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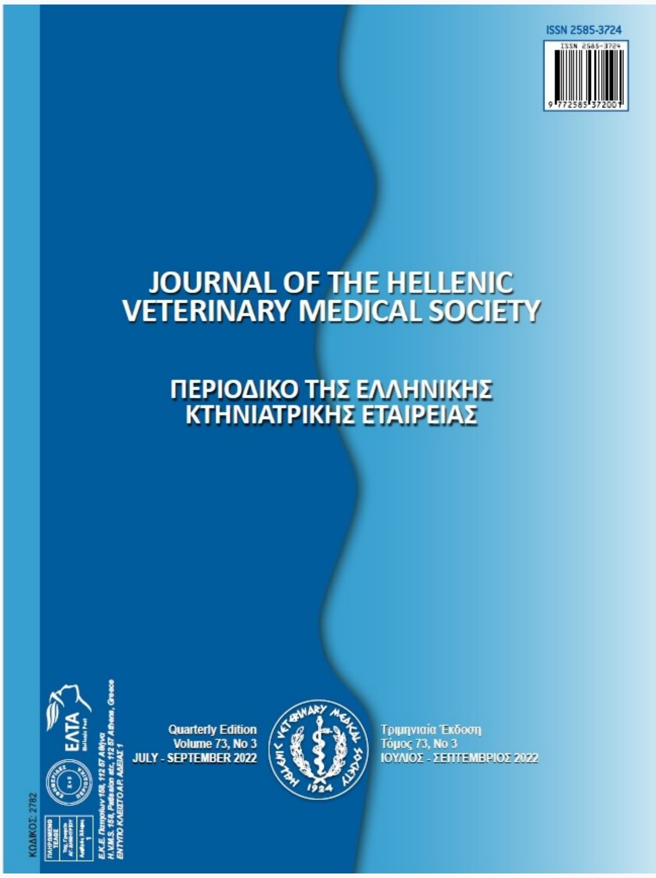

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Comparison of nano encapsulated thyme essential oils on intestinal microbial population and anti-oxidation status of broilers

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ABSTRACT: This study was conducted to evaluate the effects of non and nano encapsulated thyme essential oils with saturated and unsaturated lipids (tallow and canola oil) and two levels of diet crude protein (standard and low than standard) on intestinal microbial population and blood anti-oxidation status of broilers. In this study, 384 Ross-308 broiler chickens in a factorial engagement of 2×2×2 (2 form of thyme medicinal plant essential oils, two sources of lipids, and 2 levels of diet crude protein) were used in a completely randomized design with 8 treatments, 4 replicates, and 12 bird in each replicate for 42 days. Experimental periods included starter (1-10 days), grower (11-24 days), and finisher (25-42 days). Canola oil significantly reduced the intestinal coliforms population, whereas tallow increased them ($P<0.05$), a similar effect was observed with thyme forms, so the lowest coliform population blogged to the nano encapsulated form of thyme. In contrast, the highest coliform population resulted in non-capsulated thyme essential oil ($P<0.05$). In an interaction between thyme essential oil form and diet crude protein level, the lowest coliform population was observed with diet contained nano encapsulated thyme essential oil and low level of diet crude protein ($P<0.05$). Regarding antioxidant status, reducing the diet crude protein increased Malondialdehyde and Glutathione peroxidase ($P<0.05$). In an interaction between diet crude protein and thyme form, a diet with low crude protein and capsulated thyme increased the level of Superoxide dismutase ($P<0.05$). In general, results of the current experiment indicated that in Ross-308, broiler chickens, using capsulated thyme essential oil with canola oil and low crude protein improves intestine microbial population and antioxidant status of broilers.

