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Comparison of Dietary Supplementation with Cumin Essential Oil and Prebiotic Fermacto on Humoral Immune Response, Blood Metabolites and Performance of Broiler Chickens

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Abstract: Five hundred day-old male Ross broiler chicks were divided into 50 groups of 10 birds each and randomly assigned to 10 dietary treatments of 5 replicates each. Two corn-soybean meal-based diets were first provided to meet 100 and 95% of recommended digestible amino acids (RDAA) for starter (1-14 d of age), grower (15-35 d of age) and/or finisher (36-49 d of age) periods. Each starter diet were subdivided into 5 parts and supplemented with 0 and 2 g kg^{-1} Fermacto and 0.2, 0.4 and 0.8 g kg^{-1} Cumin Essential Oil (CEO). The grower diets contained half of the same supplementation of the starter diets and each of the un-supplemented finisher diet fed to the corresponding birds. The blood metabolites concentration, cell differentiation and total anti-SRBC, IgG and IgM titres measured on d 26 in birds fed diet containing 100% RDAA level. Cumin essential oil and Fermacto did not have a significant effect on performance parameters in the starter and finisher periods, but higher BWG was observed in birds fed diet with the lowest level of CEO supplementation in the grower period. Birds fed diet with 100% RDAA and Fermacto had higher feed: gain ratio in the grower period when compared to those fed diet of similar AA and 0.2 g kg^{-1} of CEO. A 5% decrease in the RDAA had no adverse effect on the overall feed consumption and BWG, but feed: gain ratio was significantly (P>0.05) increased. There was no significant difference in total anti SRBC, IgG and IgM titres. The inclusion of Fermacto and/or various levels of CEO in diets did not have an influence on serum metabolite concentrations (mg/dl) at d 28, although triglyceride and VLDL concentrations was lower (P<0.05) in chicks fed starter diet contained 0.4 g kg^{-1} CEO.

Key words: Cumin Essential Oil · Digestible Amino Acids · Prebiotic Fermacto · Humoral Immune Response

INTRODUCTION

The currently used feed additives such as antibiotics, probiotics and prebiotics in broiler diets to enhance nutrient utilization by mean of diverse mechanisms. Due to possible hazards and risks of antibiotics in poultry production, the importance of using prebiotics or natural feed additives as an alternative has increased more than ever [1]. Fermacto is a commercial microbial feed supplement derived from Aspergillus Mycelium and has shown to affect the host animal through stimulation of growth and improvement in intestinal microbial balance [2, 3].

The aromatic medicinal plants are probably natural alternatives for antibiotics. The aromatic plants and essential oils extract from these plants are important growth promoter due to their antimicrobial and stimulating effects on animal digestive system. Aromatic plants have been used traditionally in the therapy of some diseases for a long time. Many Plants contain extensive variety of phytochemical compounds with antimicrobial activity [4]. Plant extracts have demonstrated to have an antimicrobial effect *in vitro*, but their influence on growth of farm animal species has not been demonstrated yet. *Cuminum cyminum Linn*. *(Cumin)* is an annual plant of the Umbelliferae family. Cumin is an important medical herb in Asia and has antioxidant, anticholesterol and antimicrobial properties. The inhibitory effect of cumin extract on *E. coli* 0:157 demonstrated *in vitro* [5]. According to Shetty *et al.* [6], fungi and yeast were more sensitive to cumin essential oil as compared to bacteria. Cumin increased activity and excretion content of bile acids [7] and also increased pancreas and small intestine digestive enzymes such as amylase, tripsine, chymotripsine

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and lipase in rats [8-11]. The oral consumption of cumin seed (%1.25) significantly decreased Gastrointestinal Transit Time (GTT) and increased retention time in rats [12]. The purpose of this study was to evaluate the effect of dietary cumin essential oil and Fermacto on immune response, blood metabolites and performance of broiler chickens. In addition the effect on organ weight, GTT and fat digestibility were determined.

