International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 22 No. 2 Jan. 2018, pp. 114-122
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http://www.ijias.issr-journals.org/

Effects of dietary thyme and oregano essential oils entrapped in chitosan and Canarium charcoal stable matrix on growth performances in broiler chickens

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ABSTRACT: Using essential oils (EOs) in animal feed is not practical due to the instability and volatility of their components. This study was designed to stabilize thyme and oregano EOs in chitosan and Canarium charcoal to mitigate their volatile and oxidative ability in poultry feed. The dietary treatments consisted of control diet (R0); control diet supplemented with chitosan film forming solution containing 0.01% of a blend of thyme and oregano EOs (R_{Th+Or}), 0.2% charcoal without EOs (Roc), chitosan film forming solution containing 0.01% of thyme EO entrapped with charcoal (Roc_{+Th}), chitosan film forming solution containing 0.01% of oregano EO entrapped with charcoal (R_{OC+Or}) and chitosan film forming solution containing 0.01% of a blend of thyme and oregano EOs entrapped with charcoal ($R_{OC+Th+Or}$). The results revealed that the entrapped EOs has no significant effect on feed intake. Weight gain markedly ($P<0.05$) increased with the blend of entrapped EOs for about 20% and 8% respectively during starter (days 1 to 21) and grower (days 22 to 49) phases compared to the control diet. The lowest feed conversion ratio (FCR) was also recorded with the blended entrapped EOs. Feeding broilers with the EOs mixture markedly ($P<0.05$) increased lactic acid bacteria count compared to the pathogens counts in both the ileum and the Cæcum. The entrapped EOs markedly decreased serum content in aspartate aminotransferase (ASAT) and creatinin, and increased in total protein and albumin. Thyme and oregano EOs can be stabilized in chitosan and Canarium seeds charcoal for a better growth in broiler chickens.

KEYWORDS: Broiler chickens, chitosan, plant charcoal, oregano, stable matrix, thyme.

1 INTRODUCTION

Essential oils (EOs) are volatiles substances that give plants their fragrance. Essential oils from a variety of plants are found to have antibacterial ability, and also exhibit antioxidant, anti-inflammatory, digestion stimulating and hypolipidemic activities [1], [2]. As an effective means of improving growth performances, their use as feed additives for poultry has become an exciting area of study [3], [4], [5]. They have been shown to stimulate feed intake and enzyme secretion related to food digestion and offer healthy performance benefits to poultry [6], [7]. However, the properties of EOs identified so far are extremely varied and their main active compounds made up by terpenoids and phenolics acids are labile and volatiles. They easily evaporates and decomposes during feed processing and storage when expose to heat, light or oxygen. In order to overcome the volatile and decomposition of bioactive compounds of EOs, nanoencapsulation which was recently applied for food industry [8], [9] could also be applied for animal feed. Encapsulation can protect and increase the physical stability of actives compounds, avoid loss of EOs and improve the bioavailability of EOs in the digestive tract of animals.

Chitosan has been widely used for the encapsulation of bioactive compounds in recent years due to its biocompatibility with various active and functional compounds, low toxicity and biodegradabiliy [8], [10], [11] [12]. Chitosan is a powder extracted from the shell of small aquatic animals such as crustaceans, squid or some mushrooms wall [8]. It is a good coating material and a selective chelating agent with preference to proteins and metals [13]. Due to his ability to bind a variety of

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compounds, chitosan could be an ideal organic support for the stabilization of EOs in animal feed. However, encapsulation of EOs in chitosan is effective only in solution and cannot be easily used as feed additive in this form. There is a need to look for a practical way and means to absorb the EOs encapsulated in chitosan solution in a solid and stable matrix for an easy incorporation in animal feed.

