PHARMACOLOGICAL SCREENING OF SOME MEDICINAL PLANTS AS ANTIMICROBIAL AND FEED ADDITIVES

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ABSTRACT

The following study was conducted to investigate the antibacterial and feed additive potential of medicinal plants. Ethanol extracts of different medicinal plants including *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Piper nigrum* (Black Pepper), *Cinnamomum cassia* (Cinnamon), *Thymus vulgaris* (Thyme), *Laurus nobilis* (Bay leaf), and *Syzgium aromaticum* (Clove) were tested using the disc diffusion method for their antimicrobial activity against the common poultry pathogens *E. coli*, *S. typhimurium*, *E. faecium*, and *E. faecalis*. Cinnamon extract (CE), at 130 mg/disk, exhibited antibacterial activity against *E. coli*, *S. typhimurium*, and *E. faecalis.* Thyme extract (TE), at 30 mg/disk, exhibited antibacterial activity against *E. coli*, *E. faecium*, and *E. faecalis* while the remaining medicinal plants extracts showed no activity. The minimum inhibitory concentration (MIC) of the cinnamon and thyme ranged from 31.25 to 250 mg/ml by the dilution method. From this in vitro antibacterial study, cinnamon and thyme were selected for a 21-d feeding trial in broilers to study their influence on feed consumption, body weight gain, and feed conversion. There were 6 dietary treatments groups: 1) negative control (NC) containing no plant extracts or antibiotic, 2) positive control (PC) containing BMD (bacitracin) at 50g/ton of feed, 3) Diet 1 plus low level of cinnamon extract (LCE) at 290 gm/100 kg of feed, 4) Diet 1 plus high level of cinnamon extract (HCE) at 580 gm/ 100 kg of feed, 5) Diet 1 plus low level of thyme extract (LTE) at 290 gm/100kg of feed, and 6) Diet 6 plus high level of thyme extract (HTE) at 580 gm/100 kg of feed. No significant changes in body weight gain were observed with the cinnamon extracts compared to

the NC or PC at 7, 14, or 21 d. The HTE reduced body weight gain compare to the NC and PC at 7, 14, and 21 d ($P < 0.02$). No difference in feed efficiency was observed with any of the treatments except LCE which reduced feed efficiency compared to other treatments. No difference in feed consumption was found among any of the treatments. These results suggest that cinnamon and thyme have antibacterial activity *in vitro*, and thyme has an activity that reduces body weight. Since cinnamon caused no significant change in body weight gain compared to positive or negative controls, it warrants further study as a substitute for antibiotics in the diet.

Key words: medicinal plant extracts, antibacterial, feed additives, broiler, MIC.

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Introduction

Antibiotics such as avoparcin, bacitracin, lincomycin, penicillin-G -procaine, chlortetracycline and virginiamycin promote growth because of an affect on the microflora in the gastrointestinal tract (Coates et al., 1963; deMan, 1975). Throughout the world, the use of these antibiotics as dietary growth promoters in poultry diets differ dramatically. Sweden now allows no use of antibiotics for growth promotion purposes whereas the USA uses a wide range of antibiotics (W.H.O., 1997).

Antimicrobial resistance in zoonotic enteropathogens including *Salmonella*, *Escherichia coli* (*E. coli*), and *Enterococci* in food animals is of special concern to human health because these bacteria are likely to transfer from the food chain to humans (Endtz et al., 1991). As a consequence, the European Commission banned 4 commonly used feed antibiotics monensin sodium, salinomycin sodium, avilamycin, flavophospholipol. To minimize this resistance, different agencies including the Centers for Disease Control & Prevention (CDC), Atlanta, USA are in favor of banning these feed antibiotics in the USA (Hileman, 2002).

The phasing out of antibiotic growth promoters (AGP) will affect the poultry and animal industry at large. To minimize the loss in growth, there is a need to find alternatives to AGP. There are a number of non-therapeutic alternatives such as enzymes, inorganic acids, probiotics, prebiotics, herbs, immunostimulant and other management practices (Banerjee, 1998).

Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity (Shelef 1983; Zaika, 1988; Beuchat and Golden, 1989; Juven et al, 1994; Chang, 1995). More recently, medicinal plant extracts were developed and proposed for use in food as natural antimicrobials (Del Campo et al., 2000; Hsieh, 2000; Hsieh et al., 2001). However, little or no work has been done on the effects of plant extracts on body weight and performance in poultry. The present study was conduced to determine the effect of different medicinal plant (herbs) extracts in broiler diets as a possible alternative to antibiotic feed additives.

Review of Literature

Subtherapeutic Use of Antibiotics as Feed Additives

Antimicrobials are powerful but controversial tools. In the United States, food animals are often exposed to antimicrobial compounds to treat or prevent infectious diseases and/or to promote growth (McEwen and Fedorka-Cray, 2002). The early history of supplementing animal feeds with antimicrobials parallels the isolation, identification and characterization of vitamin B_{12} in 1948. Further research in this arena showed that several feed ingredients, including dried mycelia of certain fungi, were more potent as growth promoters in the diet of chicks than was vitamin B_{12} alone. The active component for growth promotion in mycelia fungi was shown to have antimicrobial activity (Jones and Ricke, 2003). In 1950 antibiotics were approved for use as animal feed additives (Gersema and Helling, 1986). A total of 32 veterinary non-prescription antimicrobial compounds are approved for use in broiler feeds in the U.S. Eleven compounds are listed as growth promoters (AGP), fifteen are listed to treat coccidiosis and six are listed for other purposes. Seven of these compounds, including bacitracin, chlortetracycline, erythromycin, lincomycin, novobiocin, oxytetracycline, and penicillin are also used in human medicine (Jones and Ricke, 2003). In the poultry industry, bacitracin, chlortetracycline, penicillin, tylosin, and virginiamycin are some of the important antibiotics used as growth promoters (McEwen and Fedorka-Cray, 2002). Bacitracin is used more frequently in the starter and grower diet. Virginiamycin and other antibiotics are used most frequently in the grower and withdrawal diet (Chapman and Johnson, 2000). Antibacterial feed additives are also used for controlling *Clostridium perfringens*-associated NCrotic enteritis in broilers. However, currently immunoprophylaxis is used to control NCrotic enteritis in broilers. Feed additives thus share more than simply increasing body weight gain (Lovland et al., 2004).

Possible mechanisms of growth promoter action of antimicrobials

The mechanism by which antibacterial agents improve growth performance is not known, but several theories have been proposed: 1) Because they thin the small intestinal epithelium, nutrients are

more efficiently absorbed (Boyd and Edward., 1967; Fuller et al., 1984); 2) Nutrients are spared because competing microorganisms are reduced (Eyssen, 1962); 3) The different microorganisms responsible for subclinical infections are reduced or eliminated (Barnes et al., 1978); 4) There is a reduction in production of the growth-depressing toxins or metabolites by intestinal microflora (Dang and Visek, 1960).

Antibiotic resistance

The emergence of antimicrobial resistance has its roots in the use of antimicrobials in animals and the subsequent transfer of resistance genes and bacteria among animals, animal products and the environment (McEwen and Fedorka-Cray, 2002). Extra-chromosomal genes were found responsible for these antimicrobial resistant phenotypes that may impart resistance to an entire antimicrobial class. These resistance genes have been associated with plasmids which are large, transferable, extra-chromosomal DNA elements. Other DNA mobile elements, such as transposons and integrons, are present on plasmids. These DNA mobile elements transmit genetic determinants for antimicrobial resistance mechanisms and may cause rapid dissemination of resistance genes among different bacteria (McDermott et al., 2002). The emergence of multiresistant bacteria to antimicrobial drugs has increased the need for new antibiotics or modifications of older antibiotics (Tollefson and Miller, 2000). Yoshimura et al. (2000) showed that *enterococci* isolated from fecal droppings of chickens on broiler and layer farms were resistant to ampicillin, clindamycin, erythromycin, streptomycin, tetracycline and tylosin. This resistance was more frequent in enterococcal isolates from broiler farms than in those from layer farms. *Enterococcus faecium* (*E. faecium*) and *Enterococcus faecalis* (*E. faecalis*), isolated from cloacal cultures from three turkey flocks fed virginiamycin showed a higher percentage of quinupristin-dalfopristin-resistance with the oldest flock being 100% resistant (Welton et al., 1998).

Important pathogens in this study

The zoonotic enteropathogens such as *Salmonella* species, *Campylobacter* species, commensal bacteria such as, *E. coli, enterococci,* and bacterial pathogens of animals e.g., *Pasteurella*, and

Actinobacillus species are a few of the species reported to have developed resistance. However, the prevalence of resistance varies (McEwen and Fedorka-Cray, 2002). Resistance to penicillin was first observed in *E. faecium* in 1983, and in 1988 the first cases of resistance to vancomycin an "antibiotic of last resort", were detected in Europe. Less than 2% of *E. faecalis* were found to be resistant to ampicillin and vancomycin, whereas 83 % of the *E. faecium* isolates were resistant to ampicillin and 52% were resistant to vancomycin in a study conducted between 1995 and 1997 examining over 15,000 enterococcus isolates (W.H.O., 1997). *E. faecium* was resistant to several types of antibiotics including quinolones and aminoglycosides. Vancomycin resistant strains of *E. faecium* were reported in the US in 1989. Resistance to several antibiotics and tolerance for adverse conditions makes *E. faecium* a major concern for the medical community, which has dubbed this microbe a "supergerm". The *S. typhimurium* DT 104 is of special concern because it is causing increasing numbers of cases of salmonellosis in humans. The *S. typhimurium* DT 104 is primarily associated with cattle, but it has spread to a range of food animals, including pigs, sheep and poultry. Comminuted meat products such as sausages and burgers are the main sources of food borne infection (W. H. O., 1997). *S. typhimurium* was found to be resistant to many of the commonly used antibiotics including ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracyclines and many others and, as a result, the illness is more difficult to treat (W.H.O., 1997). For the present study gram positive bacteria *E. faecium,* and *E. faecalis* and gram negative bacteria *E. coli,* and *S. typhimurium* were evaluated.

Ban on feed antibiotics

Antibacterial substances are used in considerable amounts as growth promoters in animal husbandry, and carry incalculable risks for human health resulting from the use of particular feed additives (Witte, 2000). The indiscriminate use of antibiotics as feed additives could lead to an increased number of antimicrobial-resistant bacteria, and ultimately compromise the treatment of bacterial infections in humans (Gersema and Helling, 1986; McDermott et al., 2002). Many countries concerned about this problem have restricted and or banned the use of antimicrobial compounds in feed for food

animals to slow the development of resistance, and some groups advocate similar types of measures in the United States (McEwen and Fedorka-Cray, 2002). Major changes occurred in the use of antimicrobial agents for growth promotion during the last 6 years in different countries. In 1986, the Swedish Government banned the use of antimicrobial growth promoters (Wierup, 2001). Denmark banned the use of avoparcin in 1995 and virginiamycin in 1998. The glycopeptide-resistant *E. faecium* in broilers was decreased after the ban of avoparcin from 72.7% in 1995 to 5.8% in 2000 (Aarestrup and Jensen, 2001).

Alternatives to feed antibiotic growth promoters

There are a number of non-therapeutic alternatives to antibiotic growth promoters, including enzymes, (in)organic acids, probiotics, prebiotics, herbs, immunostimulants and specific management practices (McEwen and Fedorka-Cray, 2002). Ileal digestibility is improved by exogenous enzymes, thereby limiting nutrients to the microbial flora those chaniging the population. Acids control *in vitro* and *in vivo* growth of microbial flora. Prebiotics are 'non-digestible feed ingredients' which exert some selective effects on the intestinal microflora. The use of herbs and essential oils may relate to their anti-microbial activity against pathogenic bacteria and parasites (Banerjee, 1998).