Keywords: Broiler, Crude protein levels, Essential oil, Intestinal microbial population and Anti-oxidation status

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INTRODUCTION

Nanotechnology is a general term that refers to all advanced technologies in the field of nanoscale work. Nanocapsules, are capsules with a nanometer diameter and can be inserted and encapsulated (Elbaz*et al.,* 2020; Ji *et al.,* 2019). Nano-capsulation of aromatic substances can improve the healing properties and facilitates their access. Because of their small size, these substances increase the mechanism of cellular uptake and increase their efficiency (Bayramzadeh *et al.,* 2019). Encapsulation acts as a barrier to oxygen transfer and prevents the growth of aerobic bacteria and delays lipid oxidation. Therefore, it leads to increasing the stability and durability of essential oils (Zhaveh *et al.*, 2015). On the other hand, encapsulation controlled and sustained release of a certain amount of essential oils from the carrier (Khalili *et al.*, 2015).

The use of medical plants has recently increased in poultry diets. Medical plants are added to poultry diets for different reasons such as antibacterial, antioxidant, growth improvements, reduction of using drugs and chemical additives, improving taste, market considerations of poultry products, and improvement of blood and immune parameters (Rossi et al., 2020; Torun Kumar Paul et al., 2020; Pandey et al., 2019). Thyme is more considerable than the other medicinal plants. Its constituents (thymol and carvacrol) have been used in many experiments (Galli et al., 2020; Ghanima et al., 2020; Karam et al., 2019; Yin et al., 2017).

Naturally, antioxidant ingredients abound in various medical plants like thyme. The use of thyme essential oil and saturated and unsaturated oils in poultry ration decreases oxidation side effects. Ghasemi Shansabadi *et al*. (2014) indicated that low-density thyme powder in the ration of Japanese quails increases their growth and prevents oxidation of fats, and performs as a highly effective antioxidant. The use of Shirazi thyme powder in broiler chickens ration increased chickens' performance. It reduced the carcass's oxidation process and, consequently, increases the durability of poultry meat in the refrigerator (Salehi *et al.,* 2015). Kalanta *et al*. (2015) studied the effect of drinking essential oil of thyme on performance traits, antibody level against Newcastle virus diseases, Gumboro and bronchitis, and blood parameters in broiler chickens. Essential oils comprise mainly hydrocarbon terpenes (isoprenes) and terpenoids, which their synergistic effects could lead to antifungal and antimicrobial effects (Zhaveh *et al.*, 2015). They showed that various levels of thyme essential oil significantly influence performance indexes and blood parameters. Present experiment carried out to evaluate the effects of using normal and nano encapsulated thyme essential oils with saturated and unsaturated oil on intestinal microbial population and anti-oxidation condition of broiler.

MATERIALS AND METHODS

The present experiment was performed in broiler chicken farming of Islamic Azad University of Maragheh in March 2019 for 42 days. In this study, a total of 384 male Ross-308 broilers (one-day-old) were selected and analyzed using 2×2×2 factorial experiments consisting of two thyme essential oils (normal and encapsulated), two oil type (saturated and unsaturated), and two crude proteins (standard and 5% lower) in a random design with eight treatments and four repetitions in each treatment in 32 experimental units. The experimental rations were adjusted based on food requirements in Ross-308 broiler nutrition specification guidelines by formulated using the UFFDA program. Diet ware for the periods including a starter (1 to 10 days), growing (11 to 24 days), and finisher (25 to 42 days) (Table 1). The thyme essential oil was prepared from Barij Assurance Company (Kashan, Iran). The thyme essential oil was mixed with the fats used in the meal (Firstly dissolved in the vegetable oil component of the ration), and the final homogeneous mixture was added to the meal. For capsulation, the loading capacity of essential oil at a concentration of 0.5 - 2% sodium alginate was first investigated, then was encapsulated with biocompatible calcium alginate hydrogel (Dima *et al.,* 2013). Two fatty sources, including tallow (saturated) and canola oil (unsaturated), were used.

