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Research Article

The Effects of Thyme and Cinnamon Microencapsulated Essential Oils on Growth Performance, Intestinal Microbial Population and Meat Quality in Broiler Chicks

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Abstract

The aim of this study was to study the effects of microencapsulation of thyme and cinnamon essential oils on growth performance, intestinal microbial population and meat quality of broiler chickens. For this purpose, a total of 320 one-day-old male 308 strain chicks were randomly divided into 8 dietary treatment groups consisting of 4 replicates with 10 birds per replicate and fed until d 42. The birds fed diets supplemented with essential oils had significantly higher feed intake (FI) and weight gain (WG) and had improved feed conversion ratio (FCR) compared with the control group in total rearing period ($P < 0.05$). Dietary inclusion of encapsulated cinnamon resulted in a higher count of *Lactobacillus sp.* compared to the other groups on 42 days of age, while any treatments did not significantly affect *Escherichia coli* count ($P < 0.05$). The thiobarbituric acid values in thigh meat after 7 and 14 d of storage at 4 °C were linearly decreased in diets inclusion of essential oils ($P < 0.05$). In conclusion, the addition of thyme and cinnamon to the diet of broiler chickens especially as microencapsulated is recommended.

Keywords: Cinnamon; Meat Quality; Microencapsulation; Performance; Thyme

Abbreviations

FI: Feed Intake; WG: Weight Gain; FCR: Feed Conversion Ratio; AGP: Antibiotic Growth Promoters; EO: Essential Oils; GIT: Gastrointestinal Tract; TBA: Thiobarbituric Acid; MDA: Malondialdehyde; MRS: Man Rogosa Sharpe; EMB: Eosin Methylene Blue Agar; CFU: Colony-Forming Units; TBARS: Thiobarbituric Acid Reactive Substances; AME: Apparent Metabolisable Energy.

Introduction

The antibiotic feeding in animal leads to adverse effects in humans such as the spread of resistant bacterial strains, allergic reactions, toxic effects, prevalence of secondary infections and metabolic disorders [1]. In recent years, the animal production industries have imposed restrictions about the use of antibiotic growth promoters (AGP) in animal feed. Therefore, it is necessary to find some alternatives in animal feed industry to improve the performance and health and to control pathogenic microbes and protozoa [2]. Essential oils (EO) have been proposed as alternatives to antibiotic growth promoters. EOs are volatile and aromatic compounds synthesized by plants. They have antimicrobial, antifungal, antiviral properties and immune enhancer in poultry so improve the immune system [3]. Moreover, they play an important role in stimulating of digestive enzymes and affect lipids metabolism. Thus, EOs leads to the gut health and better performance of poultry feed from antibiotic-free diets [4]. Because of having lipophilic features, EOs able to accumulate between two phospholipids layers of the cell membrane of bacteria. EOs damage to membrane integrity through affect pH homeostasis and equilibrium of inorganic ions. Thus, they inhibit the growth of bacterial cells and the production of toxic bacterial metabolites [5].

Thyme and cinnamon oils are two of EOs with high potential for use in the diets of chicks. Thyme (*Thymus vulgaris L.*) contains many active principles such as phenolic antioxidants and antimicrobial. These compounds are health promoting and prevent from disease. Thyme plant contains 0.8 to 2.6 % (usually 1 percent) essential oil. The major compounds in Iranian thyme oil are Carvacrol and Thymol including about 80% of essential oil compounds [6]. Cinnamon is an aromatic condiment with desirable properties such as antioxidants, antiviral, blood purifier and helper to food digestion. The most properties of cinnamon is related to cinnamaldehyde as the principal component (60.41%) [7].

Despite the desirable properties of EOs, there are several limitations in using of them. EOs are highly volatile, unstable and sensitive to oxygen, light and heat consequently quickly decomposed [8]. Also, most essential oils are quickly absorbed after oral, pulmonary or dermal administration [9]. Therefore, without proper protection, most orally administrated essential oils may not reach to the lower gastrointestinal tract (GIT), where most foodborne pathogens reside and propagate [10]. The lack of an effective and practically feasible method for delivery of essential oils is of the major obstacles.

