Buttin, 2002).

Birds' hindguts, particularly the cecum, are a primary colonization site for harmful bacteria. Because gastric acidity can be harmful to some of the food borne pathogens that live in the hindgut, keeping the pH of the hindgut and ceca low is critical for gut health (Jhones & Ricke, 2003). Changes in ileal and cecal digesta pH were seen in one study after feeding *Artemisia annua* to meat-type chicken. Dietary *Artemisia annua* is thought to lower the pH of the hindgut digesta and lipid oxidation products in white and dark meat (Cherian et al., 2013).

Khalaji et al. (2011) used dried *Artemisia sieberi* leaves as a top dressing in broiler feeds and found that the jejunal pH increased significantly. However, there is little information on the effect of *Artemisia annua* on pH modulation in the hindgut, which is the primary location of pathogen colonization in poultry. Because gastric acidity can be harmful to some of the food borne pathogens that live in the hindgut, keeping the pH of the hindgut and ceca low is critical for gut health (Jhones & Ricke, 2003).

Brisibe et al. (2008) investigated the efficacy of feeding 20% dried pulverized *Artemisia annua* leaves against *Eimeria tenella* in broiler and layer chickens, finding a decrease in lesion scores and oocyst in feces. A recent study found that feeding *Artemisia annua* to coccidiosis-infected free-range broilers resulted in a significantly lower amount of excreted oocysts (de Almeida et al., 2012).

In pigs, Straw et al. (1991) found that acidification of meals did not result in lower digesta pH but did increase growth performance, suggesting that lower gut pH is not always a response to diet acidification. The prebiotic properties of these additions may

aid in the proliferation of beneficial microorganisms and increased VFA production, lowering the pH of the digesta. When formic acid was supplied at 5 or 10 g/kg of feed in one investigation, the pH of the jejunum was not altered (Hernandez, 2006).

The pH of monogastric animals' diets is commonly reported to range between 5.5 and 6.5, and it varies when digesta passes through various GIT segments. As expected, digesta in the proventriculus is acidic, with the lowest pH in birds fed low *Moringa oleifera* leaf meal (MOLM) or negative control diets, and somewhat less acidic digesta in birds fed antibiotics and high MOLM levels, respectively. The pH of digesta drops steadily when it reaches the proventriculus, or glandular stomach, where hydrochloric acid and pepsinogen are released and combined with digesta through gizzard muscular contractions (Svihus, 2014).

2.9.4. Intestinal Microflora

For many years, essential oils from Artemisia spp. have been widely used for antimalarial, antibacterial, antiviral, nematicidal, and fungicidal applications (Ahameethunisa & Hopper, 2010). A great number of researchers from all around the world have looked into the effects of plant extracts on microorganisms (Juteau et al., 2002).

Antimicrobial activity of the essential oil derived from *Artemisia annua* cultivated in West Cameroon was found to be active against most isolates tested in a study by Désirée et al. The sizes of the inhibition zones ranged from 6 mm (*Pseudomonas aeruginosa* and *Shigella flexneri*) to 45 mm (*Pseudomonas aeruginosa* and *Shigella flexneri*) (*Vibrio cholerae*). In vitro cultivated plantlets of *Artemisia annua* can be employed as an alternate approach for producing artemisinin and its precursor with antibacterial activity, according to Appalasamy et al. (2014) and Verdian-Rizietal. in Iran and Li et al. (2003) in China tested *Artemisia annua's* antibacterial effectiveness against *S. aureus* and E. coli. *Artemisia annua* essential oil suppressed the growth of Gram-positive bacteria *Enterococcus*hirae and both tested fungi in a study done by Juteau et al (*Candida albicans* and *Saccharomyces cerevisiae*).

According to Lopes-Lutz et al. (2008), the oils of different *Artemisia* species inhibited the growth of bacteria such as *Escherichia coli, Staphylococcus epidermidis*, and *Staphylococcus aureus* to varying degrees. In another study, *Artemisia* reduced the populations of *coliform* and *E. coli* in the cecum of birds, but had no effect on the population of *Lactobacillus*. Several essential oils of *Artemisia* have been shown to have significant antibacterial action against bacteria, yeasts, dermatophytes, and *aspergillum* strains in previous studies (Janssen et al., 1987).

In another trial, extracts of *Artemisia annua* presented antimicrobial action against *C.perfringens*. Based on extraction with n-hexane and dichloromethane, the Minimum inhibitory concentration (MIC) values of *Artemisia annua* plant extracts were 0.185 mg/ml and 0.270 mg/ml, respectively. With a MIC value of more than 0.670 mg/ml, the methanol extract had the lowest antibacterial activity. The n-hexane extract showed the best antibacterial action in vitro against *C. perfringens* strain 200302-1-1-Ba, so it was chosen as a feed supplement in the subsequent broiler trials, where it was incorporated at a concentration of 250 mg/kg feed. (Engberg et al., 2012).The majority of research examining the antimicrobial activity of various essential oils against various bacteria agrees that essential oils are slightly more active against Gram positive bacteria than Gram negative bacteria (Brenes & Roura, 2010).

According to another research trial, at 35 and 42 days*, lactobacilli* populations in the intestinal and caecal contents were measured by adding *Artemisia annua* (essential oil and powder) to broiler diets. Ghazanfari et al. (2015) showed similar findings using *Artemisia sieberi* oil (300 mg/kg-1) administered to Ross 308 broilers (1-42 days). Khalaji et al. (2011) found that giving Ross 308 broilers 1 percent *Artemisia sieberi* leaves had no influence on the caecal *lactobacilli* populations. The rise in *lactobacilli* populations in the gut of broilers fed an *Artemisia annua* (essential oil and powder) diet kept the bacteria populating the broilers' gastrointestinal tract in check.

The essential oils have been shown to have antibacterial properties against Gram-negative bacteria such as *Campylobacter jejuni, Escherichia coli, Mycoplasma*

gallisepticum, *Mycoplasma synoviae, Pseudomonas aeruginosa, Salmonella enteridis, and Klebsiella sp(*Solorzano-Santos & Miranda-Novales, 2012).

Herbs and the essential oil derived from them have been shown to have antibacterial properties (Burt, 2004; zer et al.). Helander et al. (1998) established the antibacterial mode of action, concluding that the essential oil has the potential to dissolve the bacterial membrane, allowing membrane-associated substances from the cell to escape to the external medium. The results of this investigation demonstrated that antibiotic or essential oil treatments had no effect on the internal microbial population, which could imply optimal housing circumstances. Cross et al. (2007) found that adding oregano to a chicken feed had no influence on the populations of lactic acid bacteria, coliforms, anaerobes, and *Clostridium perfringens* in the intestinal microflora.

Kirkpinar et al. (2011) also found that oregano essential oil supplementation had no effect on total organism, Streptococcus, Lactobacillus spp., or Coliform counts in the ileum of broilers.

2.9.5. Gut Morphology

At hatch, the digestive system of chicks has an immature anatomy and functional capacity. Morphological and physiological changes to the GIT only take place after hatch, including a rise in the surface area for digesting and absorption (Panda et al., 2006).

According to research performed by Hong et. al., ileum of chicks fed with the antibiotic virginiamycin had the smallest total villous area, shortest villous height, and shallowest crypt depth (Hong et al., 2012). On the other hand, M. oleifera leaves have glutathione, which is essential for preserving mucosal integrity. Glutathione is a conjugate ingredient of glutamate, the most prevalent amino acid in blood (Rao & Samak, 2012).

According to one study, adding pulverized M. oleifera leaves to broiler diets up to 25 g/kg as a phytogenic feed addition had a noticeable favorable impact on intestinal morphology, digestive organ size, and pH (Nkukwana et al., 2015). According to Li et al. (2015), the deeper crypts and shorter villus resulted in decreased food absorption,

increased electrolyte and water production in the gastrointestinal tract, and decreased performance.