When chickens lived for 42 days, two chickens from each repetition (total n = 64) were selected randomly (Hamidi *et al.,* 2021), and after slaughter, 1 cm long pieces samples was taken from the bottom of the ileum (Ghazanfari *et al.,* 2015). The number of *Lactobacillus* bacteria in the culture environment (MRS-Agar) was reported, then numbering of *Escherichia coli* (Eosin methylene-blue) was done in the culture environment. After preparation of the sample from ileum content, consecutive dilutions (from 10-1 to 10-6) were prepared. Then to count the total bacteria, they were cultured in specific cultivation environments (Heir *et al.*, 2018). The total number of bacteria in every dilution of ileum was calculated, and the

Table 1. Nutritional contents of diets fed to broilers during the starter, growing and finishing periods

Meal content (%)	Starter period 1-10 days		Growing period 11-24 days		Finishing period 25-42 days	
	Standard CP	5% Lower	Standard CP	5% Lower	Standard CP	5% Lower
Corn	75.47	77.51	93.54	71.58	75.56	55.60
Soybean meal	50.44	7.40	90.37	77.34	71.36	63.33
Oil	26.3	71.3	39.3	94.2	05.3	58.2
Bone meal	26.2	3.2	18.2	06.2	87.1	75.1
Oyster powder	29.0	27.0	29.0	35.0	33.0	34.0
Salt	46.0	43.0	47.0	44.0	42.0	39.0
Vitamin supplement *	0.25	0.25	0.25	0.25	0.25	0.25
Mineral supplement*	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.37	0.32	0.34	0.22	0.38	0.25
Sum	100	100	100	100	100	100
Chemical compounds of nutrient meals						
Metabolic energy (Kilocalorie/kg	3000	3000	3000	3000	3000	3000
Crude protein (%)	27.50	26.12	20.95	19.90	20.64	19.60
Calcium (%)	1.05	0.99	0.88	0.83	0.80	0.76
Usable phosphorus(%)	0.50	0.47	0.43	0.41	0.39	0.37
Sodium (%)	0.32	0.30	0.21	0.20	0.19	0.18
Lysin (%)	1.67	1.59	1.26	1.20	1.23	1.17
Methionine + Cysteine (%)	1.18	1.12	0.99	0.94	0.85	0.81
Tryptophan (%)	0.35	0.33	0.28	0.27	0.28	0.27

*Provides per kg of diet:trans-retinol 3784mg, cholecalciferol 125mg,-tocopherol acetate 100 mg, vitamin K33 mg, vitamin B12 mg, vitaminB25 mg, vitamin B64 mg, vitamin B120017 mg, niacin 40 mg, folic acid 18 mg,D-biotin 015 mg, calciumD-pantothenate 15 mg, Mn 100 mg,Fe 80 mg, Zn 80 mg, Cu 8 mg, I 2 mg, Co 05 mg, Se 015 mg.

mean of them as the total number of bacteria in digestive content per gram was reported in every treatment repetition (He *et al.*, 2021).

To specify an antioxidant condition of serum blood was collected before slaughtering of the birds, the Total Antioxidant Capacity of Serum (TAC.S) and the level of Malondialdehyde (MDA.S) were identified. The liver samples (washed with PBS (Sigma), and minced with scissors) were centrifuged after homogenization in the buffer solution (1.15% potassium chloride, pH=7.4, centrifuged in 4°C for 15M/5000R), and the surface solution was used for measuring the activity of Glutathione peroxidase enzymes (GPX.L), Superoxide dismutase (SOD.L), and the level of Malondialdehyde (MAD.L). GPX.L activity and SOD.L were measured using the method applied by Ishaq *et al.* (2021) using every unit per mg protein. MDA level, an index of lipid peroxidation, was measured in the serum and liver of broiler chicken based on Mousavi *et al.* (2018)'s method.

The statistical analysis of data was analysed using GLM software (9.1) SAS, and the mean levels were compared with the Tukey method (P ≥0.05) (SAS, 2008). The statistical model of the design was as follows:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijkl}$$

where: Y_{ijkl} = a dependent variable, μ = overall mean, A_i = the effect of Thyme essential oil, B_j = the effect of oil type, C_k = the effect of crude protein levels, AB_{ij} = Interaction of Factors A×B, AC_{ik} = Interaction of Factors A×C, BC_{jk} = Interactivity of Factors B×C; ABC_{ijk} = Interaction of Factors A×B×Cand ε_{ijkl} = the random error.