Many studies demonstrated that biocharcoals from plant and other organic origins can bind a variety of compounds with different compositions and shapes in their pores, and charcoals can also used as feed additive to improved growth in poultry [14], [15], [16]. Their use as growth promoters could be more efficient when they are combined to other actives and functional compounds like chitosan and EOs which are known to have beneficial effects on chicken health and growth. Previous studies suggested that the wide range of beneficial effects of EOs can be enhanced through synergetic effects both between individual EO's and in combination with other feed additives [1], [2].

The present study is proposed to give an overview on the potential of chitosan and plant charcoal to preserve the properties of essential oils by limiting their oxidation and volatilization, so as to contribute to their efficient use in poultry feed to offer intestinal health advantages and improve growth performances in broiler chickens.

2 MATERIALS AND METHODS

2.1 SITE OF STUDY

This study was conducted at the poultry unit of the Teaching and Research Farm of the University of Dschang, Cameroon. This farm is located at 5°26' North and 10°26' EST and at an altitude of 1420 m above sea level. Annual temperatures vary between 10°C and 25°C. Rainfall ranges from 1500-2000 mm per annum over a 9 months rainy season (March to November).

2.2 ESSENTIAL OILS AND CHARCOAL

Essential oil of Thyme and Oregano were obtained from Barij Esans.Co., Tehran, Iran. Mature black fruit seeds (*Canarium schweinfurthii* Engl.) were collected in the villages around the University Experimental Farm. These seeds were burnt on a wire netting using firewood to black charcoal, quenched with water and sun-dried. After drying, the charcoal was ground and sieved to pass a 1-mm mesh, and used to absorb EOs encapsulated in chitosan solution.

2.3 PREPARATION OF CHITOSAN FILM FORMING SOLUTION CONTAINING EOS ENTRAPPED IN CHARCOAL

Analytical grade water-soluble chitosan 0820a® used in this experiment was provided by Shandong Guanghao biological product Co.Ltd (Shandong, China). Chitosan film forming solution was prepared according to a method modified from the procedure reported by Chi et al. (2006). Briefly, chitosan stock solution (2% (w/v)) was prepared under magnetic stirring by dissolving 2 g of chitosan in 100 ml of distilled water at room temperature overnight.

The Oregano (10 g), Thyme (10 g) or a blend (1/1) of thyme and oregano EOs (10 g) was mixed to 10 ml of Tween 20. The mixture EO-Tween 20 was vortex and introduced in the Chitosan stock solution under constant stirring. After 1 hour under stirring, 200 g of charcoal were added to this EO-loaded chitosan film forming solution, and homogenized by hand shacking to allow charcoal to absorb all the solution. This charcoal was finally dried in an oven at 55^0C for 72 hours and used as feed additive during the starter and the grower phases in this experiment. The final concentration of EO in the feed was 0.01%.

2.4 ANIMAL

Three hundred and twenty (320) day old Cobb500 broiler chicks acquired from a local hatchery were divided into 5 experimental groups of 64 chicks each. Chicks were litter-brooded to 21 days of age at a density of 20 chicks/m². Vaccination and other routine poultry management practices were maintained.

2.5 DIETARY TREATMENTS

The dietary treatments consisted of control diet (R0); control diet (table 1) supplemented with chitosan film forming solution containing 0.01% of a blend of thyme and oregano EOs (R_{Th+Or}), 0.2% charcoal without EOs (Roc), chitosan film forming solution containing 0.01% of thyme EO entrapped with charcoal (Roc_{+Th}), chitosan film forming solution containing 0.01% of oregano EO entrapped with charcoal (R_{OC+Or}) and chitosan film forming solution containing 0.01% of a blend of thyme and

oregano EOs entrapped with charcoal ($R_{OC+Th+Or}$). Each experimental ration including the control was fed at random in a completely randomized design to 16 chicks (8 males and 8 females) replicated 4 times. Feed and water were offered *ad libitum*.