MATERILS AND METHODS

Five hundred day-old male broiler chicks (Ross 308) were randomly divided into fifty groups of equal weights and assigned to 50 floor pens. The ten dietary treatments were randomly fed to 5 replicates pens of 10 birds each. Corn-soybean meal-based broiler diets were prepared according to the digestible nutrients recommended grown to 2.5 kg by Aviagen Company [13] and ingredient composition of Leeson and Summers [14] report. Two diets were first provided to meet 100 and 95% of recommended digestible amino acids (RDAA) for either of the starter (1-14 d of age), grower (15-35 d) and finisher (36-49 d) periods. The starter diets with each level of RDAA were subdivided into 5 parts and supplemented with 0.0 and 2.0 g kg^{-1} Fermacto, 0.2, 0.4 and 0.8 g kg^{-1} cumin essential oil (CEO). The grower diets contained half of the same supplementation of the starter diets and each of the un-supplemented finisher diet was fed to the corresponding birds. The composition of experimental diets is shown in Table 1. The CEO and Fermacto were provided from Herbal Exire and Javaneh Khorasan Companies (Mashhad, Iran), respectively. The CEO was first mixed with vegetable oil and then was added to the basal diet. Birds were exposed to 24 h light and had free access to feed and water throughout the experiment. The body weight (BW) and feed intake (FI) in each group of birds were determined four h after feed removal and feed: gain ratio was calculated during 1-14, 15-35 and 36-49 d of age. Daily mortalities were recorded and used to correct performance criteria. Gastrointestinal transit time (GTT) of feed in diets contained 100% RDAA was measured by covering the pens with clean paper 4 h after feed withdrawal and the time between offering the chromic oxide (0.3%) diet and appearance of average three spotted green excreta in each pens at d 16 was recorded. The pens excreta samples were collected for lipid digestibility determination after birds consumed the chromic oxide diet for 32 h. A sample of diet and excreta from each pen were stored in-20° C for further analysis. Lipid content of the diet and excreta samples were determined by soxhlet

Table 1: Composition (g/kg) of starter, grower and finisher diets contained 100 or 95 percent recommended crude protein and digestible amino acids (RDAA)

	Starter[1] (g/kg)		Grower[2] (g/kg)		Finisher[3] (g/kg)	
Ingredients	100% RDAA	95% RDAA	100% RDAA	95% RDAA	100% RDAA	95% RDAA
Composition						
Corn	507	534.6	543	580	609	633
Wheat Bran	35	40	24	21	23	28.9
Soybean meal	375.5	345	332	302.7	270	241.6
Vegetable oil	38.5	36	60.5	55	59	57
Limestone	18.5	18.5	16.6	16.5	16	16.5
Dicalcium phosphat	14.5	15	12.8	13.4	12	12
Nacl	2.9	3	2.9	3	3.1	3
Methionin	2.1	1.9	2.2	2.1	1.9	1.7
L-Lysine	0	0	0	0.3	0	0.3
Vitamin premix[4]	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix[4]	2.5	2.5	2.5	2.5	2.5	2.5
Vitmin E	1	1	1	1	1	1
calculated composition						
AME,kcal/kg	3009	3011	3176	3176	3225	3226
Crude protein	230.1	218.48	210	199.4	186.12	175.62
Calcium	10.09	10.15	8.99	9	8.5	8.61
Available phosphor	5.02	5.09	4.51	4.56	4.23	4.2
Lysine	11.73	10.92	10.4	9.93	8.86	8.35
Methionin	5.4	5.06	5.27	5.06	4.7	4.38
Methionin+Cystine	8.155	7.68	7.81	7.47	6.97	6.52
Linoleic acid	30.98	30.21	42.3	40	42.54	41.98

[1]The levels of 0, (control) 0.2 g kg^{-1} Fermacto, 0.2, 0.4 and 0.8 g kg^{-1} CEO were added to starter diets with either DAA levels.

[2]The grower diets contained half of the same supplementation of the starter diets.

[3]Each un-supplemented finisher diet was fed to the corresponding birds.

[4]Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3. 9800 IU, vitamin E. 121 IU; B12, 20 ìg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg, niacin, 22 mg; cholin, 840 mg; biotin, 30 ìg, thiamine, 4 mg; zinc sulphate, 60 mg; manganese, 60 mg.

extraction (Soxtec System HT 1043 Extraction unit) according to standard procedure of analysis [15]. The diet and excreta samples chromium were determined using dry ashing procedure of Miller-Ihli and Greene [16]. One chick close to the average replicate weight from each pen fed 100% RDAA was selected, weighed and killed by cervical dislocation after 4 h fasting to determine the breast, legs, abdominal fat pad, liver (with gall bladder), ceca, heart, spleen, bursa of Fabricius and total tract at d 28 and 49.