RESULTS

Intestinal microflora

The effects of experimental treatments on intestinal microbiology are presented in Table 2. Concerning the results of experimental diets on the small intestine's microbial population in chickens, the type of oil and form of essential oil have significant effects on the population of coliform in the intestine (P <0.05). The protein level did not have significant effects on the microbial population of the small intestine (P >0.05). This result was also observed in the effects of thyme essential oil so that the use of encapsulated thyme es-

Table 2. The effects of experimental treatments on intestinal microbiology

Effects			TAB	Coliform	Lactobacillus
Main effects					
Fat					
Canola			8.63	6.32[b]	5.00
Tallow			8.53	7.29[a]	4.36
SEM			0.4709	0.2845	0.3045
p-value			0.8799	0.0238	0.1499
Thyme					
Capsulated thyme			7.69	6.08[b]	3.93
Thyme			9.47	7.53[a]	5.43
SEM			0.4709	0.2845	0.3045
p-value			0.0131	0.0014	0.0019
Protein					
Normal			8.29	6.65	4.55
Low			8.87	6.95	4.82
SEM			0.4709	0.2845	0.3045
p-value			0.3897	0.4548	0.5271
2. Interactions					
Fat × thyme					
Canola capsulated			7.72	5.55	4.41
Canola thyme			9.54	7.09	5.60
Tallow capsulated			7.66	6.60	3.46
Tallow thyme			9.40	7.97	5.27
SEM			0.6660	0.4023	0.4306
p-value			0.9527	0.8312	0.4737
Fat × protein					
Canola normal			8.59	6.28	4.84
Canola low			8.67	6.35	5.17
Tallow normal			7.98	7.02	4.26
Tallow low			9.07	7.55	4.47
SEM			0.6660	0.4023	0.4306
p-value			4529/0	0.5745	0.8878
Thyme × protein					
Capsulated normal			7.15	5.14[d]	4.03
Capsulated low			8.22	7.01[b]	3.84
Thyme normal			9.42	8.16[a]	5.06
Thyme low			9.52	6.90[c]	5.81
SEM			0.6660	0.4023	0.4306
p-value			0.4705	0.0007	0.2885
3. Interactions					
Fat ×	**Thyme ×**	**Protein**			
Canola	Capsulated	Normal	7.79	4.45	4.69
Canola	Capsulated	Low	7.64	6.64	4.12
Canola	Normal	Normal	9.39	8.11	4.98
Canola	Normal	Low	9.69	6.06	6.22
Tallow	Capsulated	Normal	6.51	5.83	3.57
Tallow	Capsulated	Low	8.80	7.38	3.55
Tallow	Normal	Normal	9.45	8.21	5.15
Tallow	Normal	Low	9.35	7.73	5.39
SEM			0.9418	0.5690	0.6090
p-value			0.2952	0.1833	0.3167

Means with different Latin letters in each column has significant difference (P<0.05).

sential oil reduced the level of intestinal coliforms in chickens (P <0.05). In the mutual effects of the form of thyme essential oil and the crude protein level in the diets, the use of encapsulated thyme essential oil and the common crude protein in the meals of chickens reduced intestinal coliforms population (P <0.05).

Antioxidant compounds

The effects of experimental treatments on the levels of antioxidant compounds in the blood serum of chickens are shown in Table 3. Decreasing the level of crude protein in the meals increased MDA.L and GPX.L in chickens (P <0.05). In triple mutual effects of fat source type, essential oil form and crude protein level of meals, the use of tallow, normal thyme essential oil, and low protein meal increased SOD.L (P <0.05) in chickens. The experimental treatments had no effect on serum TAC.S and MDA.S levels (P <0.05) of chickens.