Medicinal plants (herbs)

Ancient use of medicinal herbs

Culinary herbs and their essential oils have been used extensively for many years in food products, perfumery, and dental and oral products due to their different medicinal properties (Suppakul et al., 2003). However, secondary plant metabolites are largely unexploited in 'conventional' animal production systems. In the past, plant metabolites were generally considered as a source of antinutritional factors. Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plant source as alternative performance enhancers (Greathead, 2003). In contrast to their regulated status in India, China, and other countries, herbal medicines are

regarded as dietary supplements for humans in the US and are widely used. It is reported that approximately one quarter of adults used herbs to treat a medical illness within the past year in the US (Bent and Ko, 2004). Herbs contain some complicated mixtures of organic chemicals that may vary depending upon many factors related to the growth, production, and processing of the herbal product. Though herbs with antimicrobial properties are reported, their use in broiler diets has not been studied extensively.

Use of medicinal plants in poultry research

No significant differences in body weight or feed efficiency were observed after dietary administration of 5 or 20 ppm of capsaicin in broilers. However the *Salmonella enteritidis* (S. enteritidis) positive culture rate for cecal tonsils was significantly lower ($P < 0.05$) in the treatment groups receiving 5 ppm or 20 ppm dietary capsaicin than in the untreated control group. Capsaicin administration increases resistance to S. enteritidis colonization and organ invasion without detrimental effects on growth in broiler chickens (McElroy et al., 1994). Adding high levels of some varieties of sweet lupines in broiler diets decreased feed intake and growth rate in broilers and specific signs of acute and chronic toxicity in some individuals were observed (Olkowski et al., 2001).

Medicinal plants with antimicrobial activity

Cinnamon (Cinnamomum cassia)

Essential oils of cinnamon (*Cinnamomum cassia*), were found to possess antimicrobial properties in-vitro and shown to inhibit the growth of *B.cereus* (Kalemba and Kunicka, 2003; Valero and Salmeron, 2003). Alcoholic extracts of cinnamon were found most effective against *Helicobacter pylori,* in reducing its growth (Tabak et al., 1996). It was found that a combination of cinnamon and nisin accelerated the death of *S. typhimurium* and *E. coli* O157:H7 in apple juice, and hence enhanced the safety of the product (Yuste and Fung, 2004). A study by Mau et al.(2001) on the antibacterial activity of extracts of chive (Allium tuberosum), cinnamon and corni fructus (*Cornus officinalis*) against common

foodborne microorganisms, alone and in combination, showed that the mixed extract, consisting of three extracts in equal volumes possessed an antimicrobial spectrum and had excellent stability to heat, pH, and storage on growth of *E. coli* at 2-5 mg/ml. The mixed extract also inhibited the growth of *Pichia membranaefaciens* at 2 mg/ml. When the mixed extract was used in foods, an expected antimicrobial effect in orange juice, pork, and milk was observed. Overall, the mixed extract has promising potential for incorporation into various food products for which a natural antimicrobial additive is desired.

H. pylori is associated with the pathogenesis of gastritis, duodenal ulcers, and gastric lymphoma. The cinnamon extract, at a concentration of 80 mg /day as a single agent, was found ineffective in eradicating *H. pylori* infections in an experiment carried out in human subjects (*in-vivo*). However, a combination of cinnamon with other antimicrobials, or cinnamon extract at a higher concentration, may prove useful. The cinnamon extract was well tolerated and side effects were minimal (Nir et al., 2000). Acute (24 hours) and chronic (90 days) oral toxicity studies on an ethanol extract of cinnamon in mice at the dose rate of 0.5, 1.0 , or 3 g/kg for acute and 100 mg/kg/day for chronic studies showed that the extracts caused no significant acute or chronic mortality compared to the control during the study (Shah et al., 1998).

A 67% ethanol/water extract of cinnamon bark inhibited the activity of bacterial endotoxin. This was the first report, which states that an inhibitor of bacterial endotoxin exists in a plant (Azumi et al., 1997). The bark of *Cinnamomum zeylanicum* (*C. zeylanicum*) was found effective against fluconazole-resistant candida species, which is an emerging problem. The minimum inhibitory concentrations (MIC) of the bark of *C. zeylanicum* bark ranged from < 0.05-30 mg/ml and were slightly better than commercially available cinnamon powder. Cinnamon candies and gums have MIC ranging from 25-100 mg/ml (Quale et al., 1996). A comparative bacteriostatic study showed that the bacteriostatic effect of oleum of *Perilla frutescens* and *Cinnamomum cassia*, was superior than benzoic acid (Zhang et al., 1990).

Buffered methanol (80% methanol and 20% PBS) and acetone extracts of edible plants of 26 species including cinnamon screened for their antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella infantis* by the disc

assay showed that the MIC of extracts determined by the agar dilution method ranged from 165 to 2640 mg/ml. *B. cereus* was the most sensitive microorganism to extracts from *Cinnamomum cassia*, *Azadirachta indica*, *Ruta graveolens*, *Rumex nervosus*, *Thymus serpyllum* and *Zingiber officinale* with MIC of 165 to 660 mg/ml. The inhibitory activity against *E. coli* and *S. infantis* was produced only by *Cinnamomum cassia* extract at the highest MIC of 2640 mg/ml (Alzoreky and Nakahara, 2003). The phytochemical analysis of an essential oil (CC-oil) of *C. cassia* stem bark by GC-MS (Gas chromatography and mass spectrometry) led to the identification of cinnamaldehyde (CNA, 1), 2-hydroxycinnamaldehyde (2-CNA), coumarin (2), and cinnamyl acetate as chief component (Choi et al., 2001). The *C. cassia* bark-derived cinnamaldehyde, when tested using 1 or 0.5 mg/disks, revealed potent inhibition against *Clostridium perfringens* and *Bacteroides fragilis.* The growth of *Bifidobacterium bifidum* was significantly inhibited at the dose of 1 and 0.5 mg/disk, whereas weak or no inhibitory activity was obtained against *Bifidobacterium longum* or *Lactobacillus acidophilus*. In contrast, tetracycline and chloramphenicol showed an inhibitory effect against all test bacteria at doses as low as 0.01 mg/disk (Lee and Ahn, 1998).

Curcuma longa (Turmeric)

Curcuma longa (*C. longa*), a perennial herb, is a member of the *Zingiberaceae* family and has a long tradition of use in the Chinese and Ayurvedic systems of medicine. Curcuminoids, a group of phenolic compounds isolated from the roots of *C. longa*, exhibited a variety of beneficial effects on health and has the ability to prevent certain diseases (Joe et al., 2004). In East Asia, the rhizomes from *C. longa*, are considered to have natural medicinal properties, including antibacterial, anti-inflammatory, antineoplastic, and analgesic activities because they contains a number of moniterpenoids, sesquiterpenoids, and curcuminoids (Tang and Eisenbrand, 1992; Fang et al., 2003). It is also reported to have insecticidal activity (Chander et al., 1991a, b). In addition, wound healing and detoxifying properties of curcumin have also received considerable attention (Joe et al., 2004). A study by Limtrakul

et al. (2004) using RT-PCR showed that all three curcuminoids isolated from *C. longa* inhibited multidrug resistance -1 (MDR-1) gene expression. Fraction II of the oil extract from the turmeric oleoresin containing ar-Turmerone, turmerone, and curlone showed antibacterial activity by the pour plate method against *Bacillus cereus*, *Bacillus coagulans, Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Negi et al., 1999). Methanol extract of the dried powdered turmeric rhizome and curcumin inhibited the growth of all strains of *H. pylori in vitro* with a MIC range of 6.25-50 µg/ml (Mahady et al., 2002). Extracts of C. longa greatly reduced aflatoxin production aflatoxin production by *Aspergillus parasiticus* in vitro (more than 90%) at concentrations of 5-10 mg/ml. Curcumin, an active antioxidant from C. longa did not produce any effect on aflatoxin production by *Aspergillus parasiticus* (Soni et al., 1992).

Laurus nobilis (Bay Leaf)

Bay leaf oil tested for its bactericidal activity showed to be active against *Salmonella* enterica (*S. enterica*) obtained from food and clinical sources showed that it is most active against *S. enterica*. It was also found effective against *E. coli*. (Friedman et al., 2002). n-Hexane, ethanol and water extracts of bay leaves were evaluated for cytotoxic properties using the brine shrimp bioassay. This study indicated that only the n-hexane extract exhibited cytotoxic activity (Kivcak and Mert, 2002). The microbial growth inhibitory properties of an essential oil of *Laurus nobilis* were studied by the determination of the MIC. against five bacterial strains, one fungus and two yeasts. The essential oils extracted from bay leaf had antimicrobial activity (Raharivelomanana et al., 1989).

Piper nigrum (Black pepper)

Black pepper (*P. nigrum*) is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus congestion, fever, intermittent fever, cold extremities, colic, gastric ailments and diarrhea. It has been shown to have antimicrobial activity (Perez and Anesini, 1994; Dorman and Deans, 2000). Both aqueous

and ethanol extracts of black pepper screened for antibacterial activity against a penicillin G resistant strain of *Staphylococcus aureus,* showed antibacterial activity, which was determined by the agar-well diffusion method, using cephazolin as a standard antibiotic (Perez and Anesini,1994). Piperine, [1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2,4, pentadienyl piperidine, a pungent alkaloid present in *P. nigrum,* enhanced the bioavailability of various structurally and therapeutically diverse drugs. A concise mechanism of its bioavailability enhancing action is poorly understood. However, data suggests that piperine is absorbed very fast across the intestinal barrier; it may form non-polar complexes with drugs and solutes thus increasing permeability across the barriers (Khajuria et al., 1998). Piperine exerted significant protection against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity in mice. Silymarin, a known hepatoprotective drug, was also tested simultaneously for comparison. Piperine showed lower hepatoprotective potency than silymarin (Koul and Kapil, 1993).

Platel et al. (2003) showed that the spice mix of coriander, turmeric, red chilli, black pepper and cumin favorably enhanced the pancreatic lipase, chymotrypsin and amylase activity when consumed via diet. In addition, these spice mix brought about a pronounced stimulation of bile flow and bile acid secretion. Activities of pancreatic lipase, amylase and chymotrypsin were elevated by 40, 16 and 77%, respectively. The higher secretion of bile, especially with an elevated level of bile acids, and a beneficial stimulation of pancreatic digestive enzymes, particularly lipase, could be two mechanisms by which these combinations of spices aid in digestion and increased performace.

***Syzgium aromaticum (Clove)* (*Syn-Eugenia caryophyllus Eugenia caryophyllata, Eugenia aromatica, Caryophyllus aromaticu*m)**

Essential oils of clove possess antimicrobial properties (Kalemba and Kunicka, 2003). Clove oil was effective against *E. coli*, *L monocytogenes*, *S. enterica* (Friedman et al., 2002). The antibacterial activity of clove against two gram-negative bacteria, such as *Pseudomonas fluorescens* and *Serratia liquefaciens*, and four gram-positive bacteria, such as *Brochothrix thermosphacta*, *Carnobacterium piscicola*, *Lactobacillus curvatus*, and *Lactobacillus*, involved in meat spoilage was found effective.

The 1/100 dilution of clove oils inhibited the bacterial growth of five of the six tested bacteria mentioned above.

A relationship between the inhibitory effect of essential oils and the presence of eugenol and cinnamaldehyde was found (Ouattara et al., 1997). A crude methanol extract of *S. aromaticum* exhibited growth-inhibitory activity against gram-negative anaerobic pathogens, including *Porphyromonas gingivalis* and *Prevotella intermedia*. The chromatographic analysis of clove isolated eight active compounds identified as 5,7-dihydroxy-2-methylchromone 8-C-beta-D-glucopyranoside, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid, and oleanolic acid. The flavones, kaempferol and myricetin, active compounds from clove, demonstrated potent growth-inhibitory activity against the periodontal pathogens *Porphyromous gingivalis* and *Porphyromous intermedia* (Cai and Wu, 1996).