Glucose, triglyceride, cholesterol, HDL, LDL and VLDL concentration in serum of chicks fed 100% RDAA were measured enzymatically by an auto-analyzer (Selectra E, Vital Scientific, Netherland) and the RBC and Lymphocyte, Monocyte, Heterophile and Eosinofile count were determined based on the procedure of Gross and Siegel [17] at d 28. Five chicks per replicate fed 100% RDAA were injected intramuscular with SRBC (10% suspension in PBS, 1 ml/chick) at 28 day of age. Blood samples were collected at d 7 and 14 of injection. Total anti-SRBC, IgM and IgG were measured according to the method of Cheema *et al.* [18].

Statistical Analysis: The data were subjected to ANOVA as a completely randomized design using the GLM procedures of SAS software [19]. Tukey's test was applied to compare the treatment means when the treatment effect was significant at P=0.05. Orthogonal contrasts were used to compare the RDAA levels, Fermacto and CEO effects on all criteria. All data were tested for normality prior to analysis. Abnormal data were first transformed and then analyzed.

RESULTS AND DISCUSSION

Performance: The effects of different levels of CEO and Fermacto on performance parameters and their orthogonal contrasts on broiler chicks are shown in Tables 2 and 3, respectively. Regardless of RDAA level, the levels of CEO and Fermacto did not have a significant effect on feed consumption and feed: gain ratio in starter and finisher periods, although feed consumption in CEO fed birds increased (P<0.05) as compared to those fed diet containing Fermacto in the grower period. The feed: gain ratio was the lowest in birds fed diet with the lowest level of CEO (0.2 g kg^{-1}) when diets were formulated to contain 100% RDAA during the growing period. Birds fed diet contained Fermacto and/or various levels of CEO at either levels of RDAA did not significantly influence final BWG. Regardless of RDAA levels, the BWG up to 35 d were showed to be higher in birds fed starter and grower diets containing 0.2 and 0.1 g kg^{-1} CEO, respectively.

The orthogonal contrast showed that the feed consumption of broilers fed diets supplemented with Fermacto was decreased in the grower (P<0.001), finisher (P=0.003) and overall periods (P=0.053) as compared to those fed control diet, whereas feed: gain ratio improved (P=0.009) in the finisher and/or the entire experiment (P<0.001) periods. Body weight gain was increased (P=0.026) in birds fed diet containing Fermacto than those fed control diet in the growing period.

The orthogonal contrast revealed that the CEO fed birds had higher feed consumption than those fed Fermacto contained diets during growing (P<0.001), finishing (P=0.009) and the entire experimental (P<0.029) periods, but was lower than control birds (P=0.048 and P=0.026 in grower and finisher periods, respectively).

A 5% decrease in RDAA in diet significantly increased feed consumption of birds fed starter (P= 0.019) and grower (P= 0.009) diets but not in the finisher and/or the entire experimental periods. The feed: gain ratio was similar in birds fed either levels of starter and/or grower RDAA diets, but birds fed 100% RDAA diet improved their feed: gain ratio in the finisher and/or entire experiment. The orthogonal comparison showed that birds fed diets containing CEO had higher BWG as compared to those fed diet contained Fermacto in the grower period (P<0.001) and/or those fed control diet in the starter period (P<0.001).

Growth of chicks fed diet contained different levels of CEO was similar to those fed Prebiotic Fermacto contained diet and this was in agreement with Hernandez *et al*. [20] who observed an improvement in the body weight gain of chicks fed diets supplemented with plant extracts and/or essential oils extracted medical herbs. Lee *et al.* [21] investigated the effect of Thymol and Carvacrol on female broiler chick performance. The dietary Carvacrol reduced BWG, whereas feed: gain ratio improved as compared to control and/or Thymol fed birds. Cross *et al*. [22] reported that Thyme oil and Yarrow herb has positively influenced growth performance of broilers as it occurred with our birds fed CEO diets.

Although the levels of CEO and Fermacto in starter diets were two times as those in the grower diets, but the feed digestibility is usually higher in the growing period, because chicks endogenous enzymes system are not completed in the early stage of life. The improved feed: gain ratio in birds fed diet containing Prebiotic Fermacto in our study is in agreement with those reported by Khaksar *et al*. [2] and Tangendjaja [3].