The negative effects of heat stress on broilers' intestines can be explained in a few different ways. Among these, heat stress triggers an inflammatory response in the gut and causes oxidative stress, which produces reactive oxygen species (ROS) and pro-inflammatory cytokines (Yi et al., 2016). However, as evidenced by the increased villus height, decreased crypt depth, and greater villus height-crypt ratio, EA supplementation could mitigate the intestine morphological damage of heat-stressed broilers.

In one study, the duodenum of birds treated with essential oils exhibited longer villus lengths, although the jejunum and illeum's villus heights and cryptal depths were unaffected. Sehm et al. (2007) found that foods high in flavonoids increased the height of intestinal villi in pigs. Michiels et al. found that broilers consuming carvacrol in the diet had smaller villus heights that were higher (Michiels et al., 2010).

In a different experiment, birds supplemented with n-hexane extract had lower scores for small intestinal lesions and lower counts of ileal and caecal C. perfringens, demonstrating that the dietary addition of *Artemisia annua* extract modifies the severity and course of the disease by delaying the onset of the illness and promoting a quicker recovery in the birds (Engberg et al., 2012).

The effects of additive supplementation on the villus height, crypt depth, and villus height-to-crypt depth ratio in the duodenum and jejunum were insignificant in different studies. According to Garcia et al., (2007), Awad et al., (2006) and Baurhooet al. (2007) supplementation with organic acids, a probiotic, and a prebiotic, respectively, significantly altered intestinal villus height. Longer villi were associated with improved performance in several studies. Dietary energy level had a substantial impact on the height of villus in the jejunum and the ratio of villus height to crypt depth. It follows that dietary energy can have an impact on intestinal shape. Broilers fed high-protein, low-energy diets exhibited longer villi than those on low-protein, high-energy diets, according to Yamauchi et al (1993) research

3. Materials and Methods

3.1. Material

3.1.1. Preparing of Farm

This study was carried out in unit of Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Kırıkkale University. Washing and cleaning of farm including disinfection of water tanks, disinfection of drinkers and feed utensils, cleaning and disinfection of floor and cleaning of surrounding areas of research unit were done one week before bird's arrival. Fumigation was done by using paraformaldehyde 10% for 8 hours, before 48 hours from birds' arrival. During fumigation, farm kept close for 24 hours. Infrared 200W bulbs installed in farm for maintaining temperature 33-34C for chicks' arrival.

3.1.2. Birds

In this study, 96 (Ross 308) newly hatched unsexed broiler birds were used as animal material. Birds purchased from a commercial hatchery were brought to poultry feeding unit of Department of Animal Nutrition and Nutritional Diseases. Birds were weighed one by one and divided into three groups. Groups were created as; 1) control group consumed basal diet (C), 2) antibiotic group consumed basal diet containing 5mg/kg *lincomycin* (L) (Naser et al., 2017) and 3) *Artemisia* group consumed basal diet containing 5g/kg *Artemisia annua L.* plant (Durrani et al., 2006). Each group consisted of 4 subgroups with 8 chicks each and a total of 32 chicks. The study was conducted after getting approval from Kırıkkale University Animal Experiments Local Ethics Committee (2021/10).

3.1.3. Feed Materials

For this study, a sample of *Artemisia annua L.* plant, in order to determine the plant species, delivered by Prof. Dr. Yusuf MENEMEN belong to, Biology Department, Kırıkkale University. Study samples were collected after plant species approval from Adapazarı district of Sakarya province, Turkey. Plants were kept in open place for drying. After 2 weeks of drying, plants were crushed by using grinder. Later on, for making equal particle size, plants were grinded again in small grinding machine. *Lincomycin* antibiotic having 99.5% was received from Net farma® Medicine and Premix Company, Turkey

Firstly, all birds fed with starter diet in 0-21days. After that, chickens divided into three groups. Control group fed basal finisher feed while antibiotic and plant group fed basal diet with 5mg/kg *Lincomycin* and 5g/kg *Artemisia annua L.* plant, respectively.

3.2.   Methods

3.2.1.   Care and management of birds

The study continued for 42 days and during study. Birds of all groups received *ad-libitum* feed and water. During the study, feeding was provided with hanging feeders and water with nipple drinkers. The house temperature was maintained 34ºC at day 0 and then decreased 2ºC each week, gradually. For heating system, electric heaters and infrared 250W bulbs were used. Light was provided unstoppable for 24 hours, each day. Ventilation was managed by automatic fan.

3.2.2.   Proximate analysis of feed and plant extract

3.2.2.1.   Determination of Dry Matter

A clean petri plate was taken and weighed then feed sample added on it and was weighed again. Later petri plate was kept in hot air oven for 2 hours at 135ºC. Then it was removed and placed in desiccators for cooling. After cooling, final weight was weighed and noted. Moisture level was determined by putting values in formula.

$$\textbf{Moisture \%} = \frac{\text{Weight of Plate with Dried Sample} - \text{Weight of Empty Plate}}{\text{Weight of Plate with Moisture Sample}} * 100$$

3.2.2.2. Determination of Ash

A clean porcelain crucible was taken, weighed and tare. Sample was added, weighed again, and its weight was noted. After that crucible were put in furnace at 600ºCfor 6 hours. Then crucible was taken out and left in desiccator for cooling. After cooling, final weight weighed and noted. Value was added in following formula for checking ash level in samples.

$$\textbf{Ash, \%} = \frac{Weight of Crucible with Ash - Weight of\ Empty Crucible}{\text{Weight of Plate with Dried Sample}} * 100$$

3.2.2.3. Determination of Crude Fiber

Crude fiber is known as the part of carbohydrate in food called non-soluble carbohydrate (Insoluble carbohydrates), which It's not digested by the digestive juices and do not degrade at the treatment by (acids and bases) diluted and in specific concentrations for a period of time is limited.

Method
- Weighed out 2 to 3 g of defatted, dry sample.
- Placed in the flask and added 200 ml boiling Sulphuric acid solution concentration 1,25).
- Added (50 ml) of acid and (150 ml) of distilled water until the concentration reduces.
- Attached the condenser and brought to boiling point in one minute.
- Boiled for exactly 30 minutes, maintaining the volume of solution constantly.
- Lined the Buchner funnel with the filter paper and boiling water.
- At the same time, at the end of the boiling period, flask were removed, rested for one minute and filtered the contents using suction or vacuum.
- Washed the filter paper with boiling water.
- Transferred residue to the flask using a retort containing 200 ml of boiling NaOH solution and boiled for 30 minutes same like acid.

- Preheated the filtration crucible with boiling water and carefully filtered the hydrolyzed mixture after letting it rest for 1 min.
- Washed the residue with boiling water, with the HCI solution and then again with boiling water, finishing with three washes
- Placed the crucible in oven set at 105°C for 12 hours then cooled in dryer.
- Quickly weigh the crucible with the residue inside and placed in the crucible furnace at 550° C for 3 hours. Leaved to cool in a dryer and weighed again.