The technique of microencapsulation is a promising strategy for overcoming EOs limitations. It could be used as a target delivery carrier in poultry nutrition for enhancing the intestinal delivery of essential oils [10]. Microencapsulation can control the release of the microparticles at a specific time and place as well as at a specific rate of digestive system. In this technique, the target material is surrounded by a coating of polymer, organic or mineral substance. Alginates were used as wall materials that chemically occurring polycarbohydrates consisting of copolymers of α-L-glucuronic acid (G) and β-D-mannuronic acid (M). The structure and specific properties of alginates such as biodegradability, mechanical strength, gel formation and cell affinity cause to combine with other biological materials [11].

The amount of thiobarbituric acid (TBA) is considered as a good indicator of organoleptic spoilage grading, which occurs as a result of oxidation [12]. It should be noted that malondialdehyde (MDA) is considered as the most important carbonyl produced from the auto-oxidation of long-chain poly unsaturated fatty acids. TBA measurement is based on the reaction between a malondialdehyde molecule and two TBA molecules, which leads to the formation of a pink color, and is carried out by utilizing spectrophotometry [13].

The contradictory results have reported from numerous studies done using different essential oils in *in vitro* conditions. Al-Kassie [14] reported that chicks fed with thyme and cinnamon EO were higher in feed intake and body weight gain as well as improved in feed conversion ratio in comparison with control group. Hernandez., *et al.* [15] examined the effect of two plant extracts on broilers performance and did not observed any significant differences in feed intake or feed conversion. The objective of this study was to survey of the effects of thyme and cinnamon microencapsulated essential oils on performance, intestinal microbial population and meat oxidative stability in broiler chicks.

Materials and methods

Ethical statement

All institutional and national guidelines for the care and use of laboratory animals were followed.

Preparation of microencapsulated essential oils

First, 30 g sodium alginate powder was added to 1 liter distilled water. Then, 1% thyme or cinnamon essential oils (obtained from Shahid Beheshti University of Tehran) was added to the mixture. The solution was heated on a hot plate with constant stirring until completely dissolved and clear and then cooled to room temperature. Because sodium alginate coating is brittle, 2% glycerol was added as a softener. 20 g $CaCl_2$ powder was added to 1 liter distilled water and added to the above solution for formation of gel and coherence [16].

Diet and chick's management

In this study, 320 male broiler chicks from 308 strain were assigned into 8 treatments and 4 replications and 10 chicks per replicate for a period of 42 days. The rearing period was carried out in the faculty of Agriculture, University of Guilan. The treatments included: T1 (basal diet+antibiotic), T2 (basal diet), T3 (basal diet+100 mg/kg free thyme essential oil), T4 (basal diet+100 mg/kg microencapsulated thyme essential oil), T5 (basal diet+100 mg/kg free cinnamon essential oil), T6 (basal diet+100 mg/kg microencapsulated cinnamon essential oil), T7 (basal diet+100 mg/kg free thyme and cinnamon essential oils), T8 (basal diet+100 mg/kg microencapsulated thyme and cinnamon essential oils). The basal diet was formulated to cover the nutrient requirements of chicken [17]. Ingredients and the composition of the basal diet are shown in table 1.

Ingredients	Experimental periods		
	Starter (0-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Corn, 8 g CP/kg	540.4	570.8	625.6
Soybean meal, 44 g CP/kg	390.8	355.4	301.4
Fiber	392	374	349
Soybean oil	23.3	32.9	34.9
Dicalcium phosphate	20.3	18.1	16.5
Calcium carbonate	10.4	9.4	8.6
Vitamin supplement [1]	2.5	2.5	2.5
Mineral supplement [2]	2.5	2.5	2.5
dl-Methionin	0.3	0.26	0.25
l-Lysine	2.1	1.6	1.6
l-Threonine	0.9	0.5	0.4
NaCl	2.3	2.7	2.5
Metabolizable energy (Kcal/kg)	2870	2970	3060
Crude protein	220	206	186.5
Linoleic acid	2.53	3.07	3.28
Calcium	9.6	8.7	7.9
Available phosphorus	4.8	4.3	3.9
Sodium	1.5	1.5	1.5
Chloride	2.2	2.3	2.2
Lysine	12.2	12.1	10.8
Methionine	3	2.6	2.5
Methionine+ cystine	9.1	9.1	8.3
Threonine	8.2	8.3	7.4

Table 1: Ingredients and nutrient composition of basal diets (g/kg as-fed basis).