Table 1 Composition of experimental diets

Ingredients (%)	Starter	Finisher
Maize	54	64
Wheat bran	5	1
Soybean Meal	22	16
Coton seed meal	5	5
Fish meal	5	5
Bone meal	1	1
Oiester shell	1	1
Palm oil	2	2
Premix 5%*	5	5
Total	100	100
Calculated chemical composition		
Metabolizable energy (kcal/kg)	2928.66	3042.76.80
Crude Protein (%)	23.00	20.40
Lysine (%)	1.43	1.19
Methionine (%)	0.48	0.44
Calcium (%)	1.17	1.35
Phosphore (%)	0.53	0.56
Crude fibre (%)	5.20	5.14

**Premix 5%: crude proteins 400mg, Lysin 33mg, Methionin 24 mg, Calcium 80 mg, Phosphorous 20.5 mg, metabolizable energy 2078kcal/kg, Vitamins: Retinol 10 000 000 IU, Cholecalciferol 3 000 000 UI, Tocopherol 2500 IU, Phylloquinon 4000 mg, Thiamin 5000 mg, Riboflavin 500 mg, Pyridoxin 2500 mg, Cyanocobalamin 5 mg, Folic acid 10 000 mg and Niacin 2000 mg.*

2.6 GROWTH, SERUM BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS

Feed intake, weight gain and feed conversion ration were evaluated on a weekly basis in starter and finisher phases of the study. At the end of the feeding trial at 49 days of age, 10 birds (5 males and 5 females) from each treatment group were randomly selected, fasted for 24 hours and slaughtered for carcass evaluation. From each slaughtered chicken, blood was collected in 02 test tubes of which one contained an anticoagulant. Blood with anticoagulant was used for the haematological analysis using Genius electronic haematocymeter (Model KT-6180, S/N 701106101557, Hong Kong, China). Hematological parameters included white blood cell (WBC), red blood cell (RBC), haemoglobin (HB), haematocrit (HCT) and platelets (PLT). Meanwhile, after centrifugation of blood free from anticoagulant, serum was collected and preserved at -20°C for the evaluation of biochemical parameters including total protein, albumin, globulin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total cholesterol, cholesterol HDL and LDL, triglyceride, urea and creatinin using colorimetric method as prescribed by the Chronolab® commercial kits.

2.7 MICROBIAL COUNT

The ileum and the cæcum from four slaughtered birds per treatment were sampled and pooled by intestinal segment. The numbers of lactic acid bacteria, *Escherichia coli* and salmonella were counted on appropriate specific culture medium (MRS Agar for lactic acid bacteria, Mac Conkey AGAR for *E. coli* and SS AGAR for salmonella respectively) as proceeded by Pineda *et al*. [17].

2.8 STATISTICAL ANALYSIS

All the data were submitted to analysis of the variance using Statistical Package for Social Science (SPSS 21.0) software. Significant differences between treatment means were separated using Duncan's multiple range tests at 5 % threshold significance.

3 RESULTS

Table 2 summarizes the live body weight (LBW), weight gain (WG), and feed conversion ratio (FCR) of broilers as affected by entrapped chitosan film forming solution containing thyme and oregano EOs in *Canarium* charcoal. Whether blended or not, the entrapped EOs has no significant effect on feed intake. Weight gain markedly (P<0.05) increased with the entrapped blend EOs for about 20%, 8% and 8% respectively during starter phase (days 1 to 21), finisher phase (days 22 to 49) and throughout the study period (days 1 to 49) compared to the control diet. Irrespective to study phase, the lowest feed conversion ratio (FCR) was also recorded with the blend of entrapped EOs.