  Calculations;

  Crude fiber content % = 100 (A-B/C)

  Where:
- A = weight of crucible with dry residue (g)
- B = weight of crucible with ash (g)
- C = weight of sample (g)

3.2.2.4. Determination of Crude Protein

One gram sample was taken in digestion flask and 15.4gram digestion mixture was weight and added. Then 30ml commercial H_2SO_4 were added and flask placed on heater for 2.5hours. Flask was left for cooling and then distill water was added slowly until to volume 250ml. Then 10ml diluted volume was taken in distillation unit and 10ml 40% NaOH was added. After heating, titration was done against 10ml 2% Boric Acid solution in a 100ml beaker. After making volume to 40ml, titration was done against 0.05N H_2SO_4until color turned pink. Reading was noted and CP was measured by putting values in following formula.

$$\textbf{CP} = \frac{Reading*10.9375}{SampleWeight} *100$$

Here 10.9375 is a constant factor for 0.05N H_2SO_4 solution, which is obtained by following formula;

$$\textbf{CP Factor} = \frac{0.014*0.05*100*6.25*250}{10}$$

Where,

- 0.014= Atomic Weight of Nitrogen
- 0.05= Normality of used Sulfuric Acid
- 6.25= Nitrogen in Protein
- 10= Used Sample

3.2.2.5.   Determination of Crude Fat

Brazilian filter paper was taken and weighed. Then 3g sample was added, and sample was folded in Brazilian filter paper and stapled in tweeze form. Then it was kept in Soxhlet tube for 3 hours. After that,hexane was added above the level of internal columns. Due to movement of hexane oil emerged and moved in bottom tube. After 3 hours, crude fat was separated and collected in cylinder. Then it was kept in oven for 30minutesat 135ºC. After that, it was left for cooling in desiccators. At the end, weight was noted and crude fat amount was determined by using following formula:

$$\text{Ether Extract, \%} = \frac{Weight\,of\,beaker\,with\,crude\,fat - Weight\,of\,empty\,beaker}{Sample\,of\,weight} * 100$$

3.2.3. Determination of Body Weight and Weekly Body Weight increase

Chicks were divided by weighing at 0, 7, 14, 21, 28, 35, 42nd days. Weights of chicks were determined by weighing balance-having sensitivity 1g on first week and later on with 5g sensitivity weighing balance. Then, increased in body weight gain for all groups at each week were checked. Average body weight gains were determined by calculating the difference between the average body weights obtained on a weekly basis and the body weight averages of the previous week (Wan, 2017).

3.2.4. Determination of Feed Consumptions and Feed Conversion Ratios

Feed were provided with bucket to each subgroup. Every week, feeds were placed in all buckets and at the end of week; weights of remaining feeds were weighed separately for each subgroup. Feed consumptions were determined by calculating differences between given feeds and remaining feeds. Feed consumption for each chick was determined by calculating average of total feed consumption of related sub group. Feed conversion

ratio (FCR) for each subgroup was calculated by dividing average feed consumption by average body weight gain at each week.

$$\textbf{FCR} = \frac{\text{Average feed consumption}}{Average body weight gain}$$

3.2.5. Slaughtering Process

After body weights of all the animals were weighed on the 42nd day of the experiment, three animals from each subgroup were randomly separated and their pre-slaughter weights were determined. At the beginning of the slaughtering process, blood was taken from the vena jugularis for digestive enzyme activity analyzes. After then, slaughter of the animals was done by decapitating the broilers and separating them from their bodies. After slaughter process, their abdomens were cut along the midline, and duodenal feces were taken out for intestinal pH measuring. Ceca contents were taken to determine the intestinal microflora status.

3.2.6. Digestive Enzyme Activities

Alpha-amylase and lipase were tested using the respective kit (Alpha-amylase, Lipase; Otto Scientific), and enzymatic activity was assessed using an enzymatic colorimetric method in accordance with the manufacturer's instructions.

Figure 2: Digestive enzyme kit.
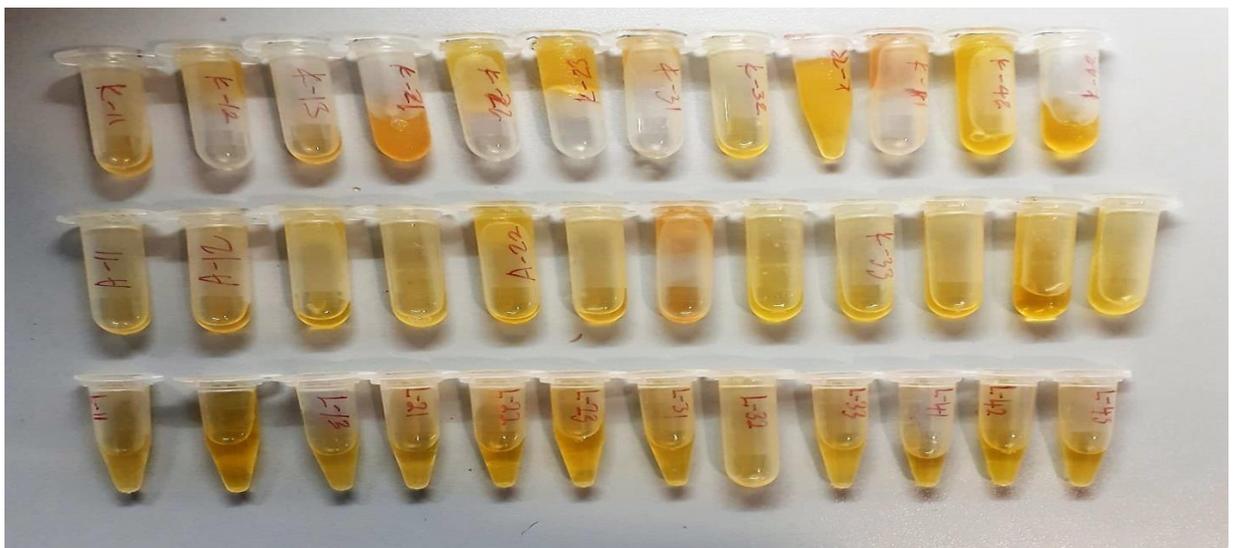
1. LİPASE TEST PROTOCOL:

Lipase measurement was done with the Otto scientific kit.

Test Principle

This method is based on the cleavage of a specific chromogenic lipase substrate 1,2- O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester emulsified with bile acids. The pancreatic enzyme activity is determined specifically by the combination of bile acid and colipase used in this assay. The chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6- methylresorufin) ester is cleaved by the catalyticaction of alkaline lipase solution to form 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaricacid-(6- methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methyl resorufin. The colour intensity of the red dye formed is directly proportional to the lipasea ctivity and can be determined photometrically.

Components:

Each reagent ready use.

R1: Buffer/ Colipase/ Cholate BICIN buffer Colipase. Na-deoxycholate calcium chloride

R2: Emulsion / ChromogenicSubustrate / Cholatetartrate buffer, 1,2-o-dilauryl-rac-glycerol 3 glutricacid-(6-methyl resorufin), taurodeoxycholated etergent preservatives.

Sample working:

Samples; biochemical analysis of the Lipase activity made with using BS400 automatic analyzer.

Stepstoworking:

- Add 200 ul of reagent 1 into the cuvette. And than add 4 ul of sample into the path cuvette, mix fully.
- Incubate at 37 °C for 5 min.
- Add 50 ul of reagent 2 into the cuvette, mix fully.
- Mix, read initial absorbance after 2 minute. Read absorbance (580 nm) again after 3, 4 and 5 min.

- Calculate A/min.

2. α-AMYLASE TEST PROTOCOL:

α-AMYLASE measurement was done with the Otto scientific kit.

Test Principle:

These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically and amyloclastically or measure the formation of degradation products saccharogenically or kinetically with the aid of enzyme- catalyzed subsequent reactions. The kinetic method described here is based on the cleavage of 2-chloro-4-nitrophenyl-aDmaltotrioside (CNP-G3) by a-amylase. Colorimetric test with 2-chloro-4-nitrophenyl-a-D-maltotriose (CNP-G3) as direct substrate. Colour is released directly as a result of a cleavage at the aglycone.

The increase of absorption of chloro-nitrophenol is directly proportional to the a-amylase concentration. The hydrolysis pattern in the formulation of the reagent show about less than 10 % CNP-G2 andlessthan 1% CNP-G4 as byproducts.