Thymus vulgaris (Thyme)

The oil of thyme and its different components are becoming increasingly popular as a naturally occurring antimicrobial and also as an antioxidant agent (Dursun et al., 2003). Thyme showed broad antibacterial activity by inhibiting the growth of both gram-positive and gram-negative bacteria. However, gram positive bacteria *Clostridium botulinum* and *Clostridium perfringens* appeared to be more sensitive than the gram-negative organisms (Nevas et al., 2004). The alcohol and ethanol extracts of thyme, thyme essential oil, thymol and carvacrol were found to have strong inhibition activity against *Bacillus subtilis*, *S. sonnei, E. coli* (Fan and Chen, 2001). Aqueous extracts of thyme significantlt inhibited the growth of *H. pylori*, reducing its growth (Tabak et al., 1996). The essential oil of thyme, or its constituent thymol, decreased viable counts of *S. typhimurium* on nutrient agar (NA) (Juven et al., 1994). Thymol showed antagonistic effect against *S. sonnei* in anaerobic conditions in vitro (Juven et al., 1994). Carvacrol, a compound present in the essential oil fraction of oreganum and thyme showed a dose-related inhibition of growth of the pathogen *Bacillus cereus* (Ultee et al., 2000). The lowest

minimum inhibitory concentrations were 0.03% (v/v) thyme oil against *C. albicans* and *E. coli* (Hammer et al., 1999). Thyme extracts exerted no microbicidal activity against *Porphyromous aeruginosa* (Thuille et al., 2003). However, antibacterial growth-inhibitory effect of thyme on *Shigella sonnei* (*S. sonnei*) was noted.

The addition of basil and thyme to spaghetti sauce prior to autoclaving and *S. sonnei* inoculation indicated that basil and thyme contributed to the reduction of *S. sonnei* after 16 days at 12^0 C, but not at 4^0C temperatures. This study indicated that pH and NaCl concentrations affect the activity of thyme (Bagamboula et al., 2003).

Thyme essential oil exhibited bacteriostatic and bactericidal properties against the non-toxigenic strain of *E. coli* O157:H7 in a broad temperature range. It was found that lecithin diminished the antibacterial properties (Burt and Reinders, 2003). In an *in vitro* antibacterial study, thyme showed greatest inhibition against *A. hydrophila* compare to other psycrotrophic food-borne bacteria such as *Aeromonas hydrophila*, *Listeria monocytogenes* and *Yersinia enterocolitica.* Inhibition of growth was tested by using the paper disc agar diffusion method, while the MIC was determined by the broth microdilution method (Fabio et al., 2003). Thyme oil was tested for its antibacterial activity against *Campylobacter jejuni* (*C. jejuni*), *E. coli* O157:H7, *Listeria monocytogenes*, and *S. enterica* obtained from food and clinical sources and was found most effective against *E. coli*, *L monocytogenes S. enterica,*and *C. jejuni* (Friedman et al., 2002). When extracts of garlic (*Allium sativum*), sage (*Salvia officinalis*), caraway (*Carum carvi*), peppermint (*Mentha piperita*), fennel (*Foeniculum vulgare*), thyme (*Thymus vulgaris*), paprika (*Capsicum annuum*), marjoram (*Majorana hortensis*), cardamom (*Elettaria cardamomum*) were fed layers, a well seasoned taste was obtained to the eggs from birds fed with garlic, fennel, peppermint and marjoram (n = 705) (Richter et al., 2002). Feeding thyme leaves to male Wistar rats at 2 or 10% of standard a diet for 6 weeks showed that thyme leaves were not toxic to rats (Haroun et al., 2002).

Zingiber officinale (Ginger)

Zinziber officinale (*Z. officinale*) has been shown to have antimicrobial activity (Habsah et al., 2000; Srinivasan et al., 2001). Ethanolic extract of the rhizomes of *Z. officinale* showed significant inhibition of growth of both certain gram-positive and gram-negative bacteria. It also displayed anti-inflammatory, analgesic, antipyretic and antimicrobial activities. In rats, the extract reduced carrageenan-induced paw swelling and yeast-induced fever. The extract reduced blood glucose in rabbits (Mascolo, 1998). The essential oils of *Z. officinale* showed antimicrobial activity against gram-positive and gram-negative bacteria using the agar diffusion method (Martins et al., 2001). Toxicity studies conducted on *Z. officinale*, used as aphrodisiacs in Arab Medicine showed no toxicity during acute toxicity test. The percent lethality was insignificant as compared to the control (Qureshi, 1999).

The safety and efficacy of herbal remedies is a concern for many people. Ginger, when subjected to clinical trials among pregnant women, was found clinically effective against chemotherapy-induced nausea and vomiting. While safety concerns exist in the literature for this herb with regards to its use by pregnant women, no clinical evidence of harm was observed (Westfall, 2004). Methanol extract of the dried powdered ginger rhizome and the isolated constituents, 6-, 8-,10-gingerol and 6-shogoal were tested against 19 strains of *H. pylori.* It inhibited growth of all 19 strains *in vitro* with a minimum inhibitory concentration range of 6.25-50 µg/ml. The crude extract, containing gingerols, inhibited the growth of all strains of *H. pylori* with an MIC range of 0.78 to 12.5 µg /ml and with significant activity against the CagA+ strains (Mahady et al., 2003). The extracts of ginger exhibited antibacterial activity against the pathogens *S. aureus*, *S. pyogenes*, *S. pneumoniae* and *H. influenzae*. The MIC of extracts ranged from 0.0003 µg/ml to 0.7 µg/ml for ginger, while MBC ranged from 0.135 µg/ml to 2.04 µg/ml for ginger. Results indicated that extracts of ginger and *Garcinia kola* roots may contain compounds with therapeutic activity (Akoachere et al., 2002).

Material and Methods

Selection of medicinal plants for this study

Seven medicinal plants including *Zinziber officinale* rhizomes (Ginger), *Cinnamomum cassia* bark (Cinnamon), *Piper nigrum* fruits (Black Pepper), *Curcuma longa* rhizomes (Turmeric), Thymus *vulagaris* leaves (Thyme), *Laurus nobilis* leaves (Bay leaf), *Syzgium aromaticum* fruits (Clove), were utilized in this studies. These plants have previously been reported to have antibacterial activity against different bacterial strains.

Preparation of Extracts

Grinding of the selected plant materials

After drying at 37^0C for 24 h the plant material was ground in a grinding machine (Thomas Wiley laboratory mill, model # 4, screen size-1mm) made for the laboratory. Exposure to sunlight was avoided to prevent the loss of active components.

Extraction of selected plant material powder by maceration method

One liter of an 80 % ethanol extraction fluid was mixed with 200 g of powdered plant material. The mixtures were kept for 2-5days in tightly sealed vessels at room temperature at 22^0C, protected from sunlight, and mixed several times daily with a sterile glass rod. This mixture is filtered through muslin cloth and the residue, if necessary, adjusted to the required concentration (500 ml of 80% ethanol for the residue of 200 g of powdered plant material) with the extraction fluid for further extraction. Further extraction of the residue was repeated 3-5 times until a clear colorless supernatant extraction liquid was obtained indicating that no more extraction from the plant material was possible.

The extracted liquid was subjected to rota-evaporatoration (Brinkmann rotavapor, Model # R) or water bath evaporation (Precision shaking water bath, model #25) to remove the ethanol. Either method is good depends on the quantity of extraction fluid, for more quantity, water bath evaporation is used. To concentrate the larger quantity of aliquote, water-bath evaporation was used. Rota evaporation was used

to concentrate the smaller quantity of extract. A 250 ml aliquot of extracted liquid was subjected to rota-evaporatoration for 3-4 h. The water bath temperature was adjusted to 70^0 C. The semisolid extract produced was kept in the deep freezer at -80^0C overnight and then subjected to freeze drying for 24 hrs at -60^0C at 200 millitorr vacuum.

For water bath evaporation, 3000 ml of liquid extract material was placed into a 3500 ml beaker. It was subjected to water bath evaporation at 70^0C temperature for 7-10 hrs daily for 2-3 days until a semisolid state of extracted liquid was obtained. Continuous evaporation was not done to avoid the charring of the extract constituents. The approximate volume of semisolid liquid by that time was 200-300 ml. The level of the water in the water bath was adjusted to 1/4 of the beaker height while shaker speed was adjusted to 27 to 32 oscillations per minute. The semisolid extract produced was frozen at -80^0C and then freeze dried to completely remove ethanol and water from the extract at -60^0C at 200 millitorr vacuum. Extract from this method was then weighed and stored at 22^0C in desiccators until further use.

In vitro antibacterial studies

Antimicrobial susceptibility studies

Inhibition of microbial growth was tested by using the paper disc agar diffusion method (Kirby-Bauer Method; Drago et al., 1999) (Appendix-B), while the MIC was determined by the dilution (both micro and macro) method (de Paiva et al., 2003) (Appendix-C). Standard aseptic microbiological methods were followed throughout this antibacterial study.

Microorganisms

ATCC strains of, *E. faecium* and *E. faecium, S. typhimurium* (Microbiology teaching culture collection, Department of Biology, Virginia Tech, USA), were obtained. In addition, clinical isolates of *E. coli* were obtained from the Shandon valley, VA, USA through School of Veterinary Medicine, Virginia Tech, U.S.A.

Disc diffusion method for antibacterial activity (Mukherjee et al., 1995a, b)

This method was used to assay the plant extracts for antimicrobial activity. The procedure, as explained in detail in Appendix B was followed. In brief, the test quantity of specific extract as shown in table 3 was dissolved in either distilled water or tween-80, depending upon the solubility of the extract. In order to detect potential antimicrobial activity in the plant extracts, paper discs (diameter 12 mm) were soaked in an extract solution containing different concentration as mentioned in table 3. The plant species and type of extract tested are shown in Table 1, while the bacteria are listed in Table 2. Entire surface of agar plate was inoculated with the culture of bacteria. The paper discs soaked in each of the test solutions containing different extract solutions at varying concentrations, as well as the standard drug solution (an antibiotic which is used as a feed additive) and the control-blank (sterile water discs or sterile tween 80 discs) were placed separately in each quarter of the plate under aseptic conditions. Multiple plates were (four replications) done for each of the extract was done. The plates were then maintained at room temperature for 2 h allowing for diffusion of the solution. All plates were then incubated at 37^0C for 24 h and the zones of inhibition were subsequently measured in mm (Mukherjee et al., 1995a, b).

Dilution method for MIC (de Paiva et al, 2003).

Of the 7 plants tested, only those that showed antibacterial activity (Cinnamon and Thyme) against some of the selected poultry pathogens were selected for further tests to calculate their MIC by dilution method. This test was performed in sterile 96-well microplates and macroplates. The dilution procedure, as explained in Appendix C was followed. The microdilution was performed in 96-well microtiter plates with U-shaped wells while the macrodilution technique as described by the National Committee for Clinical Laboratory Standards was followed (de Paiva et al, 2003). In brief, the cultures were diluted in Müeller-Hinton broth at a density adjusted to a 0.5 McFarland turbidity. The final inoculum was 5×10^5 CFU/ml of bacterial colony. Controls with 0.5 ml of only culture medium or others with plant extracts were used in the tests. The wells were filled with 100 µl of sterile H_2O and 100 µl of the plant extracts were added to the wells by serial two fold dilution from the suspension of plant extract stock solution. Each well was inoculated with 100 µl of 0.5 McFarland standard bacterial suspension so

that each well got 5 x 10^5 CFU/ml. The plates were covered, placed in plastic bags and incubated at 37°C for 24 hrs. In this study, the MIC was the lowest concentration of plant extracts that exhibited no growth of the organism in the wells by visual reading.

Feeding trial

Male broilers (Hub bird females X Ross males) (n=512), vaccinated for Marek's only, were obtained from George's Hatchery, Harrisonburg, VA USA, on the day-of-hatch and randomly assigned to 96 Petersime battery cages (8 birds/pen). Chicks were assigned randomly to the dietary treatment groups meeting NRC requirements (NRC, 1994) as shown in Table 8.