Table 2 Growth performance of broiler chicken as affected by thyme and oregano EOs entrapped in chitosan and Canarium charcoal

Study period (days)	Treatments							
	R_0	R_{Th+Or}	R_{0C}	R_{0C+Th}	R_{0C+Or}	$R_{0C+Th+Or}$	SEM	P value
				Feed intake (g)				
01 - 21	1086.61	1097.83	1083.33	1087.33	1088.83	1081.52	18.36	1.000
22 - 49	4470.75	4460.02	4609.14	4426.78	4613.00	4495.39	128.45	0.763
01 - 49	5557.36	5557.85	5692.47	5514.11	5701.83	5576.91	118.60	0.495
				Live body weight (g)				
01 - 21	655.16^{a}	699.39^{a}	668.50^{a}	694.36^{a}	685.14^{a}	808.13^{b}	14.16	0.000
01 - 49	2589.15^{b}	2797.92^{a}	2570.06^{b}	2548.03^{b}	2749.83^{a}	2799.27^{a}	33.99	0.032
				Body weight gain (g)				
01 - 21	612.17^{b}	656.39^{b}	625.50^{b}	651.36^{b}	642.14^{b}	766.12^{a}	14.24	0.000
22 - 49	1933.98^{b}	1991.14^{ab}	1901.56^{ab}	1853.67^{b}	2064.69^{a}	2098.53^{a}	28.58	0.087
01 - 49	2546.15^{b}	2747.92^{a}	2527.06^{b}	2505.03^{b}	2706.83^{a}	2757.27^{a}	34.03	0.032
				Feed conversion ratio				
01 - 21	1.79	1.76	1.73	1.67	1.70	1.65	0.03	0.634
22 - 49	2.31^{ab}	2.24^{ab}	2.43^{a}	2.39^{ab}	2.24^{ab}	2.14^{b}	0.04	0.163
01 - 49	2.19^{ab}	2.11^{ab}	2.25^{a}	2.20^{ab}	2.11^{ab}	2.02^{b}	0.03	0.161

a.b: Means with the same superscript on the same line are not significantly different (P>0.05). P= probability.
R_0= control diet; R_{Th+Or}=R_0 + blend of EOs; R_{0C} = R_0 + 0.2% charcoal without EOs; R_{0C+Th} =R_0 + entrapped thyme EO;
R_{0C+Or}: R_0 + entrapped oregano EOs; $R_{0C+Th+Or}$= R_0+entrapped blended EOs

The effect of various treatments on carcass yield and the relative weight of cut-out is summarized in table 3. Treatments failed to induce any marked effect on carcass yield and the relative weight of head, legs and abdominal fat. Non entrapped blend of EOs significantly (P<0.05) decreased the relative weight of the liver as compared to all the treatments. Feeding broiler with Canarium charcoal without EOs markedly (P<0.05) decreased the relative weight of heart.

Table 3 Carcass traits of broiler chickens as affected by thyme and oregano EOs entrapped in chitosan and Canarium charcoal

Carcass traits	Treatments							
	R_0	R_{Th+Or}	R_{0C}	R_{0C+Th}	R_{0C+Or}	$R_{0C+Th+Or}$	SEM	P value
Carcass yield (%)	74.43	74.48	74.60	75.22	74.5	75.45	0.53	0.411
Head (%BW)	2.02	1.84	2.00	2.05	2.08	2.00	0.09	0.349
Legs (%BW)	3.50	3.46	3.42	3.60	3.53	3.25	0.19	0.991
Liver (%BW)	1.77^{a}	1.44^{c}	1.75^{a}	1.66^{bc}	1.72^{ab}	1.70^{ab}	0.07	0.002
Heart(%BW)	0.46^{ab}	0.53^{a}	0.39^{b}	0.45^{ab}	0.44^{ab}	0.45^{ab}	0.03	0.030
Abdominal fat (%BW)	1.77	1.86	1.81	1.66	1.74	2.01	0.20	0.845

a.b,c: Means with the same superscript on the same line are not significantly different (P>0.05). P= probability.
R_0= control diet; R_{Th+Or}=R_0 + blend of EOs; R_{0C} = R_0 + 0.2% charcoal without EOs; R_{0C+Th} =R_0 + entrapped thyme EO;
R_{0C+Or}: R_0 + entrapped oregano EOs; $R_{0C+Th+Or}$= R_0+entrapped blended EOs

As shown in table 4, this result revealed that thyme and oregano EOs, whether entrapped or not, blended or not failed to induced any significant (P>0.05) effect on digestive organs development in broiler chickens.