Component:

Each reagents or standard ready use.

Reagent1:   MES buffer, Nacl, Ca-Acetate, Potassiumthiocyanate CNP-G3, Stabilizers and Detergents.

Sample working:

Samples; biochemical analysis of the α-AMYLASE made with using BS400 auotomatic analyzer.

Steps to working:

- Add 150 ul of reagent 1 into the path cuvette. And than add 18ul of sample into the path cuvette, mix fully.
- Incubate at 37 °C for 1 min.
- Measure OD values 412nm.

3. Rat Trypsin Elisa Assay Procedure

- Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

- Add 50 ul standard to standard well. (Dontaddbiotinylated antibody because the standard solution contains biotinylated antibody).

- Add 40 ul sample to ample wells and then add 10 ul anti-TRP antibody to sample wells, then add 50 ul streptavidin-HRP to sample wells and standard wells. (Not blank control well).

- Mixwell.Cover the plate with a sealer. Incubate 60 minutes at 37 °C.

- Remove the saler and wash the plate 5 times with wash buffer. (Automatedwashing).

- Add 50 ul subsrate solution A to each well and then add 50 ul subsrate solution B to each well.

- Incubate plate covered with a sealer for 10 minutes at 37 °C in the dark.

- Add 50 ul Stop solution to each well, the blue color will change into yellow immediately.

- Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

3.2.7. Determination of Intestinal pH

For pH determination, fecal sample were collected from duodenum in sterile cup from 24 animals belong to 3 groups (Control, Antibiotic, Plant extract) and immediately stored at -20ºC. Next day, sample were removed from refrigerator and kept for thawing. After then, samples were mixed homogeneously by porcelain mortar. Each sample was diluted until5-foldwith distilled water and mixed at vortex until homogenous. Then digital pH probe (LaMotte, modelpH5) was placed inside the cup having digesta sample and pH were recorded for each sample (Cherian et al., 2013; Martinez et al., 2019).

3.2.8. Determination of Intestinal Microflora

The mediums used in microbiological analyses and their preparation

a) Plate Count Agar

Overall, 8.75 g of the ready-made medium was weighed and dissolved in 500 ml of distilled water, and after the pH value was adjusted to 7.0±0.2, it was sterilized in an autoclave at 121ºC for 15 minutes. After the medium removed from the autoclave was cooled to 50ºC, it was poured into sterile petri dishes.

b) MacConkey Agar

Overall,25 g of the ready-made medium was weighed and dissolved in 500 ml of distilled water, and after the pH value was adjusted to 7.1±0.2, it was sterilized in an autoclave at 121ºC for 15 minutes. After the medium removed from the autoclave was cooled to 50ºC, it was poured into sterile petri dishes.

c) Violet Red Bile Agar

Overall,19.75 g of ready-made medium was weighed and dissolved in 500 ml of distilled water and poured into sterile petri dishes after the pH value was adjusted to 7.4±0.2.

d) de Man Rogosa and Sharpe Agar

34.1 g of the ready-made medium was weighed and dissolved in 500 ml of distilled water, and after the pH value was adjusted to 5.7±0.2, it was sterilized in an autoclave at 121ºC for 15 minutes. The medium, which was removed from the autoclave, was cooled to 50ºC and poured into sterile petri dishes.

At the end of the trail, 36 birds that belong to control, antibiotic and plant group were slaughtered and fecal samples from ceca for microbial analysis were collected in sterile cups. Samples freeze at -20º C temperatures immediately for microbiological examination. On the day of microbiological examination, samples removed out from refrigerators for melting. After melting, two grams of fecal samples were homogenized with 18 ml peptone water (0.1%, CAS 91079-38-8, Merck, Germany). Subsequently, ten-fold serial dilutions were prepared in peptone water, and 0.5 ml of first, third, fifth

and seventh dilutions were inoculated on specific media: double-layered Plate Count Agar (PCA, 105463, Merck) for total anaerobic bacteria, Violet Red Bile Lactose Agar (VL, 101406, Merck) for *coliform* bacteria, de Man Rogosa and Sharpe Agar (MRS, 110660, Merck) for Lactobacillus spp., and MacConkey Agar (MC, CM0007, Oxoid) for *Escherichia coli*. PCA was incubated for 48 hours at 37°C anaerobically, VL and MC were incubated under aerobic conditions for 24 hours at 37°C. Additionally, MRS was incubated at 30°C for 48-72 hours anaerobically. After incubations, plates were counted and the differences between control, antibiotic and plant groups were analyzed statistically. Results were expressed as $\log^{10}$CFU/g cecal digesta (Oso et al., 2019).

3.2.9. Determination of Intestinal Morphology

Intestinal samples taken from 42-day-old chickens that underwent necropsy were cut 30 cm behind the cecum and ileum segments of the intestine were taken. The ileum segments were taken into a 10% buffered paraformaldehyde solution without break and waited for the detection process for 48 hours. After the process, 4 samples were taken from different parts of the ileum with 1 cm intervals and taped. Then they were washed for 12 hours under running tap water. During routine tissue tracking, the samples were processed with different concentrations of alcohol (50º, 70º, 80º, 90º, 96º and 99.5º) and also processed in xylol series for two hours. After the processes, it was embedded in paraffin and blocked. Then, the paraffin blocks were trimmed with a 25µm microtome. After the tissues began to appear in paraffin sections, 4 sections with a thickness of 5 µm were taken into the decays 1 at a time. In order for the paraffin on the lamellas to melt and for the section to be fully adhered, it was incubated overnight in a hot environment (37ºC). Then, the following steps were followed for the routine hematoxylin-eosin (HE) staining;

Deparaffinization: Tissues were passed through 3 xylols for 10 minutes each.

Dehydration: The deparafinized tissues were passed through different concentrations of alcohol (absolute, 96º, 90º, 80º, 70º, 50º) for 3 minutes series respectively.

Rehydration: It was soaked in distilled water for 2 minutes.

Hematoxylin Stage: Tissues were immersed in hematoxylin and kept for 6-7 minutes.

Washing: The tissues removed from the hematoxylin were washed under running tap water for about 5 minutes.

The Eosin Stage: The tissues were taken into eosin and left for 1 minute.

The Stage of Output Alcohols: To remove excess eosin from the tissues, the tissues are passed through different concentrations of alcohol (absolute 50º, 70º, 80º, 90º, 96º) in the same way as in the dehydration stage but in the opposite direction. In the first alcohol series, it was kept for 1 minute each and in absolute alcohol; it was kept for 5 minutes.

The Stage of Xylol Output: After the alcohol of the tissues taken from the output of alcohol was thoroughly anesthetized, it was taken to Xylol and passed through 3 xylols for 5 minutes.

Adheretion: The tissues, which were in xylol, were taken one by one and after 2 drops of synthetic resin (Entellan) were dripped on them, they were closed with the lamel, left to dry afterwards (Slaoui et al., 2011).

After drying, examination and measurement of villous height, villous surface area and villous number were done by stereo investigater.