[1] Vitamin supplement provided per kilogram of diet: vitamin A (retinyl acetate), 10000 IU; vitamin D3, 3500 IU; vitamin E (dl-α-tocopheryl acetate), 60 mg; vitamin K, 3 mg; thiamin, 3 mg; riboflavin, 6 mg; pyridoxine, 5 mg; vitamin B12, 0.01 mg; niacin, 45 mg; choline chloride, 200 mg; pantothenic acid, 11 mg; folic acid, 1 mg and biotin, 0.15 mg.

[2] Mineral supplement provided per kilogram of diet: Fe 1 mg, Mn (manganese sulphate), 100 mg, Zn (zinc sulphate), 60 mg; Cu (copper sulphate), 10 mg; I (calcium iodate), 1 mg and Co, 0.2 mg.

Sample and data collection

The parameters related to growth performance of broilers such as FI, WG and feed conversion ratio (FCR) were evaluated. Also, two chicks from each replicate were randomly slaughtered and the contents of their ileum were collected for counting the ileal microflora. For counting *Lactobacillus sp.* and *Escherichia coli* populations, ileal digesta samples were diluted and poured on the Man Rogosa Sharpe (MRS) and eosin methylene blue agar (EMB), incubated at 37°C for 24 hr in the anaerobic and aerobic conditions, respectively, and the number of bacteria was determined using an electronic colony counter [18]. The results were expressed as $\log_{10}$ colony-forming units (CFU) per gram of ileal content. Also, 10 g of slaughtered chicken thigh was removed for assessment of lipid oxidation by measuring thiobarbituric acid reactive substances (TBARS) in 0, 7 and 14 days after killing [19]. The amount of Malondialdehyde (MDA) was used for determination of the lipid peroxidation by a spectrophotometric method. The TBARS was measured as mg of MDA per kg of samples.

Statistical analysis

All data was statistically analyzed according to a completely randomized design using the general linear models (GLM) procedure of SAS software [20]. Differences between means of treatments at the significant level P < 0.05 were assessed by the Duncan's test. The statistical model was as follows:

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

Where, Y_{ij} is the observed size of the test, μ is the population mean, T_i is the treatment effect, and ε_{ij} is the error of the experiment.

Results and Discussion

Growth performance

The effects of thyme and cinnamon essential oils microencapsulated with alginate on the growth performance of broiler chicks are presented in table 2. Birds fed diets supplemented with essential oils had significantly higher feed intake (FI) and weight gain (WG) and had improved feed conversion ratio (FCR) compared with the control group in total rearing period (P < 0.05). At the end of the rearing period, all the birds fed with diets containing of EO had higher FI and better WG compared with the birds on the control treatment (P < 0.05). This increase in BW was also reflected in the improved FCR for birds fed EO compared with the control group (P < 0.05). The highest and lowest the growth performance were obtained into the treatments containing both encapsulated thyme and cinnamon and without any additive, respectively. Similar results were reported by Lee., *et al*. [21] who reported that adding of cinnamaldehyde to the diet of broilers improved their growth performance. These researchers concluded that the presence of EO in diet could encourage the secretion of endogenous digestive enzymes and increase the digestion and gut passage rate in chickens and led to the improving of growth performance [21]. The positive effects of dietary thyme oil on chick performance were reported by Cross., *et al.* [22], while this dietary had not significant differences on the intestinal microflora populations, apparent metabolisable energy (AME) and the calculated coefficients of digestibility. The negative effects of the medicinal herbs on food intake were shown in chickens fed diets containing plant extract [23]. In a conducted research by Lee., *et al*. [24] on female broiler chickens, any significant effects were not observed between the essential oil constituents (thymol and cinnamaldehyde) and growth performance. The reasons of the inconsistent results for the effects of EO on the growth performance could be the differences in levels of EO, sources of herbs of EO, basal diet composition, or the microbial environment in the birds were reared.