Table 4 Digestive organs development of broilers as affected by thyme and oregano EOs entrapped in chitosan and Canarium charcoal

Digestive organ traits	Treatments							
	R_0	R_{Th+Or}	R_{0C}	R_{0C+Th}	R_{0C+Or}	$R_{0C+Th+Or}$	SEM	P value
Gizzard (%BW)	1.37	1.38	1.30	1.36	1.44	1.34	0.07	0.090
Pancreas (% BW)	0.18	0.21	0.19	0.20	0.21	0.16	0.03	0.932
lntestin weight (g)	82.17	78.10	87.90	73.00	90.10	88.00	4.08	0.100
Intestin lenght (g)	189.92	204.60	192.70	189.80	185.70	190.10	6.54	0.674
Intestin density (g/cm)	0.40	0.38	0.47	0.41	0.49	0.48	0.34	0.113

a.b: Means with the same superscript on the same line are not significantly different (P>0.05). P= probability.
R_0= control diet; R_{Th+Or}=R_0 + blend of EOs; R_{0C} = R_0 + 0.2% charcoal without EOs; R_{0C+Th} =R_0 + entrapped thyme EO;
R_{0C+Or}: R_0 + entrapped oregano EOs; $R_{0C+Th+Or}$= R_0+entrapped blended EOs

Table 5 indicates that feeding broilers with a blend of the entrapped and non entrapped EOs markedly (P<0.05) increased lactic acid bacteria count compared to the *E. coli* and salmonella counts in both the ileum and the Cæcum. When compared to control diet without any supplement, thyme and oregano EOs individually or blended, entrapped or not significantly (P<0.05) increased the lactic acid bacteria count in the ileum and the Cæcum. The increase in lactic acid bacteria count was also significant with EOs entrapped in chitosan and charcoal compared to diet supplemented with charcoal without EOs.

Table 5 Ileal and cæcal microbial counts as affected by thyme and oregano EOs entrapped in chitosan and Canarium charcoal

Bacteria counts $\log_{10}$ ($CFUx10^{-7}$)	Traitements							
	R_0	R_{Th+Or}	R_{0C}	R_{0C+Th}	R_{0C+Or}	$R_{0C+Th+Or}$	SEM	P value
Ileon								
Lactic acid bacteria	8.04^{cB}	9.42^{aA}	8.45^{bC}	9.36^{aA}	9.36^{aA}	9.93^{aA}	0.07	0.000
E. coli	9.35^{abA}	7.81^{cC}	9.63^{aA}	9.34^{abA}	9.11^{abA}	8.98^{bB}	0.07	0.000
Salmonella	8.19^{cB}	8.37^{bB}	9.07^{aB}	8.59^{bB}	8.37^{bcB}	9.32^{aB}	0.11	0.000
SEM	0.25	0.11	0.17	0.13	0.16	0.15		
P	0.030	0.063	0.000	0.003	0.001	0.004		
Cæcum								
Lactic acid bacteria	8.08^{cA}	9.42^{abA}	6.67^{dB}	9.67^{abA}	9.32^{bA}	9.77^{aA}	0.16	0.000
E. coli	8.27^{cA}	8.64^{bB}	9.14^{abA}	8.46^{bcB}	9.27^{aA}	8.42^{cB}	0.14	0.023
Salmonella	7.75^{bA}	8.72^{abB}	9.13^{aA}	7.71^{bC}	8.56^{abB}	8.21^{abB}	0.03	0.014
SEM	0.16	0.23	0.40	0.28	0.23	0.24		
P	0.503	0.000	0.000	0.000	0.411	0.000		

a.b,c,d: Means with the same superscript on the same line are not significantly different (P>0.05). P= probability.
A,.B: Means with the same superscript on the same column are not significantly different (P>0.05).
R_0= control diet; R_{Th+Or}=R_0 + blend of EOs; R_{0C} = R_0 + 0.2% charcoal without EOs; R_{0C+Th} =R_0 + entrapped thyme EO;
R_{0C+Or}: R_0 + entrapped oregano EOs; $R_{0C+Th+Or}$= R_0+entrapped blended EOs