Figure 3: Calculation of villi numbers with the Fractionator.
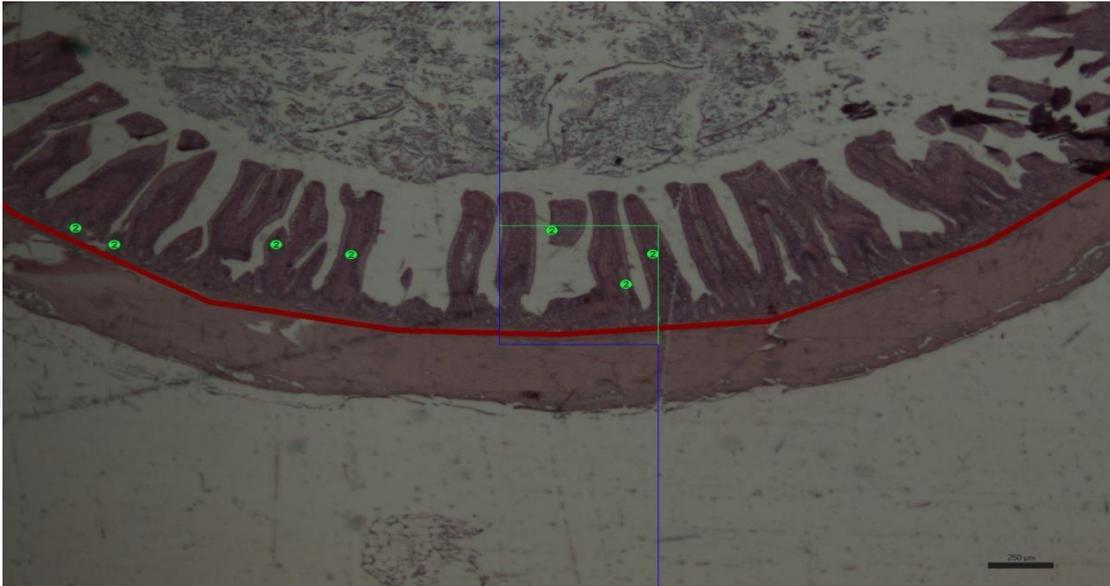
The area of interest was divided into sub-areas of 1 mm^{2}and 1/3 sampling was applied and the number of villi was estimated by counting the villi hitting the sampled areas. One side of the neutral counting frame in the figure is 0.06 mm^2 and its area is 0.36 mm^2. The green numbers in the figure represent the villi within the neutral counting frame and hitting the countable lines (green color). Bar = 250 μm.

Figure 4: Measurement of surface areas of villi by isotropic Fakir method.

Line Separation 500.00 (µm) and Volume Per Unit Length of Probe 250000 (µm³) were used to calculate the villi surface areas. Since the isotropic probe has 3 radial lines in the isotropic x, y and z axis, the data obtained are completely unbiased. While using the relevant probe, the intersection areas of the villi surface and the lines of the probe were taken as basis and calculations were made.

Figure 5: Measurement of villous heights with the help of line tool in stereo investigator.
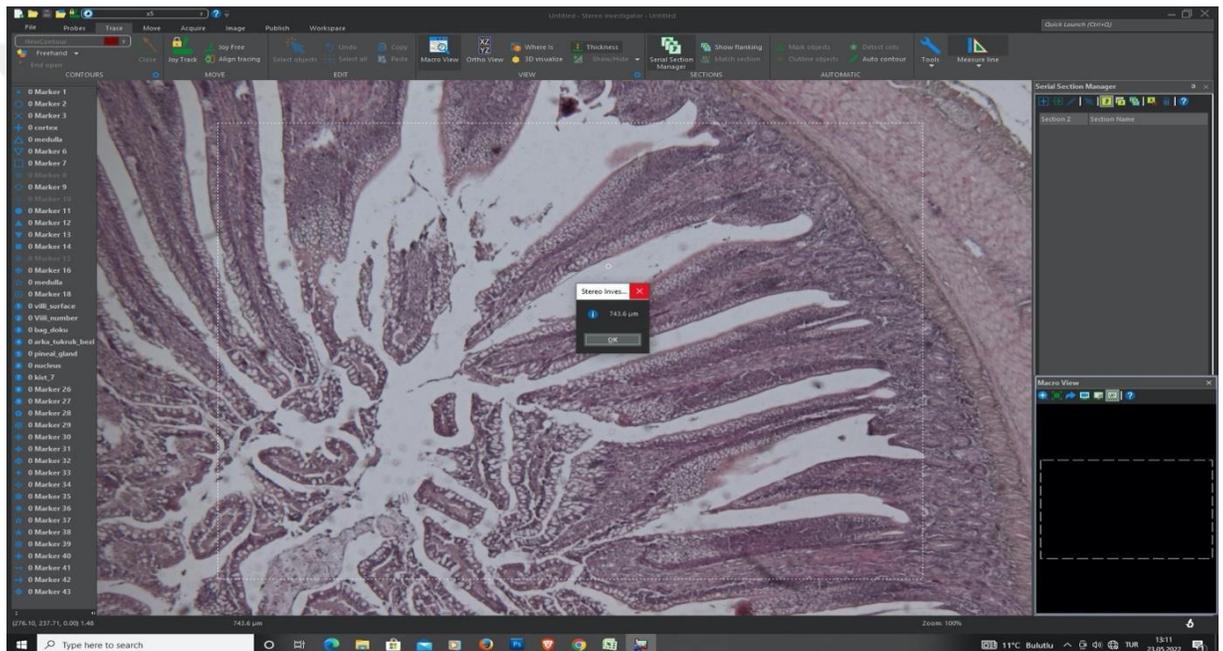
The height of the villi was calculated in the villi that were counted during the villi count. This calculation was done with the line tool from the villous tip to the villous base (crypts were ignored).

3.2.10. Determination of Mortality Rates

The deaths occurred during the experimental study were recorded daily and at the end divided by the total number of animals in the study. Mortality % was calculated by using following formula;

Mortality % = (Number of dead birds / Total number of birds) * 100

3.2.11. Statistical Analysis

All data obtained in the study were subjected to one-way analysis of variance (One-Way ANOVA) using SPSS statistical package program (SPSS 15.0 for Windows). Differences among the study groups were determined with Duncan test and the $p < 0.05$ values were considered statistical significance.

4. RESULTS

Nutrient content and metabolizable energy values of rations used in research trial are given in table 3. There are two types of rations including starter and finisher feed were used in research trial. Starter feed has 23.54 % CP and 3100 Kcal ME while finisher feed has 20 % CP and 3200 Kcal ME.

Table 2: Nutrient compositions of starter and finisher diets

Nutrients	Starter	Finisher
Crude Protein, %	23.54	20.00
Cellulose, %	2.72	2.55
Fat, %	4.78	6.65
Ash, %	6.67	6.20
Methionine, %	0.69	0.58
Calcium, %	1.07	1.02
Sodium, %	0.18	0.17
Phosphorous, %	0.63	0.61
Lysine, %	1.49	1.22
Monensin, mg/kg	0.10	0.1
Metabolizable energy, kcal/kg	3000	3150

4.1. Body Weights and Body Weight Gains

The body weight changes observed in chicks during the trial period are given in Table 4. At the start of research trial, body weights of birds were weighed and homogeneous distribution was provided between groups. In 2nd and 3rd weeks of study, among body weights of all groups were statistically similar (P>0.05), but body weights of Antibiotic and *Artemisia* groups numerically higher as compared to control(P> 0.05). At the end of research trail, body weights of control, Antibiotic and *Artemisia* groups *were* 2668.33, 2683.50 and 2752.25 g respectively (P>0.05).

Table 3: Weekly average body weights of control and experimental groups, g

Weeks of trial	Control	Antibiotic	*Artemisia*	P
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	
0	1023.62 ± 7.75	1023.47 ± 9.87	1031.72 ± 8.69	0.751
1	1636.44 ± 23.14	1648.31 ± 23.57	1646.88 ± 21.38	0.922
2	2190.63 ± 29.81	2224.34 ± 34.82	2236.13 ± 33.34	0.596
3	2668.33 ± 26.51	2683.50 ± 36.83	2752.25 ± 37.11	0.190

The weekly live weight increase observed in chicks during the trial period is given in Table 5. In the first week of study, there was no significant difference among body weight gains of groups (P>0.05). Body weight gain of antibiotic group was the highest with 624.89among all groups. In 2nd and 3rd weeks of study, among body weight gains of all groups were statistically similar (P>0.05). On the other hand, in 2nd week body weight gains of Antibiotic and Artemisia groups numerically higher as compared to control, in 3rd week only the Artemisia group was higher numerically compared to control. In 3rd week, body weight gains of all groups were lower than other weeks. There was no difference between the groups at the overall period (P>0.05), but only in the Artemisia group was observed a remarkable numerical increasing.