Diet 1 - No added plant extract or antibiotic (Negative control).

Diet 2 - Contained BMD (50 g/ton) (Positive control).

Diet 3 –Basal diet plus low level of cinnamon extract (290 gm/100 kg of feed).

Diet 4 – Basal diet plus high level of cinnamon extract (580 gm/100 kg of feed).

Diet 5 – Basal diet plus low level of thyme extract (290 gm/100 kg of feed).

Diet 6 – Basal diet plus high level of thyme extract (580 gm/100 kg of feed).

There were 16 pens assigned to both Treatments 1 and 2. Eight pens were assigned to each of the other treatments. Effectively, there were three levels of each extract: 0, low, and high. The low dose was equivalent to the in-vitro antibacterial response equivalent to the normal level (50 g/ton of feed) of bacitracin added to broiler diets while the high level was twice that dose. Body weight by pen and feed consumption were recorded at 1, 2 and 3 wks of age. Weight of the birds were determined to make sure equals betweens the treatments. Feed and water were provide *ad lib*.

Statistical Analysis

Since the readings of control (distilled water) in the *in vitro* antibacterial studies of medicinal plant were zero, the data was analyzed by simple arithmetic means of the different extracts and standard error compare to the control. No other statistical test was applied to show significance since the extracts

were either positive or negative for the antibacterial studies.

Data of the feeding trial were analyzed using ANOVA (SAS/STAT User's Guide 6.03, SAS Institute, Inc. Cary, NC) for body weight. Contrasts were used within type of diet to evaluate the effects of extract source and level. Linear equations were derived for each plant source with the basal diet (Treatment1) used for each plant source. Where significant differences were found among treatments, comparisons among means were separated using a Duncan's Multiple Range test. Calculations were made using the General Linear model of SAS program (SAS institute Inc., 1997). Significance implies $P \leq 0.05$.

Results

Antibacterial activity

The ethanol extract of the medicinal plants *C. longa*, *Z. officinale*, *P. nigrum*, *L. nobilis*, and *S. aromaticum*, showed no antibacterial activity against *E. coli*, *S. typhimurium*, *E. faecium*, or *E. faecalis*. at specific doses mentioned in Table 3.

The cinnamon extract (CE) exhibited antibacterial activity against *E. coli*, *S. typhimurium*, and *E. faecalis,* but no activity against *E. faecium* at the doses shown in Table-4. The range of the zone of inhibition was 21 to 29 mm (Table 4). The thyme extract (TE) exhibited antibacterial activity against *E. coli*, *E. faecalis*, and *E. faecium,* but no activity against *S. typhimurium* at the dose shown in Table-3 and 4, by the disk diffusion method. However, the dilution method showed antibacterial activity against *S. typhimurim* (Table 5). The range of the zone of inhibition was 17 to 21 mm (Table 4).

The MIC of CE tested against *E. coli*, *S. typhimurium*, and *E. faecalis* were found to be less than 31.25 mg/ml (Table 5). The MIC of the TE against *E. coli*, *E. faecalis*, and *E. faecium* ranged from 25 mg to 125 mg/ml (Table 5) and against *S. typhimurium* was 250 mg/ml. The MIC of bacitracin tested against *E. coli*, *S. typhimurium*, *E. faecalis*, and *E. faecium* ranged between 560 µg to 1120 µg/ml by dilution method (Table-5).

Feeding trial

In a 21 d feeding trail, the high level (HCE) or low level (LCE) of cinnamon extract had no significant effect on body weight gain compare to the remaining treatments ($P > 0.05$) (Table 7). Likewise no differences in body weight gain were found from 0-7 d, 7-14 d or 14-21 d for the LCE or HCE treatments compared to any other treatments (Table 7).

Cumulative body weight gain at 21 d for the LTE group was not different from the remaining treatments. However, HTE significantly reduced body weight compared to the NC and PC groups ($P < 0.02$) (Table 7). A significant reduction in body weight gain was observed for the HTE group at 0-7 d (P

< 0.003) and 7-14 d ($P < 0.05$), but not at the 14-21 d period ($P > 0.05$) compare to the NC and PC groups (Table 7).

Feed consumption

Cumulative feed consumption was not altered by LCE or HCE compare to the NC and PC treatments at 14 or 21 d ($P > 0.05$). Feed consumption of LCE and HCE diets was not affected at 7-14 d or 14-21 d compared to the other treatment groups ($P > 0.05$) (Table 8). There were no differences in feed consumption between the PC and NC groups at 14 or 21 d ($P > 0.05$) (Table 8). HTE did not change feed consumption compare to any other treatment group ($P > 0.05$).

Feed Efficiency

At 21 d, there was no significant difference in cumulative feed efficiency of the HCE group compared to the PC and NC groups. However the LCE group had improved feed efficiency compared to the PC and NC groups ($P < 0.03$) (Table 9). Improved cumulative feed efficiency was found at 14 d for the LCE group compared to PC group ($P < 0.02$). There were no differences in feed efficiency at 14-21 d between the LCE or HCE groups compared to the PC and NC groups ($P > 0.05$) (Table 9).

At 21 d, there was no significant difference in cumulative feed efficiency between the LTE or HTE groups compared to the PC groups ($P > 0.05$) (Table 9). In addition, no difference was noted at 14 d (Table 9). There was no difference in feed efficiency for the period of 7-14 d, and 14-21 d between the LTE or HTE groups (Table 9).

Discussion

The antimicrobial effect of the medicinal plants is well documented (Valero and Salmeron, 2003). The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (Kone et al., 2004). In this study, using the disk diffusion method it was observed that extracts of cinnamon and thyme produce antibacterial activity against both gram negative and gram positive pathogens. Results of this study confirmed the observation of earlier studies (Yuste and Fung, 2004; Fan and Chen, 2001).

The cinnamon extract (CE) was found to be effective against *E. coli*, *S. typhimurium*, and *E. faecalis*. This effect is in agreement with other researchers regarding the antibacterial effect against *E. coli*, however there is a difference in the concentration of extract of cinnamon at which we found antibacterial activity (Mau et al., 2001; Yuste and Fung, 2004). Using the disk diffusion method, the concentration at which antibacterial activity was found was much higher than that of mentioned by the above mentioned authors. The MIC results of cinnamon by dilution method in our studies support an earlier finding by Quale et al. (1996).

In our studies the thyme extract (TE) showed antibacterial activity. This result supports the findings of many authors (Dursun et al., 2003; Nevas et al., 2004; Fan and Chen, 2001). Thyme was found to be effective against *E. coli*, *E. faecalis*, and *E. faecium* but not *S. typhimurium* by the disk diffusion method. However, using the dilution method TE also had antibacterial activity against *S. thypimurium*. This variation may be due the fact that the dose applied using the disc diffusion method (30 mg) was less than in the dilution method (260 mg).

There was no antibacterial activity in extracts of *C. longa*, *Z. officinale*, *P. nigrum*, *L. nobilis*, or *S. aromaticum* against the tested pathogens at the specific dose. Our results are contradictory with some researchers who reported antibacterial activity of above plants against gram positive and gram negative bacteria (Tang et al., 1992; Dorman and Deans, 2000; Habsah et al.,2000; Fang et al, 2003; Kalemba and

Kunicka, 2003). This variation may be because of the dose used in this study, the method of extraction of medicinal plants, the method of antibacterial study, the genetic variation of plant, age of the plant or the environment.

The addition of sub-therapeutic levels of antibiotics to broiler feed causes an increase in weight gain (Jones and Ricke, 2003). The plant extracts used in the present study which showed antibacterial activity *in vitro* did not results in any significant increase in body weight gain compared to the positive or negative control. The results, however, were encouraging compared to the negative control for the HCE, since the means of the HCE are higher than the NC. Furthermore, TE was found to decrease body weight significantly.

At the conclusion of 21 d feeding trial, the high level of cinnamon (HCE) did not results in any significant change in body weight gain compare to the PC treatment. This result is encouraging since the means of 0-21d data are for NC is lower (non-significant) than the means for HCE. Although non-significant, there was a trend towards increased body weight in birds fed with the HCE diets compare to NC diet. There are no other published reports on this effect.

There was a dose-dependent effect of LCE and HCE on increasing the body weight gain at 7-14 days ($P=0.02$). This result suggests the need for further research on the effect of cinnamon as a possible feed additive to replace antibiotics in broiler diets.

There was conflicting evidence of the relationship between antibacterial activity of thyme extract (TE) *in vitro* and its ability to increase body weight gain when provided in the diet for 21 d in broilers. Thyme had antibacterial activity *in vitro*, however, when added to the broiler diet, body weight gain decreased significantly during the 21 d feeding trial ($P < 0.02$). These results are contradictory since addition of antibacterial compounds to broiler diets generally increases the body weight gain (Dang and Visek, 1965; Boyd and Edward, 1967; Barnes et al., 1978; Fuller et al., 1983). Thyme extract may possess active compounds that produce antibacterial activity *in vitro*, but it may also possess an active compound responsible for reducing the body weight *in vivo.* The thyme though produced antibacterial activity *in vitro* but when given in diet, might be losing its antibacterial activity because of action of

different enzymes while the process of its digestion and absorption.

The decrease in the body weight induced by thyme may have implications with regards to obesity. A significant reduction in weight gain was observed at 0-7 d ($P < 0.003$) and 7-14 d ($P < 0.05$) but not at 14-21 d period ($P > 0.05$). This may indicate that adding thyme in the diet from 14-21 d is not as effective in reducing body weight as it was from 0-7 and 0-21 d. The results of thyme in this study provide a strong basis for further research in obese subjects to reduce body weight. The reduction in body weight induced by thyme was observed without a change in feed consumption.

Feed consumption was not affected by the LCE or HCE compare to the PC treatment at 14 or 21 d ($P > 0.05$). Periodic feed consumption was also not affected at 7-14 or 14-21 d ($P > 0.05$). This suggests that the CE did not cause a feed aversion. Also, feed consumption was not affected by LTE or HTE at 7, 14, or 21 d. This also suggests that the thyme extract did not affect the bird's perception of taste of the diets.

Feed efficiency was found to be affected when the diet was supplied with LCE compared to the PC, NC or HCE ($P = 0.03$). These results suggest that there is dose-dependent variation in the feed efficiency of HCE and LCE. Increasing the dose of cinnamon increased feed efficiency. This finding is important basis for the dose-dependent studies of cinnamon to find an alternative to AGP since improved feed efficiency will decrease the cost of production. Since HCE showed better feed efficiency than LCE, increasing dose of HCE may increase feed efficiency. However, we did not find any scientific reports to support these views.

IMPLICATIONS

Antibiotic growth promoters (AGP) have made a tremendous contribution to the profitability of the poultry industry. However, as a consequence of the increasing concern about the potential public health problems because of antibiotic resistant strains of bacteria, poultry nutritionists are being challenged to develop an alternative for AGP. If herbal alternative to AGP can be found, poultry nutritionists could formulate a ration that would meet the needs of the commercial broiler industry without using AGP. This study showed that herbal extracts, particularly a cinaamon extract, when added in the broiler diet, may have a similar effect as that of AGP. This study also showed that adding thyme in broiler diets may decrease body weight significantly compared to the diet with AGP, while not affecting the feed consumption. This result is helpful for further research on reducing body weight in the obese subjects.

TABLE 1. List of plant material and type of extracts tested

Plants	Parts of plant investigated	Extract type
Cinnamomum cassia	Bark	80% Ethanol
Curcuma longa	Rhizomes	80% Ethanol
Laurus nobilis	Leaves	80% Ethanol
Piper nigrum	Fruits	80% Ethanol
Syzgium aromaticum	Fruits	80% Ethanol
Thymus vulgaris	Leaves	80% Ethanol
Zingiber officinale	Rhizomes	80% Ethanol

TABLE 2. List of the bacteria tested in this study

Bacterial Strains	Gram strain type	Details of the bacterial strains used
E. coli	Negative	Untyped isolates collected at Shenandoah Valley, through Vet Med School, Virginia Tech.
S. typhimurium	Negative	MTCC[2]
E. faecium	Positive	ATCC[1] 19434
E faecalis	Positive	ATCC[1] 19433

[1] American type culture collection.
[2] Microbiology teaching culture collection of Virginia Tech, USA.