Table 3. The effects of experimental treatments on the levels of antioxidant compounds in the blood serum of chickens

Effects	MDA.L	SOD.L	GPX.L	MDA. S	TAC. S
Main effects					
Fat					
Canola	1.28	16.12	2.83	4.84	1.73
Tallow	1.14	16.04	2.84	4.81	1.66
SEM	0.0639	0.4840	0.1052	0.3897	0.1203
p-value	0.1599	0.9165	0.9239	0.9642	0.7112
Thyme					
Capsulated thyme	1.25	16.07	2.90	5.08	1.69
Thyme	1.17	16.09	2.76	4.58	1.70
SEM	0.0639	0.4840	0.1052	0.3897	0.1203
p-value	0.3782	0.9813	0.3543	0.3734	0.9826
Protein					
Normal	1.11[b]	15.47	2.62[b]	4.82	1.73
Low	1.31[a]	16.68	3.05[a]	4.82	1.66
SEM	0.0639	0.4840	0.1052	0.3897	0.1203
p-value	0.0319	0.0901	0.0078	1.0000	0.6580
2.Interactions					
fat × thyme					
Canola capsulated	1.35	16.62	3.00	5.15	1.80
Canola thyme	1.20	15.61	2.66	4.51	1.65
Tallow capsulated	1.15	15.52	2.81	4.99	1.58
Tallow thyme	1.14	16.56	2.87	4.64	1.74
SEM	0.0904	0.6845	0.1488	0.5511	0.1701
p-value	0.4548	0.1497	0.1878	0.7878	0.3713
fat × protein					
Canola normal	1.25	15.53	2.67	4.79	1.78
Canola low	1.30	16.70	2.98	4.89	1.68
Tallow normal	0.96	15.42	2.56	4.86	1.70
Tallow low	1.32	16.66	3.12	4.76	1.63
SEM	0.0904	0.6845	0.1488	0.5511	0.1701
p-value	0.0971	0.9611	0.4025	0.8576	0.9652
Thyme × protein					
Capsulated normal	1.19	16.01	2.64	4.96	1.70
Capsulated low	1.31	16.13	3.16	5.19	1.69
Thyme normal	1.02	14.94	2.59	4.69	1.77
Thyme low	1.31	17.24	2.94	4.46	1.63
SEM	0.0904	0.6845	0.1488	0.5511	0.1701
p-value	0.3782	0.1244	0.5706	0.6867	0.7058

3.Interactions							
Fat ×	**Thyme ×**	**Protein**					
Canola	Capsulated	Normal	1.30	15.82b	2.79	5.15	1.72
Canola	Capsulated	Low	1.40	17.42a	3.20	5.18	1.88
Canola	Normal	Normal	1.20	15.24b	2.56	4.42	1.81
Canola	Normal	Low	1.20	15.99b	2.76	4.60	1.49
Fat	Capsulated	Normal	1.08	16.21[ab]	2.50	4.78	1.67
Fat	Capsulated	Low	1.22	14.84[c]	3.12	5.20	1.49
Fat	Normal	Normal	0.85	14.63[c]	2.62	4.95	1.72
Fat	Normal	Low	1.42	18.48[a]	3.12	4.32	1.76
SEM			0.1279	0.9680	0.2105	0.7794	0.4206
p-value			0.1599	0.0364	0.8975	0.5913	0.3173

Means with different Latin letters in each column has significant difference (P<0.05).