Table 4: Weekly average body weight increases of control and experimental groups, g

Weeks of trial	Control	Antibiotic	*Artemisia*	P
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	
1	612.81 ± 21.38	624.84 ± 7.59	615.15 ± 32.13	0.925
2	554.15 ± 27.19	576.03 ± 12.09	589.25 ± 16.11	0.470
3	477.65 ± 27.74	459.12 ± 18.59	516.11 ± 20.32	0.244

1-3	1644,62 ± 8.50	1659.99 ± 23.38	1720.52 ± 48.88	0.253

4.2. Feed Consumptions and Feed Conversion Ratios

The weekly feed consumption of birds during research trial is showed in table 6. Feed consumption increased in 2nd week of trail for all groups but decreased in final week. In 1st and 3rd week feed consumption of Artemisia group was numerically higher compared to other two groups while in 2nd week feed consumptions of antibiotic and *Artemisia* groups were higher than control group. At the overall period, feed consumption of *Artemisia* group was greater than control and antibiotic groups but difference not significant statistically (P> 0.05). Overall feed consumption during research days for Control, Antibiotic and *Artemisia* group were 2889.18g, 2873.23g and 2917.87g, respectively.

Table 5: Weekly average feed consumption of control and experimental groups

Weeks of trial	Control	Antibiotic	*Artemisia*	P
	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	
1	918.90 ± 15.31	892.65 ± 6.63	927.31 ± 31.2	0.488
2	979.19 ± 27.83	1014.59 ± 19.48	1010.75 ± 31.65	0.608
3	991.08 ± 18.56	965.96 ± 16.49	979.80 ± 10.60	0.544
1-3	2289,18 ±13,39	2873,23 ± 10,36	2917,87 ± 66,72	0.731

The weekly feed conversion ratios of groups are showed in table 7. There was no statistically difference (P>0.05) among groups FCR during the experimental period, but

numerically differences were observed among groups. Except 1st week of trial, *Artemisia* group determined best FCR at 2nd and 3rd week as compared to control and antibiotic groups. At the overall period, for control, antibiotic and *Artemisia* groups FCR values were 1.76, 1.73 and 1.70, respectively, and no difference was determined among all groups (P>0.05).

Table 6: Weekly feed conversion rates of control and experimental groups

Weeks of trial	Control	Antibiotic	*Artemisia*	P
	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	
1	1.50 ± 0.30	1.42 ± 0.11	1.49 ± 0.36	0.125
2	1.77 ± 0.46	1.76 ± 0.37	1.71 ± 0.34	0.568
3	2.09 ± 0.87	2.11 ± 0.64	1.90 ± 0.68	0.155
1-3	1.76 ± 0.01	1.73 ± 0.02	1.70 ± 0.03	0.152

4.3. Digestive Enzyme Activity of Control and Experimental groups

Data about digestive enzyme activity (Amylase, Lipase, Trypsin) of control and experimental groups is mentioned in table no. 8. There was no statistical difference among groups (P> 0.005), but numerically difference was present. For Amylase, *Artemisia* group showed maximum count (405.08 ± 26.41) as compared to control (364 ± 16.28) and antibiotic group (337.75 ± 13.99). In case of Lipase, antibiotic group had (23.78 ± 1.11) maximum count as compared to control and *Artemisia* group while *Artemisia* (21.15 ± 0.69) and control (23.10 ± 1.25) showed almost similar amount in blood. For Trypsin, again antibiotic group showed higher count (139.82 ± 21.16) then control (103.43 ± 12.05) and *Artemisia* group (114.34 ± 9.25).

Table 7: Digestive Enzyme ((Amylase, Lipase, Trypsin) Counts in Blood

	Control	Antibiotic	*Artemisia*	Significant Value
Enzymes (U/L)	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	

Amylase	364 ± 16.28	337.75 ± 13.99	405.08 ± 26.41	0.450
Lipase	23.10 ± 1.25	23.78 ± 1.11	21.15 ± 0.69	0.245
Trypsin	103.43 ± 12.05	139.82 ± 21.16	114.34 ± 9.25	0.560

4.4. Intestinal pH values of control and experimental groups

Data about intestinal pH of groups is showed in table 9. There was no statistical difference observed among groups (P>0.05). Antibiotic group has lowest pH among all groups. It was followed by control and antibiotic groups, respectively.

Table 8: Intestinal pH of control and experimental groups

	Control	Antibiotic	*Artemisia*	P
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	
pH	6.04 ± 0.31	5.99 ± 0.42	6.07 ± 0.73	0.582

4.5. Intestinal microflora of control and experimental groups

Intestinal bacterial counts determined within the study are given in table 10. In intestinal microflora, colonies of four bacteria item including *Coliform, E. coli, Lactobacillus spp.* and total anaerobic bacteria were counted. There was no statistical difference (P> 0.05) observed in the number of *coliform* bacteria among groups. But control group has higher numerically amount of *coliform* bacteria as compared to research group. Amounts of *E. coli* bacteria have statistically significant difference among groups (P<0.05). *E. coli* count was lowest in antibiotic group whereas it was maximum in control group. In case of *Lactobacillus spp.*, there was no difference statistically among groups (P>0.05) but bacterial count was numerarically higher in *Artemisia* group. In the number of total anaerobic bacteria, statistical difference was present among experimental and control groups (P<0.05). Antibiotic group has lowest anaerobic bacteria count while control group shows maximum anaerobic bacteria counts.

Table 9: Intestinal bacterial counts of control and experimental groups (log cfu/g)

	Control	Antibiotic	*Artemisia*	P
	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	
Coliform	5.62±0.39	4.92±0.36	4.94±0.32	0.305
E. coli	6.85±0.38	4.82±0.26	5.85±0.50	**0.000**
Lactobacillus spp.	5.28±0.36	4.96±0.26	5.48±0.32	0.510
Total anaerobic bacteria	7.60±0.25	5.70±0.16	7.01±0.30	**0.006**

4.6. Intestine Morphology

Readings of control and experimental groups regarding intestinal morphology are mentioned in table no. 11. In case of villous height and villous no there was no statistical difference in all groups (P> 0.05) but numerical difference was present. While in case of villous surface area statistical difference present among groups (P< 0.05). Villous height was higher in *Artemisia* group (686.86 ± 21.07) as compared to control (632.33 ± 19.42) and antibiotic (632.33 ± 19.42) group. Similar results were found in case of villi no. as *Artemisia* group had maximum villous no (44.49 ± 4.50) as compared to control (42.53 ± 2.87) and antibiotic (40.25 ± 2.00) groups.

Antibiotic group showed statistical difference in case of villous surface area (P=0.006) as compared to other two groups.

Table 10: Intestinal Morphology Parameters

	Control	Antibiotic	Artemisia	P Value
Parameters	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	$\bar{x}\pm S\bar{x}$	
Villous Height (μm)	632.33 ± 19.42	632.33 ± 19.42	686.86 ± 21.07	0.580
Villous No.	42.53 ± 2.87	40.25 ± 2.00	44.49 ± 4.50	0.650

Villous Area (mm²)	15142.97± 1739.87	7819.82± 141.78	14264.42± 1480.95	**0.006**

4.7. Mortality rates of control and experimental groups

Data on death cases seen during the trial period are presented in Table 12. No mortality was seen among all groups.