Traits	Treatments								SEM	P value
	T1	T2	T3	T4	T5	T6	T7	T8		
Feed intake (g/bird/days)	104.30^{b}	100.65^{c}	105.72^{b}	110.03^{a}	106.05^{b}	110.60^{a}	111.03^{a}	111.17^{a}	0.652	0.029
Weight gain (g/bird/days)	56.42^{b}	56.28^{b}	57.75^{b}	58.60^{ab}	56.97^{b}	58.77^{ab}	59.60^{ab}	61.06^{a}	0.423	0.033
Feed conversion ratio	1.87^{a}	1.90^{a}	1.88^{a}	1.88^{a}	1.87^{a}	1.86^{ab}	1.82^{bc}	1.79^{c}	0.005	0.045

Table 2: The effects of experimental treatments on growth performance of broilers in total rearing period (0-42 d).

^{abc} Means with different superscripts in the same row, differ significantly (P < 0.05).

T1: Basal diet+ avilamycin, T2: Basal diet without any additive, T3: Basal diet+ free thyme, T4: Basal diet+ encapsulated thyme, T5: Basal diet+ free cinnamon, T6: Basal diet+ encapsulated cinnamon, T7: Basal diet+ both free thyme and cinnamon, T8: Basal diet+ both encapsulated thyme and cinnamon.

Intestinal microbial population

Table 3 provides the results of experimental treatments effects on ileal microflora population of broiler chickens. Dietary inclusion of encapsulated cinnamon resulted in a higher count of *Lactobacillus sp.* compared to the other groups on 42 days of age, while any treatments did not significantly affect *Escherichia coli* count (P < 0.05). The highest value of Lactobacillus population was related to the treatment containing encapsulated cinnamon, while the least value was related to treatment with Avilamycin. Due to the hydrophobic properties of the essential oils, their active components can be penetrate into the cell wall of pathogenic bacteria and consequently, causes more permeability and change in their structure. Then, a large amount of ions and other critical cell contents will be secreted to the outside; therefore, it leads to bacteria's death [25]. *Bifido-bacteria* and *Lactobacilli* are known as important microorganisms existed in the intestine that can be useful for the host. These organisms produce short-chain fatty acids and may provide an optimal conditions in the intestine for more protection against pathogenic [26]. Extracts of alpha-Pinene and beta-Pinene are considered as the most important identified combinations that have anti-microbial activities against gram-positive and gram-negative bacteria; also, they have insecticide impacts [27]. Alpha-Pinene is existed in cinnamon essential oil. A study showed that carvacrol, cinnamaldehyde and capsaicin reduced coliforms and E. coli population [28]. On the other hand, dittany, marjoram, rosemary, yarrow and thyme did not have any significant impact on cecum and fecal coliforms, lactic acid bacteria, anaerobic bacteria and clostridium perfringens [22]. Increase of lactobacillus population in alimentary tract is usually beneficial for the host animal because it can prevent pathogenic factors, including salmonella and clostridium, based on the framework of competitive inhibition [29]. Nonetheless, the increased intestinal lactobacillus might not be desirable based on the nutritional overview due to its abilities in destructing bile salts and making disturbances in lipid digestion [29].

Bacteria	Treatments								SEM	P value
	T1	T2	T3	T4	T5	T6	T7	T8		
Lactobacillus sp.	6.35^{b}	6.49^{b}	6.69^{b}	6.86^{ab}	6.68^{b}	7.40^{a}	6.75^{ab}	6.70^{b}	0.16	0.026
E. coli	7.28	7.25	7.34	7.25	7.25	7.30	7.40	7.23	0.40	0.781

Table 3: The effects of experimental treatments on ileal microflora on day 42 (Log cfu g-1).

^{ab} Means with different superscripts in the same row, differ significantly (P < 0.05).