TABLE 3. Antibacterial effect of different concentrations of medicinal plant extracts on common poultry pathogens

Medicinal plants extracts	Extract dilution	Corresponding effects on microorganism			
		E .coli	*S. thyphimurium*	*E. faecalis*	*E. faecium*
Zingiber officinale	2 gm/2ml	-[1]	-	-	-
	2 gm/3ml	-	-	-	-
	2 gm/4ml	-	-	-	-
	2 gm/5ml	-	-	-	-
Curcuma longa	2 gm/2ml	-	-	-	-
	2 gm/3ml	-	-	-	-
	2 gm/4ml	-	-	-	-
	2 gm/5ml	-	-	-	-
				-	-
Piper nigrum	2 gm/2ml	-	-	-	-
	2 gm/3ml	-	-	-	-
	2 gm/4ml	-	-	-	-
	2 gm/5ml	-	-	-	-
Cinnamomum cassia	1 gm/1ml	+[2]	+	+	-
	1 gm/3ml	+	+	+	-
	1 gm/4ml	+	+	+	-
	1 gm/5ml	+	-	-	-
	1 gm/6ml	-	-	-	-
Laurus nobilis	1 gm/3ml	-	-	-	-
	1 gm/4ml	-	-	-	-
	1 gm/5ml	-	-	-	-
	1 gm/6ml	-	-	-	-
Syzgium aromaticum	1 gm/3ml	-	-	-	-
	1 gm/4ml	-	-	-	-
	1 gm/5ml	-	-	-	-
	1 gm/6ml	-	-	-	-
Thymus vulgaris	0.5 gm/3ml	+	-	+	+
	0.5 gm/4ml	+	-	+	+
	0.5 gm/5ml	+	-	+	-
	0.5 gm/6ml	-	-	+	-

[1]"-" refers to no antibacterial effect of corresponding medicinal plant to the mentioned bacterial strain at mentioned dose.

[2]"+" refers to antibacterial effect of corresponding medicinal plant to the mentioned bacterial strain at mentioned dose.

TABLE 4. Antibacterial activity of specific concentration of medicinal plant extract compare to control by disc diffusion method

		Antibacterial activity			
Medicinal Plants	Concentration/disk	*E.coli*	*S. typhimurium*	*E.faecalis*	*E. facecium*
Z. officinale	130mg	Negative[1]	Negative	Negative	Negative
C. longa	130mg	Negative	Negative	Negative	Negative
C. cassia	130mg	20.75 ± 0.144	20.73 ± 0.144	20.75 ± 0.204	Negative
S. aromaticum	66.6mg	Negative	Negative	Negative	Negative
P. nigrum	130mg	Negative	Negative	Negative	Negative
L. nobilis	66.6mg	Negative	Negative	Negative	Negative
T. vulgaris	30mg	19.25 ± 0.141	Negative	21.5 ± 0.288	20.75 ± 0.288
Tween-80	-	Negative	Negative	Negative	Negative
Distilled Water	-	Negative	Negative	Negative	Negative

[1]"Negative" refers to no antibacterial effect of corresponding medicinal plant to the mentioned bacterial strain at mentioned dose.

TABLE 5. Minimum inhibitory concentration (MIC) of different extracts and bacitracin by dilution method

Test material	Bacteria	MIC mg/ml
Cinnamomum cassia	*E. coli*	≤ 31.25 mg/ml
Cinnamomum cassia	*S. typhmuriumi*	≤ 31.25 mg/ml
Cinnamomum cassia	*E. faecalis*	≤ 31.25 mg/ml
Cinnamomum cassia	*E. faecium*	ND
Thymus vulgaris	*E. coli*	≤ 62.5 mg/ml
Thymus vulgaris	*S. typhimurium*	≤ 250 mg/ml
Thymus vulgaris	*E. faecalis*	≤ 31.25 mg/ml
Thymus vulgaris	*E. faecium*	≤ 31.25 mg/ml
Bacitracin	*E. coli*	≤ 560 µg/ml
Bacitracin	*E. faecium*	≤ 1120 µg/ml
Bacitracin	*E. faecium*	≤ 1120 µg/ml
Bacitracin	*S. typhimurium*	≤ 1120 µg/ml

TABLE-6. Percentage of the different ingredients in the diets[1]

Ingredients	Diet 1	Diet 2.	Diet 3	Diet 4	Diet 5	Diet 6
			(% in diet)			
Corn	55.52	55.52	55.52	55.52	55.52	55.52
Soybean Meal (48% CP)	36.57	36.57	36.57	36.57	36.57	36.57
Dicalcium Phosphate	1.93	1.93	1.93	1.93	1.93	1.93
Fat-feed Grade Tallow	2.96	2.96	2.96	2.96	2.96	2.96
Limestone	1.16	1.16	1.16	1.16	1.16	1.16
Thyme extract low level					0.29	
Thyme extract high level						0.58
Cinnamon extract low level			0.29			
Cinnamon extract high level				0.58		
Salt	0.39	0.39	0.39	0.39	0.39	0.39
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.28	0.28	0.28	0.28	0.28	0.28
Poultry Vitamin Premix	0.1	0.1	0.1	0.1	0.1	0.1
Poultry Trace Min Premix	0.1	0.1	0.1	0.1	0.1	0.1
0.06% Selenium Premix	0.05	0.05	0.05	0.05	0.05	0.05
Choline Chloride	0.06	0.06	0.06	0.06	0.06	0.06
BMD50		0.05				
Sand	0.63	0.58	0.34	0.05	0.34	0.05
Total	100	100	100	100	100	100

[1]Diet # 1 - No added plant extract or antibiotic (negative control); Diet # 2 - Diet #1 with BMD (50g/ton) (positive control).

Diet # 3 - Diet #1 with low level of Cinnamon extract; Diet # 4 - Diet #1 with high level of Cinnamon extract; Diet # 5 - Diet #1 with low level of Thyme extract; Diet # 6 - Diet #1 with high level of Thyme extract.

Table 7. Effects of different dietary treatments on body weight gain (g) in broilers[1]

Dietary treatments	0-7 d	0-14 d	0-21 d	7-14 d	14-21 d
Negative (no antibiotic/extract)	84.23 ± 1.98 [a]	305.85 ± 5.62[a]	665.29 ± 10.81[a]	221.62 ± 4.48 [a]	320.27 ± 6.31 [a]
Positive (50 gm/ton of BMD)	91.24 ± 1.98 [a]	310.91 ± 5.62[a]	671.34 ± 10.81[a]	219.67 ± 4.48 [a]	321.67 ± 6.31 [a]
Cinnamon low (290 gm/100 kg feed)	86.13 ± 3.13 [a]	290.54 ± 8.89[ab]	649.18 ± 17.10[a]	204.41 ± 7.09 [a]	319.82 ± 9.98 [a]
Cinnamon high (580gm/100 kg of diet)	86.36 ± 3.13 [a]	309.18 ± 8.89[a]	670.29 ± 17.10[a]	222.82 ± 7.09 [a]	321.76 ± 9.98 [a]
Thyme low (290 gm/100 kg feed)	86.36 ± 3.13 [a]	292.19 ± 8.89[ab]	641.75 ± 17.10[ab]	208.72 ± 7.09 [a]	310.55 ± 9.98 [a]
Thyme high (580 gm/100 kg of diet)	75.05 ± 3.13 [b]	279.18 ± 8.89[b]	616.41 ± 17.10[b]	204.13 ± 7.09 [a]	298.84 ± 9.98 [a]

[1] Means within column with no common superscript differ significantly ($P < 0.05$).

Table 8. Effect of different dietary treatments on feed consumption (g) in broilers[1]

Dietary treatment	0-7 d	0-14 d	0-21 d	7-14 d	14-21d
Negative (no antibiotic/extract)	134.74 ± 2.05[b]	426.20 ± 6.73 [a]	881.26 ± 13.01 [a]	291.45 ± 5.65 [a]	455.06 ± 7.57 [a]
Positive (BMD 50 gm/ton feed)	143.70 ± 2.05[a]	435.68 ± 6.73 [a]	889.76 ± 13.01 [a]	291.97 ± 5.65 [a]	454.08 ± 7.57 [a]
Cinnamon low (290 gm/100 kg feed)	143.35 ± 3.24[a]	438.39 ± 10.65 [a]	891.04 ± 20.57 [a]	295.04 ± 8.93 [a]	452.64 ± 11.97 [a]
Cinnamon high (580 gm/100 kg feed)	145.78 ± 3.24[a]	436.14 ± 10.65 [a]	895.22 ± 20.57 [a]	290.36 ± 8.93 [a]	459.08 ± 11.97 [a]
Thyme low (290 gm/100 kg feed)	142.76 ± 3.24[a]	422.99 ± 10.65 [a]	866.55 ± 20.57 [a]	280.23 ± 8.93 [a]	443.55 ± 11.97 [a]
Thyme high (580 gm/100 kg feed)	134.23 ± 3.24[b]	406.57 ± 10.65 [a]	833.11 ± 20.57 [a]	272.34 ± 8.93 [a]	426.54 ± 11.97 [a]

[1]Means within column with no common superscript differ significantly ($P < 0.05$).

Table 9. Effect of different dietary treatments on feed efficiency in broilers[1]

Dietary treatments	0-7 d	0-14 d	0-21 d	7-14 d	14-21 d
Negative (no antibiotic/extracts)	0.624 ± 0.011^{a}	0.717 ± 0.007^{a}	0.710 ± 0.005^{a}	0.760 ± 0.009^{a}	0.703 ± 0.005^{a}
Positive (50 gm/ton feed)	0.634 ± 0.011^{a}	0.712 ± 0.007^{a}	0.710 ± 0.005^{a}	0.751 ± 0.009^{a}	0.708 ± 0.005^{a}
Cinnamon low (290 gm/100 kg feed)	0.598 ± 0.018^{ab}	0.662 ± 0.011^{b}	0.684 ± 0.008^{b}	0.694 ± 0.015^{b}	0.706 ± 0.009^{a}
Cinnamon high (580 gm/100 kg feed)	0.589 ± 0.018^{ab}	0.708 ± 0.011^{a}	0.704 ± 0.008^{ab}	0.767 ± 0.015^{a}	0.701 ± 0.009^{a}
Thyme low (290 gm/100 kg feed)	0.582 ± 0.018^{ab}	0.691 ± 0.011^{a}	0.695 ± 0.008^{ab}	0.748 ± 0.015^{a}	0.699 ± 0.009^{a}
Thyme high (580 gm/100 kg feed)	0.556 ± 0.018^{b}	0.685 ± 0.011^{ab}	0.692 ± 0.008^{ab}	0.748 ± 0.015^{a}	0.699 ± 0.009^{a}

[1]Means within column with no common superscript differ significantly ($P < 0.05$).