Table 11: Mortality rate of control and experimental groups by weeks

Weeks of trial	Control	Antibiotic	*Artemisia*
1	0	0	0
2	0	0	0
3	0	0	0

5. Discussion:

Due in part to their biological characteristics, such as their antibacterial and antiseptic actions, a mixture of essential oils and herbal extract has been developed for use as antibiotic alternatives in the animal business. There is, however, little research that evaluates the potential use of *Artemisia annua L.* plant extract in place of antibiotics in the production of broiler chickens. In this experiment effect of particular supplementation to the birds or blend with antibiotics or phytobiotics over different parameters including intestinal pH, intestinal microflora, FCR and growth performance as well as the nutrient consumption responses in the broiler chickens were checked.

One of the major parameters of this study was growth performance among the group birds. The increase in weekly live weight that has been observed in the chicks during the trial period depicts the growth of birds has been increased markedly when they were offered treatment with antibiotic as compared to control group with zero addition or with *Artemisia* group, which is in accordance with the findings of Miles et al. (2006); Libby and Schaible, (1955) and Calik et al. (2009).During the very first week of the trial, body weight increase was maximum numerically for antibiotic group as assessed to control and *Artemisia annua* group but there was no significant difference among groups. During the 2nd and the 3rd week of trial, *Artemisia annua* group gained more weekly increase in live body weight gain rather than that of control and the antibiotic groups but the increase was indicated as numerically and there was no statistical difference among group. Weekly live body weight decreased from 4th to 6th week in all groups. Our results are in line with Kharde et al. (2012), who also reported the decrease in body weight from 4th to 6th week. Decreased in live body weight observed may be due to space problem. The above-mentioned values depict that at the end of the research trail, overall the weight gain has been decreased but there was a marked increase in weight gain in the *Artemisia* group as compared to the other two i.e. control and antibiotic which is in accordance with the findings of Jahan et al 2006.

In 1st and 3rd week of the treatments the *Artemisia* group consumed more feed as compared to other two groups while in 2nd week antibiotic group feed consumption was little bit higher and the similar findings were reported by Brisibe et al. (2008). *Artemisia* group overall feed consumption were greater than control and antibiotic groups, but the difference was of being not significant statistically. Increase in the feed consumption of *Artemisia* group concluded that might be *Artemisia annua* has positive effects on feed consumption of broiler but it is not significant. The non-significance here reveals that statistically it was same among all groups and the feed was consumed regardless to the treatment and it had neither affected the texture of feed nor its flavor. The findings of our study were also supported by previous studies such as Khaksafidi (2006) Sarangi et al. (2016); Rahimi and and Bagal et al., (2016).

Numerically differences were observed among groups of different treatments in this research. Except 1st week of trial, *Artemisia* group had showed best FCR at 2nd and 3rd week when compared with control and antibiotic groups which is similar to the findings of Gholamrezaie et al (2013). Results also showed that inclusion of *Artemisia annua* in broiler diet has positive but non-significant effects on FCR of the birds. Our results are supported by Engberg et al. (2012), who found positive but non-significant effect of adding *Artemisia annua* in broiler diet. Average FCR at the end of research trial for control was 1.77, antibiotic 1.76 and Artemisia groups were 1.70.

When Feed Conversion ratio which is also said to be as FCR has been determined by regular weighing the birds, the results were depicting no significant results between treatments and even in the control group as the amount of feed that has been offered was not only the same in all groups but also had been evenly fed by the birds of all three groups.

At day 42, digestive enzymes activitiy (Amylase, lipase, trypsin) were checked. There was no statistical difference among groups but numerically difference was present among groups. In case of Amylase, *Artemisia* group showed maximum count as compared to control and antibiotic group. Numerical increase may be due to agonistic effects of *Artemisia* on amylase. In case of lipase and trypsin, Antibiotic groups showed higher values as compared to *Artemisia* and control groups. There is less information

available about effects of *lincomycin* and *Artemisia* on broiler digestive enzyme performance so exact reason is unclear but possible reason may be due to improvement in nutrient utilization by using *lincomycin* and *Artemisia.*

On the other hand**,** Song et al., (2018) observed statistical differences among groups while used enzymatically treated *Artemisia annua L* for heat stressed broilers. Generally heat stress is responsible for decrease in digestive enzyme function due to impaired digestive activities and changes in gut morphology (Yi et al., 2016). According to Song et al., (2018), when bird treated with EA, digestive enzyme performance increased even during heat stress. It may be due to beneficial effects of EA on broiler gut morphology.

Popović et al., (2016) observed both statistical and numerical differences when he treated broiler birds with essential oil of thyme. It means that plant extract may be no negative effects on digestive enzyme activities but has positive effects and increase in dosage rate may enhance enzymes performance in broilers.

It has been claimed that feeding broiler hens dietary Essential Oils (EO) made from herbs enhanced the release of digestive enzymes from pancreas (Jang et al., 2004). Comparative to a control group, a study with hens showed that a combination of commercial EO components stimulated the activity and secretion of digestive enzymes like amylase (William and Losa, 2001).

Long et al., (2020) also described same facts that plant extract has beneficial effects on enzymatic activity. In his research, the addition of LBP (*Lycium barbarum* polysaccharides) boosted the activities of protease, amylase, and lipase in the small intestinal contents of broilers. These findings imply that the expression of digestive enzymes was induced by LBP supplementation. The fundamental processes by which dietary supplementation of LBP modifies digestive enzymes, particularly in the small intestine, are poorly understood. Another study found that the intestinal digestive enzymes amylase, lipase, and protease in broilers were improved by polysaccharides from *Atractylodesmem branaceus* (Wu, 2018).

Hashemipour et al., (2013) also reported similar findings like our study when he fed broiler with thaymol and carvacol. So, more researches are required to understand the deep mechanism of Artemisia on activity of enzymes.

The digesta pH in the intestine may also be influenced by the feed type. According to Svihus (2011), broiler chickens' average stomach pH ranged between 3 and 4 for typical pelleted meals and 5.5 to 6.5 for monogastric animals.

There was no significant effect over the intestinal pH when compared by statistical analysis but there was a marked difference in the pH of the Control group when compared with the Group having antibiotic treatment or *Artemisia* treated supplementations which are in accordance with the findings of Gholamhosseini, et al (2021). Decrease in the pH of digesta was detected in both of the experiments in reply to the addition of the additives in feed. The average pH of the control group was mediocre but of the group that has been treated by using antibiotic reveals minimum while of the group treated by *Artemisia* resulted into maximum pH among all groups. These results showed that by using *Artemisia* the pH was shifted towards basic nature while towards acidic in case of Antibiotic. The increase in the pH in feeds without having any organic acid or basic supplementation in both of trials directs that though addition of supplements may increase pH because of having basic property, or increased pH may also consequence by addition of the additives that may have some basic properties and similar results were reported by Kambarova et al. (2020).When a mixture of formic and propionic acids was introduced to diet at 1%, Waldroup et al. (1995) discovered a decrease in gut pH, but no antibacterial effect. However, there were no discernible variations in the duodenal pH across the groups in the current trial. It might be because formic acid has an acidic quality, but phytogenic extract is basic by nature; hence, the usage of Artemisia caused the pH to move toward basic.

Straw et al. (1991) reported that in the pigs the acidification in diets have no effect over the decreased pH, but it can produce progress in the growth performance, thus suggesting that decrease in the pH of the gut may not certainly had been a reaction to the diet acidification. Our findings were further supported by (Kurhekar, 2013) as well as by (Grajek et al., 2005) but in some other context

In case of intestinal micro flora, only four colonies of bacteria were included and counted i.e. *Coliform, E. coli*, anaerobic bacteria and *Lactobacillus spp*. In case of *coliform* bacterial count, research depicts that there was no as such statistical difference

(P> 0.05) observed in groups but numerically the control group has higher *coliform* bacterial count as compared to two other research group, that shows *Artemisia* or antibiotic has may be positive effects on *coliform* count and can decrease the colonies when used (Yakhkeshi, et al. 2011; Erener et al. 2011).