Table 2: Effect of Fermacto and cumin essential oil (g kg^{-1}) on growth performance and their orthogonal contrast between them on broiler chickens fed 100 or 95% recommended crude protein and digestible amino acids (RDAA)[1]

	100% RDAA					95% RDAA					
	control	Fermacto	Cumin essential oil			control	Fermacto	Cumin essential oil			
Starter[2]	0	2	0.2	0.4	0.8	0	2	0.2	0.4	0.8	MSE
Feed consumption	(g)										
1 to 14 d	23.9	24.2	23.6	24.1	23.5	22.3	22.3	24.4	23	23.2	0.533
15 to 35 d	106.7^{ab}	100.7^{b}	103.8^{ab}	102.9^{ab}	105.6^{ab}	110.7^{a}	101.5^{b}	108.9^{a}	106.5^{ab}	108.9^{a}	1.55
36 to 49 d	175.4	172	173.5	175.6	177.4	180.5	169	178.5	178.5	181.2	3.78
1 to 49 d	102.3	98.3	100.8	102.4	104.2	103.3	99.7	101.9	101.5	104.5	1.91
Body weight gain	(g/d)										
1 to 14 d	13.9^{ab}	14.4^{ab}	14.9^{a}	14.5^{ab}	14.2^{ab}	13.4^{b}	13.8^{ab}	14.8^{a}	14.4^{ab}	14.4^{ab}	0.277
15 to 35 d	57.9^{ab}	58.7^{ab}	61.1^{a}	57.2^{ab}	58.4^{ab}	58.7^{ab}	57.3^{ab}	60.6^{a}	57.7^{ab}	58.2^{ab}	1.21
36 to 49 d	80.7	85.2	83.9	86.6	88.2	82	82.3	81.5	85.1	83.5	3.12
1 to 49 d	51.6	53.1	54	54.4	52.2	51.8	52.4	52.3	52.3	53.5	1.18
Feed: gain ratio	(g:g)										
1 to 14 d	1.71	1.69	1.63	1.66	1.66	1.68	1.61	1.62	1.6	1.62	0.052
15 to 35 d	1.84^{ab}	1.88^{a}	1.73^{b}	1.78^{ab}	1.81^{ab}	1.88^{ab}	1.77^{ab}	1.78^{ab}	1.85^{ab}	1.81^{ab}	0.036
36 to 49 d	2.17	2.02	2.07	2.03	2.01	2.18	2.11	2.1	2.1	2.17	0.058
1 to 49 d	1.98^{ab}	1.89^{bc}	1.87^{c}	1.88^{bc}	1.89^{bc}	2.00^{a}	1.9^{abc}	1.95^{abc}	1.94^{abc}	1.96^{abc}	0.022

$^{a, b}$ Means within each row with uncommon superscript are significantly different (P< 0.05).

[1]Each un-supplemented finisher diet was fed to the corresponding birds.

[2]The Fermacto and CEO content in starter diets (g kg^{-1}), whereas the grower diets contained half of the same supplementation of the starter diets.

Table 3: Orthogonal comparisons fed the effect of recommended crude protein digestible amino acid (RDAA) levels, Fermacto and cumin essential oil (CEO) on feed consumption, body weight gain and feed: gain ratio of broiler chicks

	Feed consumption				Body weight gain				Feed: gain ratio			
	1-14	15-35	36-49	1-49	1-14	15-35	36-49	1-49	1-14	15-35	36-49	1-49
	P-Value											
100% vs. 95% RDAA	0.019	0.009	0.282	0.613	0.65	0.056	0.249	0.29	0.119	0.504	0.025	0.005
Fermacto vs. CEO	0.418	<0.001	0.009	0.029	0.087	<0.001	0.322	0.061	0.615	0.143	0.726	0.912
Control vs.CEO	0.252	0.048	0.026	0.859	<0.001	0.359	0.492	0.054	0.135	0.002	0.004	<0.001
Control vs. Fermacto	0.78	<0.001	0.003	0.053	0.12	0.026	0.801	0.966	0.41	0.166	0.009	<0.001

Table 4: Effect of cumin essential oil (CEO) and Fermacto (g kg^{-1}) on relative organ weights and carcass yields in broiler fed 100% RDAA at d 28[1]

Starter[2]											
Supplementation	(g kg^{-1})	Live weight (g)	Abdominal fat	gastrointestinal tract	Heart	Liver	Ceca	Legs	Breast	Spleen (g)	Bursa[3]
			(% of live weight)								
Control	0	779	1.68	13.48	0.9	2.67	0.84	17.9	17.2	1.035	4.25
Fermacto	2	788	1.78	11.73	0.66	2.29	0.72	19.2	19.6	0.967	5.25
CEO	0.2	810	1.32	11.02	0.72	2.56	1.06	18.3	17.1	0.991	4.75
CEO	0.4	740	1.24	13.74	0.87	2.77	1	19.3	19.4	0.99	4.5
CEO	0.8	879	1.14	11.27	0.74	2.32	0.85	20	19	0.952	4.5
MSE			0.254	0.822	0.113	0.311	0.148	1.86	2.31	0.042	1.02

[1]Each un-supplemented finisher diet was fed to the corresponding birds.