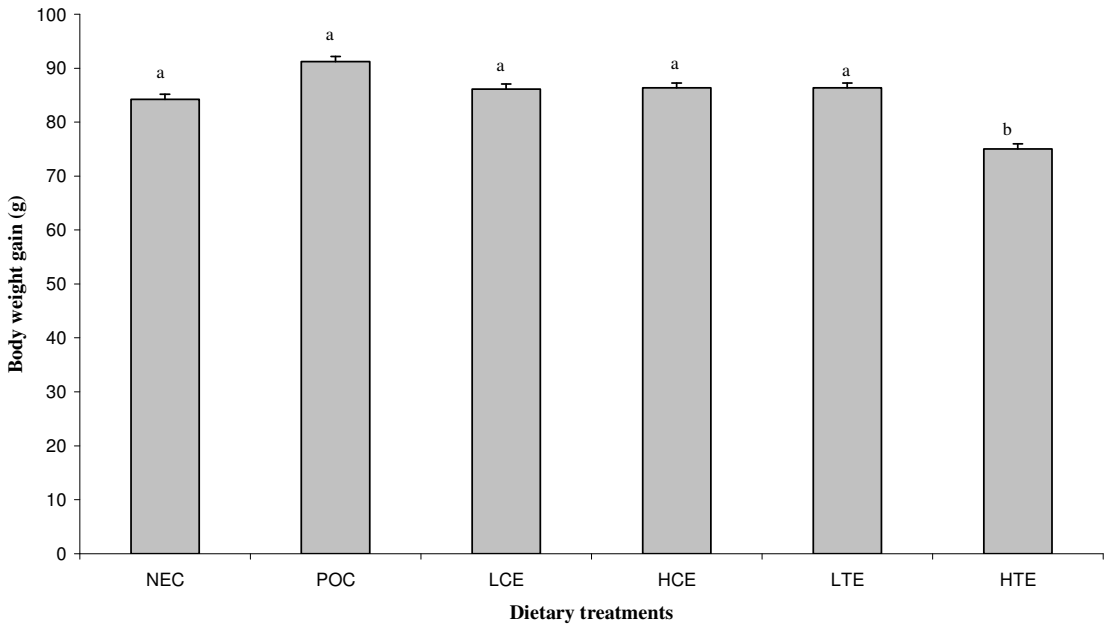

FIGURE 1. Effects of different diets on body weight gain (0-7 d) in broilers. Values are LS means with pooled SEM. NEC represents negative control with no added antibiotic or plant extracts; POC represents positive control with BMD (50 g/ton of feed); LCE represents low level of cinnamon extract (290 gm/100 kg of feed); HCE represents high level of cinnamon extract (580 gm/100 kg of feed); LTE represents low level of thyme extract (290 gm/ 100 kg of feed); HTE represents high level of thyme extract (580 gm/100 kg of feed). [a-b] Different letters above each bar indicate significant difference between means ($P < 0.05$).

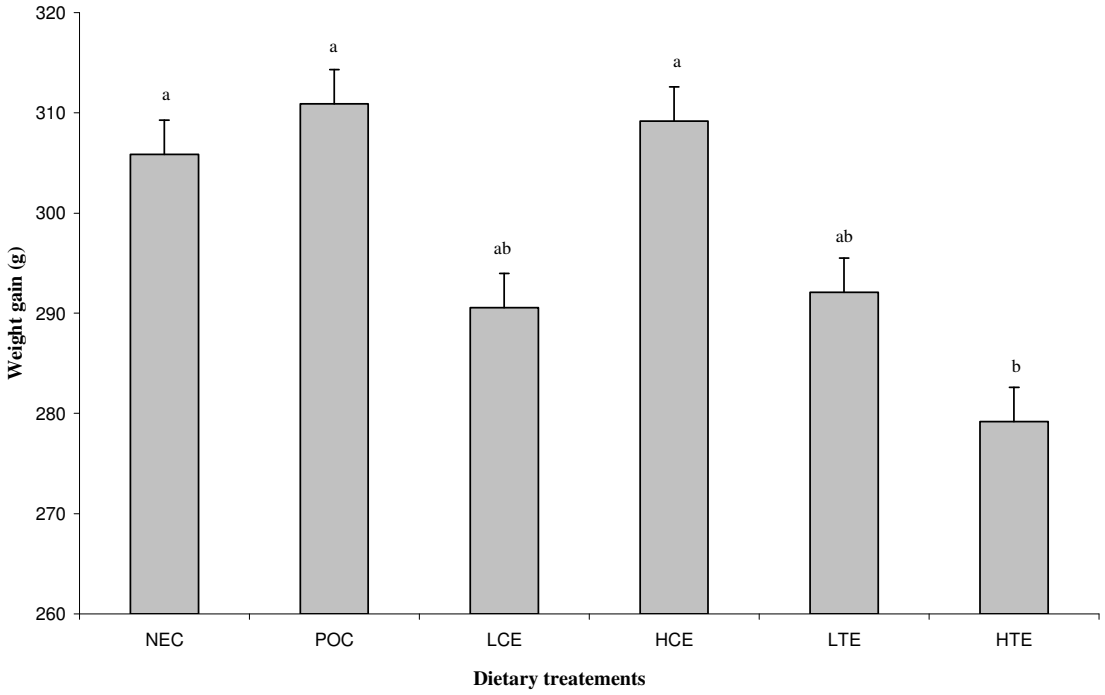

FIGURE 2. Effects of different diets on body weight gain (0-14 d) in broilers. Values are LS means with pooled SEM. NEC represents negative control with no added antibiotic or plant extracts; POC represents positive control with BMD (50g/ton of feed); LCE represents low level of cinnamon extract (290 gm/100 kg of feed); HCE represents high level of cinnamon extract (580 gm/100 kg of feed); LTE represents low level of thyme extract (290 gm/ 100 kg of feed); HTE represents high level of thyme extract (580 gm/100 kg of feed). [a-ab] Different letters above each bar indicate significant difference between means ($P < 0.05$).

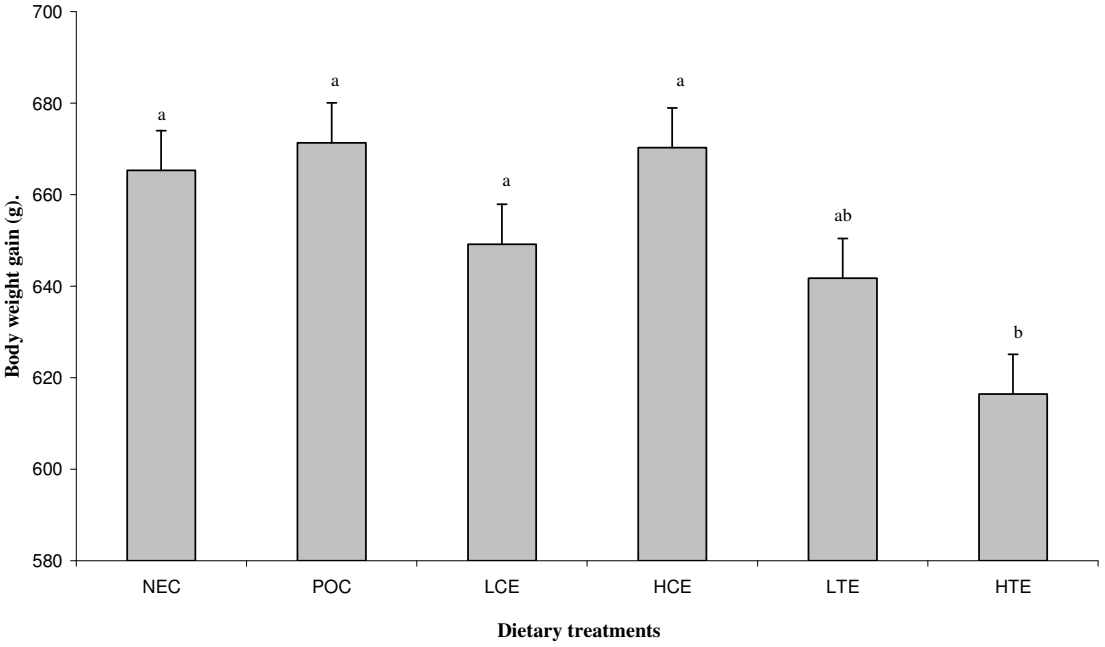

FIGURE 3. Effects of different diets on body weight gain (0-21 d) in broilers. Values are LS means with pooled SEM. NEC represents negative control with no added antibiotic or plant extracts; POC represents positive control with BMD (50g/ton of feed); LCE represents low level of cinnamon extract (290 gm/100 kg of feed); HCE represents high level of cinnamon extract (580 gm/100 kg of feed); LTE represents low level of thyme extract (290 gm/ 100 kg of feed); HTE represents high level of thyme extract (580 gm/100 kg of feed). [a-ab] Different letters above each bar indicate significant difference between means ($P < 0.05$).

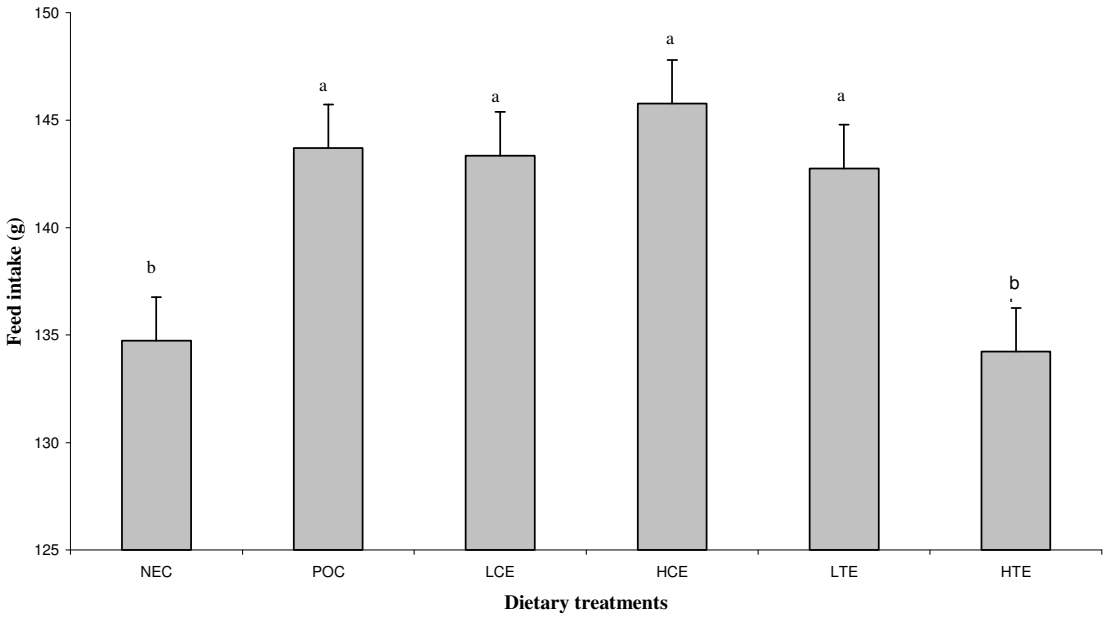

FIGURE 4. Effects of different diets on feed consumption (0-7 d) in broilers. Values are LS means with pooled SEM. NEC represents negative control with no added antibiotic or plant extracts; POC represents positive control with BMD (50g/ton of feed); LCE represents low level of cinnamon extract (290 gm/100 kg of feed); HCE represents high level of cinnamon extract (580 gm/100 kg of feed); LTE represents low level of thyme extract (290 gm/ 100 kg of feed); HTE represents high level of thyme extract (580 gm/100 kg of feed). [a-b] Different letters above each bar indicate significant difference between means ($P < 0.05$).