In the case of *E. coli* bacterial count, a marked significant difference was being observed (P<0.05) among groups that portrays using *Artemisia annua* in broiler ration has valuable effects as it lowers the amount of pathogenic bacteria i.e. *E. coli* but numerically the control group has higher *E. coli* bacterial count as compared to two other research group that shows lower bacteria count (*Artemisia* and antibiotic). It shows that *Artemisia* and antibiotic groups have may be positive effects on *E. coli* count and can decrease the colonies when considered during supplementation in feed. Chowdhury et al. 2020 and White MB 2018 also reported that *Artemisia* and antibiotic groups has positive effects on *E. coli* count and can decrease the colonies, which is in accordance with our study.

When the anaerobic bacteria count has been performed, statistical difference was presenting among experimental and control groups as well. Its means that inclusion of *Artemisia* in broiler diet has valuable effects on health of the birds as it may decreases the amount of anaerobic bacteria when added. Maximum colonies has been seen in control group while minimum had been seen in *Artemisia* and mediocre had been seen in case of antibiotics, means *Artemisia* has been involved in decreasing the colonies at a remarkable level which is similar to the findings of Cross et al. 2007 and Diaz et al 2016.

In case of *Lactobacillus*, medium range colonies had been found in control group while lowest in case of antibiotic and highest in case of *Artemisia*. That results and numerical values showed that these *lactobacilli* have quite positive correlation with *Artemisia* while it was not that much close to antibiotic treatment when compared. Anyhow, there was no as such difference statistically among groups but as said earlier bacterial count was higher in *Artemisia* group. *Lactobacillus* spp. is beneficial microbes for gut health and increase in *lactobacillus* counts depicts that *Artemisia* has may be agonistic effects on its counts. Gupta et al (2016) also reported that the *Lactobacillus* spp. are beneficial

microbes for gut health and increase in *lactobacillus* counts depicts that *Artemisia* has may be agonistic effects on its counts, which is similar to the findings of our study.

Plant extracts (lavender) showed positive effects on gram positive and gram-negative bacteria and fungi. These discoveries provide credence to the idea that phytogenic feed additives may have a positive impact on gut flora, however the findings are mixed. These variations in the outcomes may be ascribed to the kind and variety of herb, the amount of herb in the diet, and the conditions of plant processing (Alexopoulos et al., 2011; Benabdelkader et al., 2011; Djenane et al., 2012).

Dietary interventions have an impact on the parameters of intestinal morphology (Yakhkeshi et al., 2011). Acidifiers can increase the quantity and size of villi by decreasing the pH of the digestive system and suppressing harmful bacteria (Emma et al., 2013). The small intestine's function can be enhanced and optimized by the addition of an acidifier, resulting in healthy reproduction of non-pathogenic bacteria and efficient absorption in the small intestine. According to Fitasari, (2012) the small intestine is the primary site of nutrition absorption. In the ephithelial mucosa of the small intestine, there are millions of villas. The number of villi diminishes in the ileum whereas it is denser in the duodenum and jejunum.

In current study, no difference was observed among groups statistically in case of villous height but numerical difference was present. *Artemisia* group showed maximum villous height as compared to control and antibiotic which matches the findings of Djunaidi et al., (2020) while studying the effects of organic acids on broiler intestinal morphology. Increase in villous height may be due to more nutrient absorption that also responsible for more body weight gain in case of *Artemisia* group.

In case of villous no, there was no statistical difference found among groups but numerically Artemisia group showed more villous no (unit/lumen) while antibiotic group presented lowest no of villi. Similar result found by Widodo et al., (2016) in broiler. According to him, lime acid can, if hardly, enhance the number and height of villi in broilers. The total lime acid can lower ileal pH, which substantially stimulates the growth of non-pathogenic bacteria. This results in increased villi height and number.

In case of villous surface area, statistical difference was present among groups. Birds those received Artemisia plant extract showed maximum surface area while antibiotic group showed lowest area. Djunaidi et al., (2020) has done the same study and reported no significant difference among groups. According to Apriliyani, Djaelani, and Tana, (2016) the size of the height and width has an impact on the cross-sectional area of the gut, which can affect the capacity for digestion and nutritional absorption. The claim that nutrient absorption will be more effective if the absorption area grows broader and wider supports our findings (Hidayat, 2016).

Khalaji et al. (2011) reported that the ileum structure was improved by Camellia L. plant extract (CLE) and Mannan oligosaccharides (MOS), as indicated by an increase in villi length and crypt depth similar to our study, but significant differences were not found. According to Baurhoo et al. (2007), higher lactobacillus and bifidobacterial colonization of broiler intestines has been linked to an increase in villi length caused by MOS. High villi length and crypt depth may have a positive impact on nutrient absorption, although the precise mechanism by which this effect occurs is yet unknown.

The structure of the small intestine is thought to be related to its function because it is in charge of digesting and absorbing ingested meal (Yamauchi et al. 2010). In a different study, Mohiti-Asli et al. (2018) discovered that broilers fed 300 ppm of individual oil of oregano had greater villus surface area, VH, and VH to CD (Crypt depth) ratios in the jejunum than those fed control diets. This observation agreed with the conclusions reached by Sarica et al (2014). VH enlargement is associated with an increase in villus surface area, which allows for increased absorption of nutrients that are available (Awad et al. 2008).

It is well recognized that antimicrobial medicines lower the intestinal microbial burden, which in turn lowers the presence of toxins. Changes in internal morphology, such as shorter villi and deeper crypts, are related to an increase in toxins. Similar findings to those of our study were also reported by Garcia et al. (2007).They found no appreciable variations in VH and CD between birds in the control group and a group that received a combination of plant extracts.

Gunal et al., (2006)'s research, however, demonstrated that a probiotic treatment significantly enhanced the VH in the jejunum and ileum at 21 or 42 days compared to a non-supplemented basal diet. Furthermore, Pelicano et al. (2005) reported that probiotic-containing meals considerably enhanced the VH and CD in all parts of the small intestine, while in our investigation, only numerical differences were seen. A large dose of plant extract or improved management could be to blame for the noticeable variation that was noticed.

6. Conclusion

Antibiotics were commonly used in the poultry industry as a growth promoter. Excessive use of antibiotics in animals causes antibiotic resistance for many microbes. In human and veterinary medicine, the effects of antibiotic resistance in microorganisms are essentially the same. The loss of effective antibiotic therapies due to resistance will result in suffering for the affected individual, whether human or animal. Increased treatment expenses in animal and human health care will also have economic effects. For avoiding resistance issues, poultry professionals started looking for a replacement product. One of the best alternative active herbal products is *Artemisia Anna L*. The leaf extract of this plant contains bioactive compounds such as flavonoids and essential oils. The extract of this plant was used in China for 2000 years as an anti-malarial, anti-coccidial, anti-inflammatory, and anti-oxidative in animals and humans. In current study, extract of plant was used to check effects on growth performance, digestive enzyme activities, intestinal pH, intestinal microflora and intestinal morphology. *Artemisia* showed significant differences for anaerobic bacteria and surface area of villous while in other parameters *Artemisia* showed only numerical differences. Growth performance, FCR, pH, microflora and villous height and no showed clear numerical changes over antibiotic and control group. In conclusion, *Artemisia annua* showed only positive effects on bird's health, microflora, pH and morphology of intestine. There were no side effects observed in this study. Increase in dose rate may show better positive result of *Artemisia annua* used in broilers. So, it is the good alternative product for avoiding anti-microbial resistance in the poultry industry without compromising growth and production.

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