T1: Basal diet+ avilamycin, T2: Basal diet without any additive, T3: Basal diet+ free thyme, T4: Basal diet+ encapsulated thyme, T5: Basal diet+ free cinnamon, T6: Basal diet+ encapsulated cinnamon, T7: Basal diet+ both free thyme and cinnamon, T8: Basal diet+ both encapsulated thyme and cinnamon.

Meat oxidative stability

The effects of experimental treatments on lipid oxidation, measured as TBARS values (mg MDA/kg of meat), of thigh meat on the 0, 7 and 14 d of storage at 4°C are shown in table 4. TBARS values were higher (P < 0.05) in the thigh meat from broilers fed on the basal diets containing antibiotics and without additives than those from broilers fed on the EO-containing diets on the 7 and 14 days after the slaughter. The minimum values of TBARS were observed into the treatments containing both free thyme and cinnamon and both encapsulated thyme and cinnamon. No significant difference was observed between the TBARS values of the groups on the 0 day. Radwan., *et al*. [30] reported that there is a reduction in malonaldehyede of egg yolk in chickens fed with a basal diet containing to oregano or rosemary extract. A linear relationship between antioxidant activities of herbal extracts and the amount of phenolic compounds as inhibitors of lipid oxidation was reported by Luximon-Ramma., *et al.* [31]. According to impacts of environmental conditions on quantities and qualities of compounds existed in various herbal extracts, the presence of lower antioxidant properties might be due to lower amounts of potent antioxidant, including eugenol, and high amounts of cinnamaldehyde in some cases [32]. It was also reported that phenolic compounds including thymol and carvacrol play the most significant role in the formation of antioxidant properties [33]. Also, A study reported that thyme reduces the production of malondialdehyde in blood serums of broiler chickens [34]. In an investigation, the minimum amount of malondialdehyde was observed in the group fed by 1% thyme, while the presence of 1% and 2% cinnamon led to the increase of produced malondialdehyde [35]. In a study was observed that the presence of thyme with densities less than 1% can lead to the reduction of TBARS among Japanese quails [36]. Mentioned results are in accordance with our results in this study.

Time	Treatments								SEM	P value
	T1	T2	T3	T4	T5	T6	T7	T8		
Day 0	0.28	0.27	0.23	0.27	0.24	0.29	0.23	0.22	0.06	0.391
Day 7	1.75[a]	1.75[a]	1.49[b]	1.47[b]	1.43[b]	1.40[b]	1.34[b]	1.31[b]	0.13	0.042
Day 14	1.91[a]	1.97[a]	1.72[b]	1.71[b]	1.70[b]	1.72[b]	1.63[b]	1.61[b]	0.18	0.033

Table 4: The effects of experimental treatments on oxidative stability (mg MDA1/kg meat) of thigh meat stored at 4°C in broilers.

[1]MDA, malondialdehyde acid.

[ab] Means with different superscripts in the same row, differ significantly (P < 0.05).

T1: Basal diet+ avilamycin, T2: Basal diet without any additive, T3: Basal diet+ free thyme, T4: Basal diet+ encapsulated thyme, T5: Basal diet+ free cinnamon, T6: Basal diet+ encapsulated cinnamon, T7: Basal diet+ both free thyme and cinnamon, T8: Basal diet+ both encapsulated thyme and cinnamon.

Conclusions

Using the essential oils in diet of broiler chickens led to promoting health, as well as preventing and potentially treating some diseases. However, the low water solubility and stability, together with the high volatility limited their application in animal feed industry. Encapsulation of EOs with alginate represent a hopeful strategy for overcoming EOs limitations. The results from the present study showed that adding the encapsulated thyme and cinnamon to basal diet improved broilers growth performance and FCR at the end of the rearing period. Feeding the chickens with the diets containing microencapsulated cinnamon resulted in increased counts of fecal *Lactobacillus*, which is useful for gut health. The treatments with EO had more meat stability compared to other treatments. Based on the results of present research, the addition of microencapsulated thyme and cinnamon to the diet of broiler chickens is recommended.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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