As summarized in table 6, feeding broilers with individual or blended thyme and oregano EOs entrapped in chitosan and charcoal markedly (P<0.05) decreased serum content in aspartate aminotransferase (ASAT) and creatinin, and induced a significant (P<0.05) increase in total protein and albumin. These treatments failed to have any significant (P>0.05) effect on serum content in globulin and LDL-cholesterol.

Table 6 Effect of thyme and oregano EOs entrapped in chitosan and Canarium charcoal on biochemical parameters

Biochemical parameters	Treatments							
	R_0	R_{Th+Or}	R_{0C}	R_{0C+Th}	R_{0C+Or}	$R_{0C+Th+Or}$	SEM	P value
ASAT (U/L)	136.28^{a}	126.25^{c}	112.70^{b}	103.43^{b}	103.98^{b}	114.63^{b}	7.14	0.000
ALAT (U/L)	120.75^{a}	115.31^{b}	118.50^{a}	129.00^{a}	139.13^{a}	121.92^{a}	6.12	0.000
Urea (mg/dl)	0.51^{b}	0.87^{c}	0.27^{a}	1.58^{d}	0.90^{c}	0.63^{b}	0.43	0.000
Creatinin (mg/dl)	0.91^{a}	0.63^{ab}	0.77^{ab}	0.48^{b}	0.67^{ab}	0.54^{b}	0.11	0.000
Total cholesterol (mg/dl)	52.92^{b}	51.82^{b}	62.18^{a}	42.15^{b}	49.70^{b}	52.75^{b}	3.40	0.008
Triglycerides (mg/dl)	51.26^{bc}	19.03^{d}	37.27^{bc}	36.36^{c}	72.22^{a}	54.14^{b}	3.68	0.000
HDL- cholesterol (mg/dl)	11.59^{b}	29.37^{a}	14.07^{b}	16.12^{b}	12.57^{b}	13.16^{b}	1.34	0.000
LDL- cholesterol (mg/dl)	23.95	19.23	14.60	8.41	12.22	16.81	2.19	0.544
Protein total (g/dl)	1.31^{b}	2.34^{a}	2.26^{a}	2.22^{a}	2.10^{ab}	2.44^{a}	0.12	0.050
Albumin (g/dl)	0.50^{a}	1.36^{b}	3.94^{c}	1.17^{b}	1.52^{b}	1.44^{b}	0.32	0.011
Globulin (g/dl)	0.92	1.18	0.82	1.17	1.10	1.11	0.15	0.301
Albumine/ globuline	*0.54^{c}*	*1.15^{b}*	*4.80^{a}*	*1.00^{b}*	*1.38^{b}*	*1.29^{b}*	*0.16*	*0.000*

a.b,c,d: Means with the same superscript on the same line are not significantly different (P>0.05). P= probability.

Apart for the white blood cells count which significantly (P<0.05) increase with the blend of non-entrapped EOs, hematological parameters were not significantly affected by the treatments (Table 7). However, red blood cells, hemoglobin and percentage of hematocrit tend to increase compared to the control diet without any supplement, but this increase did not reach the significant threshold with entrapped EOs.