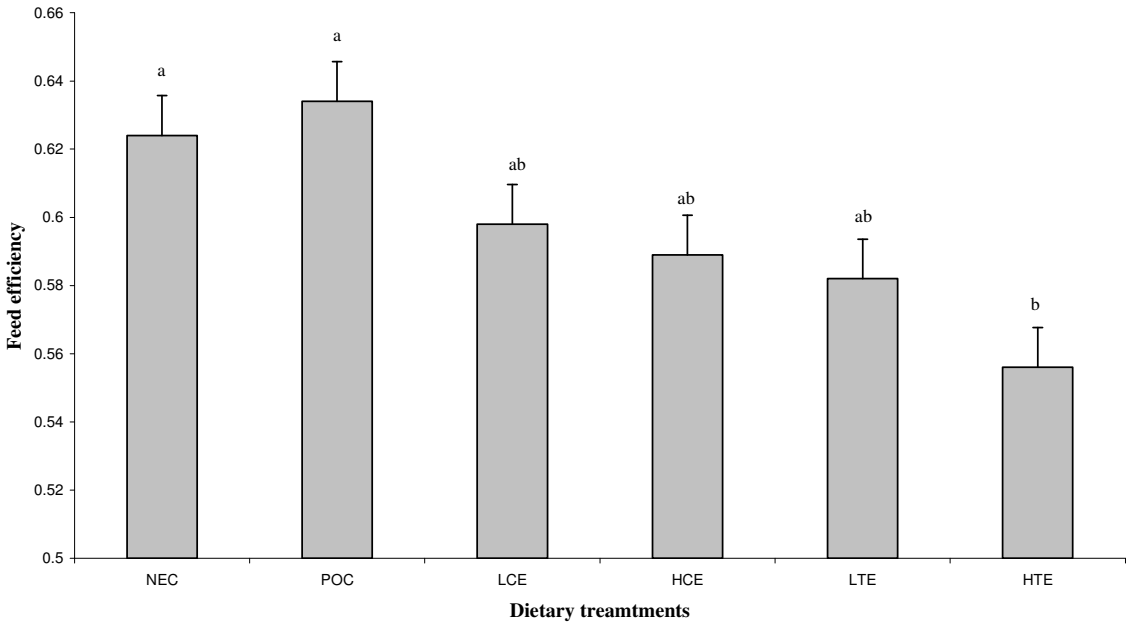

FIGURE 5. Effects of different diets on body weight gain (0-7 d) in broilers. Values are LS means with pooled SEM. NEC represents negative control with no added antibiotic or plant extracts; POC represents positive control with BMD (50g/ton of feed); LCE represents low level of cinannamon extract (290 gm/100 kg of feed); HCE represents high level of cinnamon extract (580 gm/100 kg of feed); LTE represents low level of thyme extract (290 gm/ 100 kg of feed); HTE represents high level of thyme extract (580 gm/100 kg of feed). [a-ab] Different letters above each bar indicate significant difference between means ($P < 0.05$).

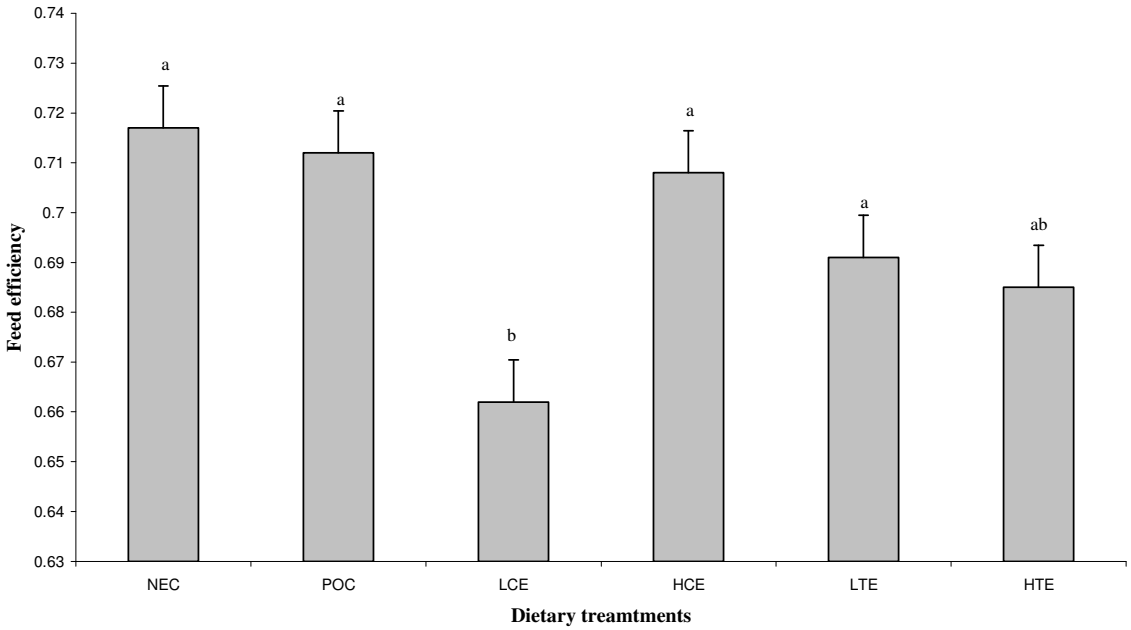

FIGURE 6. Effects of different diets on body weight gain (0-14 d) in broilers. Values are LS means with pooled SEM. NEC represents negative control with no added antibiotic or plant extracts; POC represents positive control with BMD (50g/ton of feed); LCE represents low level of cinnamon extract (290 gm/100 kg of feed); HCE represents high level of cinnamon extract (580 gm/100 kg of feed); LTE represents low level of thyme extract (290 gm/ 100 kg of feed); HTE represents high level of thyme extract (580 gm/100 kg of feed). $^{a\text{-}ab}$ Different letters above each bar indicate significant difference between means ($P < 0.05$).

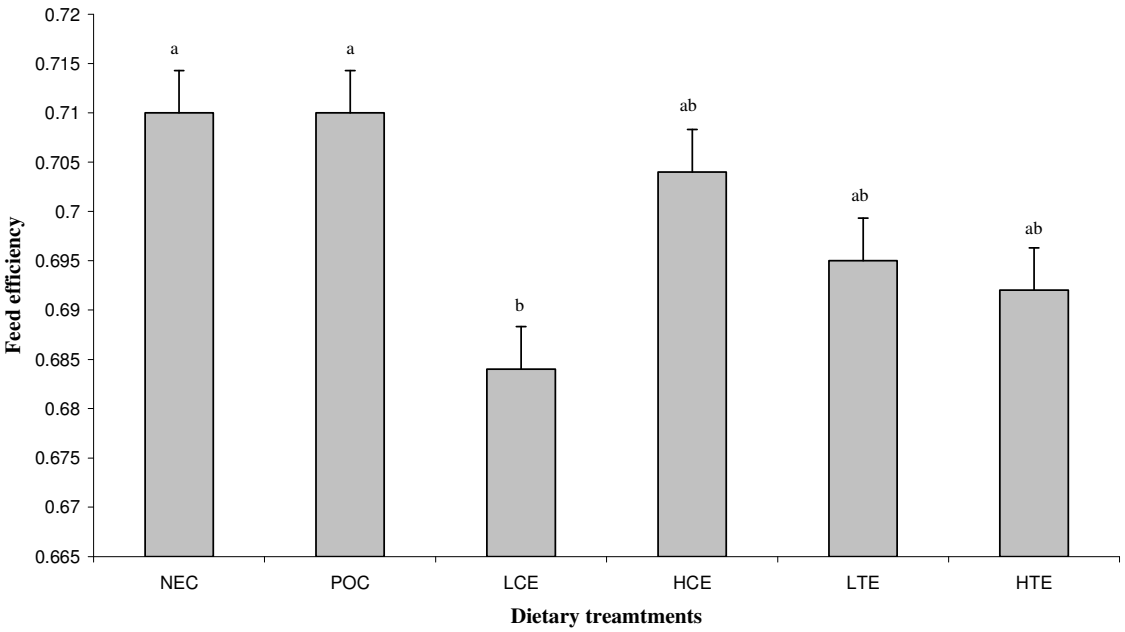

FIGURE 7. Effects of different diets on feed efficiency (0-21 d) in broilers. Values are LS means with pooled SEM. NEC represents negative control with no added antibiotic or plant extracts; POC represents positive control with BMD (50 g/ton of feed); LCE represents low level of cinnamon extract (290 gm/100 kg of feed); HCE represents high level of cinnamon extract (580 gm/100 kg of feed); LTE represents low level of thyme extract (290 gm/100 kg of feed); HTE represents high level of thyme extract (580 gm/100 kg of feed). [a-ab] Different letters above each bar indicate significant difference etween means ($P < 0.05$).

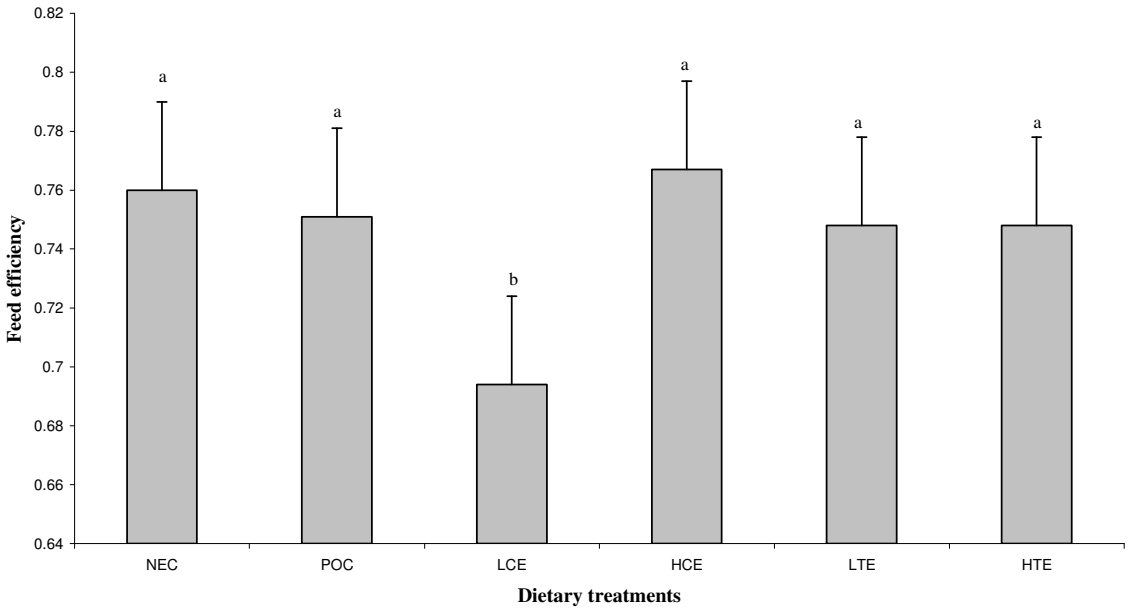

FIGURE 8. Effects of different diets on feed efficiency (7-14 d) in broilers. Values are LS means with pooled SEM. NEC represents negative control with no added antibiotic or plant extracts; POC represents positive control with BMD (50 g/ton of feed); LCE represents low level of cinnamon extract (290 gm/100 kg of feed); HCE represents high level of cinnamon extract (580 gm/100 kg of feed); LTE represents low level of thyme extract (290 gm/100 kg of feed); HTE represents high level of thyme extract (580 gm/100 kg of feed). [a-b] Different letters above each bar indicate significant difference between means ($P < 0.05$).

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Appendix A

Flow chart for the experiment

PHASE –I (Preparation of extracts)

Selection of Medicinal Plants having

Antimicrobial activity on the basis of literature

↓

Grinding of plant material to powder form.

↓

Extraction of selected plant material powder by Maceration method

↓

Preparation of extract

PHASE –II (*In-vitro* Antibacterial studies)

Antimicrobial Screening by Agar Gel Diffusion Method .

↓

Confirmation of Anti- bacterial activity of the selected plant material.

↓

Selection of the plants on the basis of its invitro antibacterial activity

↓

MIC of the selected plant material and antibiotics

↓

Calculation of approximate dose of extract for feeding trails

PHASE-III (in-vivo studies)

Preparation of different diets for broilers

↓

21d experiment on Broilers of these plant extracts.