DISCUSSION

Intestinal microflora

Thymol and carvacrol found in thyme plant and EOs has antimicrobial and antibacterial effects; therefore, in the intestines of broilers, it eliminates pathogenic elements (such as *Salmonella*, *Enterobacter sakasaki*, and *Clostridum difficile*) and thereby promotes better growth and productivity of the herd (Takaloli *et al.,* 2006; Mitsch *et al.,* 2004; Bart and Reinderz, 2003). Intestinal microflora can hydrolyze bile salts essential for the digestion and absorption of fats (Wang *et al*., 2022). Intestinal microflora, by reducing bile acids' availability, disrupts fat digestion in broilers; therefore, adding thyme-with its antimicrobial effects may reduce the microbial dissolution of bile acids by reducing the intestinal microbial load. Differences in results can be due to the type of bird, the production level, health condition, and other food items used in the meals. Our findings showed that increasing encapsulated thyme essential oils reduced the colony of pathogenic bacteria (*coliforms*) but have no significant effect on beneficial bacteria (*Lactobacillus*) in the cecal contents of chicks. These results indicated that the PF (positive effects of bacteria in the digestive system) had a positive impact on regulating the intestinal flora. In previous research, thyme essential oils have been shown to suppress harmful bacteria, stimulate beneficial bacteria by controlling the intestinal microbial ecosystem, inhibit colonization, and favor healthier microbial groups (Lin *et al*., 2020). Encapsulations have improved the efficiency of their use in various fields, including reducing the population of harmful microbes such as coliforms, by optimally delivering the active ingredients to the target tissues, compared to conventional sources. In the present experiment, the use of thyme encapsulated form in comparison with the usual form has reduced the total microbial population in the small intestine. Unsaturated fatty acids in vegetable oils such as linolenic, linoleic and arachidonic have many benefits for the proper functioning of the gastrointestinal tract, including reducing harmful microbial populations, compared to the saturated fatty acids such as oleic acid and myristic acid, which are abundant in saturated fats. Microbial populations beneficially increased by using this types of fatty acids. The reduction in the number of coliforms in the intestines of broilers in this experiment could also be related to the positive effects of high unsaturated fatty acids in canola oil compared to tallow fat. High dietary nutrients such as high protein stimulate the growth of gastrointestinal microbes while reducing the level of crude protein in the diet can have the opposite effect. The decrease in intestinal coliforms by the reduction in the level of crude protein in the diet was probably due to the lack of amino acids necessary for the growth and multiplication of the microbial population. In general, it can be stated that by using the encapsulated form of thyme essential oil, along with canola oil and reducing the level of crude protein in the diet, it is possible to reduce the microbial population of the intestine, improve the health of broilers, reducing mortality and improve the performance. The herd economy increased and production of healthy and customer-friendly products is possible.

Antioxidant compounds

Oxidative stress is an ordinary action in life, which arises out the imbalance between the production of free radicals and the body's antioxidant system's ability to remove these reactive species (Mohebodini *et al*., 2021). Glutathione peroxidase and malondialdehyde enzymes are the first defenses against free radicals (El-Senousey *et al.*, 2018). Total antioxidant capacity is an indicator of anti-radical activity, enzymatic and non-enzymatic antioxidants (Sen *et al.,* 2010). Therefore, according to the liver's measured parameters, reducing the level of crude protein in meals, using caw tallow and thyme essential oil improves the antioxidant condition of the liver and the serum of broilers. By improving antioxidant condition, the level of malondialdehyde-which is an indicator of lipid peroxidation in the body- decreases. Results of the study were in line with the findings of Youdim and Deans (2000), Hoffman-Pennesi and Wu (2010), and Roofchaee *et al*. (2011). Youdim and Deans (2000) found that supplementing the mouse meal with 42.5 mg/day with thyme and thymol essential oils improves glutathione peroxidase activity, superoxide dismutase, and total antioxidant capacity of the brain. Antioxidant activities of essential oils such as thyme and oregano occur due to antioxidant compounds of essential oils (Hoffman-Pennesi and Wu, 2010; Roofchaee *et al.,* 2011). Thymol and carvacrol in medicinal plant oils such as thyme have strong antioxidant properties and can prevent the oxidation of dietary nutrients such as unsaturated fatty acids in vegetable oils such as rapeseed oil and increase the antioxidant properties of serum. This may be more effective by lowering dietary amino acid levels in low-protein foods. Positive results of improving serum antioxidant parameters in the present experiment can also be due to the above causes.

CONCLUSIONS

Based on this experiment's results, it can be stated that in broilers, the capsulated form of dietary thyme essential oil and oil type has affected the intestinal microbial population on behalf of coliform. Also, a reduction in the level of crude protein in the diet had no adverse effects on measured traits and should be recommended.

CONFLICT OF INTEREST

The authors declare that they have no known compering financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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