Table 7 Hematological parameters of broilers as affected by thyme and oregano EOs entrapped in chitosan and Canarium charcoal

Blood parameters	Treatments							
	R_0	R_{Th+Or}	R_{0C}	R_{0C+Th}	R_{0C+Or}	$R_{0C+Th+Or}$	SEM	P value
WBC (10^3/µl)	78.00^{b}	90.17^{a}	77.10^{b}	81.50^{ab}	81.00^{ab}	74.17^{b}	1.43	0.016
RBC (10^6/µl)	3.00	3.00	2.76	3.14	3.28	3.00	0.06	0.687
Hb (g/dl)	14.00	14.50	13.20	14.14	15.57	14.33	0.34	0.560
HCT (%)	32.80	36.17	30.55	33.71	37.29	33.83	0.88	0.349
PLT (10^3/µl)	47.00	46.83	33.50	42.43	46.00	39.67	2.72	0.795

a.b: Means with the same superscript on the same line are not significantly different (P>0.05). P= probability.
WBC: white blood cells; RBC: Red blood cells; Hb: Hemoglobin; HTC: Hematocrit; PLT: Platelets

4 DISCUSSION

Irrespective to the study phase, thyme and oregano EOs and their mixture entrapped in charcoal did not have any significant effect on feed intake in broilers. This result contradicted the findings of Amerah *et al.* [7], Weber *et al.* [18] and Khattak *et al.* [5] who reported an increase in feed intake with commercial feed additives containing thymol, carvacrol and cinnamaldehyde which are present in the EOs used in the present study [5]. In the same trend, Cross *et al.* [19] and Khattak *et al.* [5] also revealed that EOs stimulated feed intake, improve weight gain and offer health advantages to poultry. The difference between the present result and the results recorded by the above authors can be due to the fact that EOs was entrapped in charcoal in this study. In fact, EO created a strong complex formation between chitosan and EOs via electrostatic interaction [8], [9]. This strong complex formation might impact the beneficial effects of the EOs compounds on diet appetite. Another explanation of the non significant effect of treatments on feed intake recorded in this study can be the better feed efficiency since chickens consumed little feed and gained more weight.

Many studies revealed that EOs promote the activity of digestive enzymes leading to an efficient use of nutrients and growth performances [6], [20]. Irrespective to study phase, live body weight and weight gain markedly increased with the entrapped blended EOs compared to the control diet. The lowest feed conversion ratio (FCR) was also recorded with the blend of entrapped EOs. Many in vitro studies suggested that combination of individual EOs has greater effect than individual EOs alone, indicating a synergy between single activities [1], [2]. One of the main mechanisms which explain the improvement of poultry performances is the ability of EOs to balance the gut microbiota by inhibiting pathogens bacterial growth due to their antibacterial properties. The antibacterial activity of thyme and oregano EOs is due to the presence of several active

compounds such as the carvacrol, thymol, eugenol or cinnamaldéhyde [21]. These compounds interact and increase the permeability of the bacteria cell membrane, deteriorate the enzymatic systems and inhibit or destroy the genetic material of the bacteria [2]. Another mechanism could be the stimulation of the secretion of the digestive enzymes of the host [1] and the modulation of the immune system which offer healthy performance benefits to poultry [5], [6], [7], [22]. The present result is similar to the findings of Alkassie [23], Malayoglu *et al.* [24] and Fotea *et al.* [25] who recorded a better FCR with the inclusion of cinnamon, oregano and thyme EOs in broilers diets. This observation suggests that nutrients utilization in broiler fed on EOs entrapped charcoal is better compared to chickens fed on control diet and diet supplemented with charcoal without EOs. Previous results reported by Amerah *et al.* [7], Krishan and Narang [2] and Khattak *et al.* [5] recognized that EOs do not only improve feed efficiency, but also act as an antioxidant, anti-inflammatory and immune system stimulator in chickens [27], [28].