↓

Data

↓

Statistical Analysis

↓

Results

Appendix B

Kirby –Bauer method (Disk diffusion method) (Drago et al., 1999)

1. Label Mueller –Hinton agar (MH) plates with name, date of inoculation, experiment number, and the name of bacterium.
2. Inoculate each agar plate with a different organism in this study as follows-
    a. Pipette 2 ml of sterile distilled water into sterile tube. Label each according to the type of bacteria and specific strain i.e. wild or ATCC
    b. Aseptically remove the cotton swab from packet and pick up 3-5 isolated colonies, from the pure culture.
    c. Emulsify the culture in labeled 2 ml H_2O tube to match the turbidity of sample to the 0.5 McFarland standard.
    d. Thoroughly mix the broth culture.
    e. Aseptically remove another cotton swab from packet and immerse its tip in the turbid broth culture. Lift the cotton tip above the broth, and roll the tip against the inside tube so as to squeeze the excess fluid from the cotton swab.
    f. Inoculate the entire surface of one agar plate with this culture moistened cotton tip. Streak the agar surface from edge to edge from top to bottom. Then rotate the plate 90^0 to the right and repeat the procedure. The plate is again rotated 45^0 to the right and streaked from top to bottom back and forth. Rub the cotton tip around the outside edge of the agar surface. This should provide a fine lawn of growth.
    g. Discard all material as appropriate for biohazard waste.
    h. Wait 5- 6 minutes after inoculation to allow the liquid culture to soak into the agar surface.
3. Using sterile forceps, remove the discs containing the different concentrations of the different plant extracts used in this study. Apply this disc to plates so that each organism is tested against all plant extracts.

4. Incubate the inoculated plates for 24h at 37^0C
5. Measure the zone of inhibition in millimeters. Record all findings.
6. Properly discard all plates as biohazard material.

Appendix: C

Dilution method for antibacterial testing to calculate MIC *(de Paiva et al, 2003)*

1. Label the Mueller –Hinton broth tubes with name, date of inoculation, experiment number, and name of bacterium.
2. The microdilution was performed in 96-well microtitre plates with U-shaped wells, label with name, date of inoculation, experiment number, and name of bacterium.
3. Add 0.1 ml (100 µL) of sterile H_20 in each well in rows A, C, E, and G.
4. Add 0.1 ml (100 µL) of sterile H_20 in 2 wells in row H for control (Total wells=50).
5. A two-fold dilution reduces the concentration of a solution by a factor of two that reduces the original concentration by one half. A series of two-fold dilutions is described as two-fold serial dilutions. Use the micropipette to dispense 100 µL of test material (cinnamon, thyme, bacitracin) to the first well and mix. This is the first two-fold dilution. Use the micropipette with the same tip to carry out a second two-fold dilution. Continue the series of two-fold dilutions until the last well of the microwell plate. Discard the quantity in the micropipette from this well.
6. Add 100 µL of cinnamon in row A in each of 12 wells by two fold serial dilution. Add 100 µL of thyme extract in row C in each of 12 wells by two fold serial dilution as explained as above in #5.
7. Add 100 µL of Bacitracin n each of 12 wells in E row by two fold serial dilution as explained as above in # 5.
8. Add 100 µL of tetracycline in each of 12 wells in G row, by two fold serial dilution as explained as above in # 5.
9. Add 100 µL of 0.5 McFarland bacterial suspension in respective wells and also in control wells. The bacterial suspension was prepared in T-soy broth .
10. The plates were sealed, placed in plastic bags and incubated at 37^0C for 24 h.
11. Measure the MIC which is defined as the lowest concentration of extract that exhibited no growth by visual reading. It is expressed in mg or µg/ml.
12. Discard the plates by appropriate procedure.

Appendix D

Calculation of final dose of both the extracts

1. Thyme

MIC of Thyme

Bacterium	per mL
E.coli	62.5 > 31.25 mg
E.faecium	≤ 31.25mg
E.faecium	≤ 31.25mg
S. typhimurium	> 125 mg

MIC of Bacitracin = 560 mcg/mL

Basic of calculation -

We need 1200 gm of feed/bird for first 3 wks of expt.
Total number of birds per treatment = 64
64 birds require = 76.8 kg of feed
We add 50 gm of bacitracin in 1000 kg of feed
So for 77.8 kg of feed we need to add 3.84 ~ 4 gm of bacitracin

Going through results we know that MIC of 560 mcg/mL of Bacitracin is equal to MIC of 31.26 gm/mL of Thyme

So required dose of Thyme extract for this expt.-

	By MIC method	
	Low level	High Level
MIC of Thyme		
If we consider 31.26 mg/mL	223.25 gm	446.50 gm
If we consider 62.5 mg/mL	446.42 gm	892.85 gm

Total extract needed = 669.75 gm

2. Cinnamon

MIC of Cinnamon

Bacterium	MIC per mL
E.coli	≤ 31.25 mg
E.faecium	≤31.25 mg
E.faecium	ND
S. typhimurium	≤ 31.25 mg

MIC of Bacitracin = 560 mcg/mL

Required dose of Cinnamon extract

	By MIC method	
	Low level	High Level
MIC of Cinnamon 31.26 mg/mL	223.25 gm	446.50 gm

Total extract needed = 669.75 gms

Appendix- E

ANOVA TABLE

Table 10. Analysis of variance for parameter of feed consumption according to the diets at 7 d (0-7 d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	152.385794	2.27	0.0123
Error	43	67.240300		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	1308.397832	261.679566	3.89	0.0054
Rep	15	1739.318041	115.954536	1.72	0.0819

Source of variation	DF	Type II SS	Mean Square	F-value	P
Trt	5	1308.327245	261.665449	3.89	0.0054
Rep	15	1739.318041	115.954536	1.72	0.0819

Table 11. Analysis of variance for parameter of feed consumption according to the diets at 7 d (0-7 d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	0.00362491	1.67	0.0781
Error	43	0.00216509		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	0.03040709	0.00608142	2.81	0.0279
Rep	15	0.04209110	0.00280607	1.30	0.2462

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	0.03290668	0.00658134	3.04	0.0195
Rep	15	0.04209110	0.00280607	1.30	0.2462

Table 12. Analysis of variance for parameter of body weight gain according to the diets at 7 d (0-7 d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	157.632069	2.51	0.0057
Error	43	62.794500		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	1323.863249	264.772650	4.22	0.0033
Rep	15	1828.778125	121.918542	1.94	0.0453

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	1332.887757	266.577551	4.25	0.0032
Rep	15	1828.778125	121.918542	1.94	0.0453

Table 13. Analysis of variance for parameter of avg feed consumption according to the diets at 14 d (7-14d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	466.31608	0.91	0.5753
Error	43	511.21822		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	3848.382986	769.676597	1.51	0.2081
Rep	15	5477.938643	365.195910	0.71	0.7565

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	3049.940151	609.988030	1.19	0.3285
Rep	15	5477.938643	365.195910	0.71	0.7565

Table 14. Analysis of variance for parameter of feed efficiency according to the diets at 14 d (7-14d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	0.00237710	1.57	0.1083
Error	43	0.00151856		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	0.02717501	0.00543500	3.58	0.0086
Rep	15	0.02036698	0.00135780	0.89	0.5755

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	0.02724463	0.00544893	3.59	0.0084
Rep	15	0.02036698	0.00135780	0.89	0.5755

Table 15. Analysis of variance for parameter of periodic body weight gain according to the diets at 14 d (7-14d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	518.31461	1.61	0.0946
Error	43	321.80741		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	6717.43995	1343.48799	1.85	0.1234
Rep	15	10092.89067	672.85938	0.93	0.5433

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	6118.37447	1223.67489	1.68	0.1588
Rep	15	10092.89067	672.85938	0.93	0.5433

Table 16. Analysis of variance for parameter of cumulative feed consumption according to the diets at 14 d (0-14 d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	840.51653	1.16	0.3341
Error	43	726027102		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	6717.43995	1343.48799	1.85	0.1234
Rep	15	10092.89067	672.85938	0.93	0.5433

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	6118.37447	1223.67489	1.68	0.1588
Rep	15	10092.89067	672.85938	0.93	0.5433

Table 17. Analysis of variance for parameter of cumulative feed efficiency according to the diets at 14 d (0-14d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	0.00177488	2.00	0.0286
Error	43	0.00088802		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	0.01709066	0.00341813	3.85	0.0057
Rep	15	0.01840687	0.00122712	1.38	0.1997

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	0.01708223	0.00341645	3.85	0.0057
Rep	15	0.01840687	0.00122712	1.38	0.1997

Table 18. Analysis of variance for parameter of cumulative weight gain according to the diets at 14 d (0-14d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	987.74551	1.95	0.0333
Error	43	506.57194		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	7588.00663	1517.60133	3.00	0.0209
Rep	15	12166.90354	811.12690	1.60	0.1138

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	6478.62744	1295.72549	2.56	0.0411
Rep	15	12166.90354	811.12690	1.60	0.1138

Table 19. Analysis of variance for parameter of cumulative weight gain according to the diets at 14 d (0-14d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	911.09098	0.99	0.4893
Error	43	918.14400		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	7736.76213	1547.35243	1.69	0.1587
Rep	15	10485.05743	699.00383	0.76	0.7102

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	5685.83613	1137.16723	1.24	0.3079
Rep	15	10485.05743	699.00383	0.76	0.7102

Table 20. Analysis of variance for parameter of average feed efficiency according to the diets at 21d (14-21d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	0.00040797	0.75	0.7566
Error	43	0.00054633		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	0.00281716	0.00056343	1.03	0.4115
Rep	15	0.00534219	0.00035615	0.65	0.8146

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	0.00064778	0.00012956	0.24	0.9439
Rep	15	0.00534219	0.00035615	0.65	0.8146

Table 21. Analysis of variance for parameter of periodic body weight gain according to the diets at 21d (14-21d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	629.51451	0.99	0.4942
Error	43	637.42174		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	6187.262143	1237.452429	1.94	0.1071
Rep	15	6403.028130	426.868542	0.67	0.7985

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	3374.465328	674.893066	1.06	0.3963
Rep	15	6403.028130	426.868542	0.67	0.7985

Table 22. Analysis of variance for parameter of periodic body weight gain according to the diets at 21d (14-21d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	2913.0032	1.08	0.4066
Error	43	2708.3442		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	27084.68701	5416.93740	2.00	0.0978
Rep	15	31175.37614	2078.35841	0.77	0.7041

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	22217.81121	4443.56224	1.64	0.1698
Rep	15	31175.37614	2078.35841	0.77	0.7041

Table 23. Analysis of variance for parameter of cumulative feed efficiency according to the diets at 21 d (0-21 d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	0.00051442	1.05	0.4346
Error	43	0.00049159		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	0.00652144	0.00130429	2.65	0.0355
Rep	15	0.00376700	0.00025113	0.51	0.9208

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	0.00430352	0.00086070	1.75	0.1436
Rep	15	0.00376700	0.00025113	0.51	0.9208

Table 24. Analysis of variance for parameter of cumulative body weight gain according to the diets at 21d (0-21 d)

Source of variation	DF	Mean Square	F Value	P
Diet	20	2532.2805	1.37	0.1922
Error	43	1853.1432		
Total	63			

Source of variation	DF	Type I SS	Mean Square	F-value	P
Trt	5	26287.58533	5257.51707	2.84	0.0267
Rep	15	24358.02487	1623.86832	0.88	0.5935

Source of variation	DF	Type III SS	Mean Square	F-value	P
Trt	5	17863.65083	3572.73017	1.93	0.1094
Rep	15	24358.02487	1623.86832	0.88	0.5935

VITA

Mohan Nagorao Thakare was born on November 3, 1975 at a small village Kalambi, Dist- Akola, Maharashtra, India. He earned his professional degree in Veterinary Medicine from Nagpur Veterinary College, Nagpur, India. He also earned his Master's degree in Veterinary Pharmacology from the same institute. He worked as a Veterinarian with the Government in India for 2 yrs. He also worked as an Assistant Professor in Veterinary Pharmacology in Nagpur Veterinary College, Nagpur, India for a short period of time. In January-2002 he started working on his second master's working on the natural product pharmacology research at Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA and received a M.S. in Animal and Poultry Sciences in August 2004 under the supervision of Dr. D. M. Denbow.

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Mohan Thakare