Treatments failed to induce any marked effect on carcass yield and digestive organs development. However, non entrapped blended EOs significantly decreased the relative weight of the liver as compared to other treatments. These results are similar to those of Kana *et al.* [16] who concluded that including plant charcoal in the chicken feed did not have any significant effect on the carcass yield. The decreased in the relative weight of the liver in the present study contradicted the findings of Fotea *et al.* [25] and Toghyani *et al.* [29] who reported that thyme did not induce any significant effect on the relative weight of the liver, head and heart in broilers.

Feeding broilers with thyme and oregano EOs individually or blended, entrapped or not markedly increased the lactic acid bacteria count (beneficial bacteria) both in the ileum and the Cæcum compared to the salmonella and the E. coli counts (pathogens). The present result is in agreement with the findings of Murry *et al.* [30] who observed an increased in lactic acid bacteria population in the gut of healthy chickens. When the living conditions in the intestine is favorable, the lactic acid bacteria multiply and eliminate pathogenic bacteria (salmonella and Escherichia coli) by acidifying the milieu and producing antibacterial substances like organic acids [2]. Although the proliferation of lactic acid bacteria stimulates the immune system of the chickens [5], [6], [7], it was shown that EOs prevents the adhesion of the pathogens bacteria on their intestinal mucosa by stimulating the secretion of mucus [31]. The increase in lactic acid bacteria count was also significant with EOs entrapped in charcoal compared to charcoal without EOs, suggesting that enrichment of charcoal with EOs can be more benefic to poultry.

Feeding individual or blended thyme and oregano EOs entrapped in charcoal to broiler induced a significant increase in serum protein and globulin content. This result suggest the capacity of EOs to improve digestion and absorption of proteins as previously reported by Bento *et al*. [1] and Krishan and Narang [2] allowing a better use of protein in broiler chicken and thus an improvement of the weight gain as recorded here. This study also revealed that feeding broilers with EOs entrapped in chitosan and stabilized in charcoal markedly decreased serum content in ASAT and creatinin. The present result contradicted the findings of Khattak *et al.* [5] who recorded no significant effect on the serum content in ASAT and ALAT in broilers fed on commercial product containing thymol, carvacrol, cinnamaldehyde, oregano, peppermint and pepper. According to Pitel *et al*. [32] and Pritchard *et al.* [33], a decrease in creatinin concentration may be a sign of cachexia (state of severe weight lose caused by illness or undernourishment) and very high protein intake can cause chronic renal failure or acute renal failure which results in an increase in serum creatinin.

The EOs entrapped in chitosan and charcoal induced a significant decrease in LDL-cholesterol. The present result contradicted the findings of Bolukbasi *et al*. [34] who reported that feeding thyme EO to broilers leads to an increase in serum content in LDL and HDL-cholesterol. The decrease in cholesterol levels recorded in this study may be due to the inhibitory effects of thymol and carvacrol, the major active compounds found in thyme and oregano EOs on HMG-CoA reductase which is the key enzyme to cholesterol synthesis [35].

Red blood cells, hemoglobin and the percentage of haematocrit were not significantly affected but tend to increase with entrapped EOs compared to the control diet without any supplement. In fact, blood parameters reflect the healthy state of an organism and any changes happening to it could be an indication of unbalance feeding or disease attack. The present result is in close agreement with the findings of Kana *et al.* [16] and Toghyani *et al.* [29] who respectively reported that supplementing broiler chickens with *Canarium* charcoal without EOs and thyme powder did not have any marked effect on red blood cells count, hemoglobin content and haematocrit percentage.

5 CONCLUSION

The present results suggested that thyme and oregano EOs can be stabilized in chitosan and *Canarium* seeds charcoal as a stable matrix for a better used in poultry feed. Blend of thyme and oregano EOs entrapped in charcoal has more beneficial effect on lactic acid bacteria growth in the intestine and offer better healthy performance benefits to broiler in both starter and grower phases.

ACKNOWLEDGEMENTS

The authors would like to extend their sincere appreciation to Dr Meimandipour Amir, Department of Animal Biotechnology, National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran who graciously provided the chitosan and the essential oils used in this study.

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