[2]The Fermacto and CEO content in starter diets (g kg^{-1}), whereas the grower diets contained half of the same supplementation of the starter diets.

[3]The bursa of Fabricius size was determined by special ruler which there was some round holes with diameter ranged from 3, 6, 9, 13, 16, 19, 21 and 26 mm, respectively.

Table 5: Effect of cumin essential oil (CEO) and Fermacto (g kg^{-1}(on relative organ weights and carcass yields in all groups of broiler chichens fed 100 or 95% recommended crude protein and digestible amino acids (RDAA) at d 49 d[1]

	Starter[2]									
RDAA	Supplementation (g kg^{-1})		Live weight (g)	Abdominal fat	gastrointestinal tract	Heart	Liver	Ceca	Legs	breast
				(% of live weight)						
100 %	Control	0	2633	2.44	8.89	0.47	1.86	0.68	21	20.5
	Fermacto	2	2320	2.36	9.26	0.55	1.99	0.89	22.5	22.2
	CEO	0.2	2439	2.26	8.2	0.47	1.98	0.68	22	21.6
	CEO	0.4	2679	2.14	7.92	0.45	1.81	0.89	21	20.5
	CEO	0.8	2690	2.14	7.84	0.44	1.89	0.7	21.5	22.1
95 %	Control	0	2735	2.44	8.3	0.52	1.84	0.7	23.3	22.8
	Fermacto	2	2675	2.18	8.44	0.52	2.09	0.7	22.5	23.3
	CEO 0.2	0.2	2512	1.91	8.14	0.48	1.72	0.84	23.6	20.8
	CEO 0.4	0.4	2736	1.84	8.68	0.52	2	0.73	21.7	20.5
	CEO 0.8	0.8	2805	2.08	8.77	0.49	2	0.82	22	22
	MSE			0.257	0.533	0.03	0.128	0.115	0.99	1.34

[1]Each un-supplemented finisher diet was fed to the corresponding birds.

[2]The Fermacto and CEO content in starter diets (g kg^{-1}), whereas the grower diets contained half of the same supplementation of the starter diets

Organ Weights: The effect of Fermacto and various levels of CEO on relative organ weights of broiler chicks at d 28 and 49 are shown in Tables 4 and 5, respectively. The Fermacto and different levels of CEO in 100% RDAA diets did not have an effect on carcass yield and organ weights of broiler chicks at d 28. Similar results observed in birds fed either levels of RDAA at d 49. The abdominal fat proportion was numerically decreased in chickens fed diets contained the mid level of CEO irrespective of RDAA levels. Abdominal fat pad was uneffected by dietary Fermacto supplementation and/or level of RDAA, which is in contrary to the results reported by Khaksar *et al.* [2]. Over growing of liver, spleen and bursa of Fabricius in broiler fed diets contained CEO or Fermacto may indicate a subclinical infection, whereas these did not have an effect on mortality. Even though, the breast meat yield, clearly responded to the different digestible amino acids supply reported by Rostagno *et al*. [23], but was not influenced by the dietary level of digestible amino acids and/or Fermacto and CEO in the present study. Miorka *et al*. [24] added probiotics in broiler diets and did not find any effects on carcass, legs and breast yields which are corroborating the results of this study. However, according to Khaksar *et al*. [2], breast yield was significantly increased with Fermacto supplementation of diet at either level of 100, 95 and/or 90% RDAA. Djouvinov *et al*. [25] and Khaksar *et al.* [2] reported an increase in ceca weight of birds fed 100% RDAA diet contained Fermacto as compared to control birds and this was in contrast with the results of our experiment. The live weight of birds fed diet containing CEO in the current study were in agreement to those reported by Lee *et al.* [21] who observed that the supplementation of feed with carvacrol and thymol did not have an effect on the liver proportion.

Fat Digestibility and Gastrointestinal Transit Time: Effect of Fermacto and CEO levels on fat digestibility and GTT in chicks fed 100% RDAA is shown in Table 6. The Mean of feed GTT (minute) and fat digestibility were not influenced by Fermacto and CEO concentration at d 16. The results of GTT were contrary to the report by Khaksar *et al*. [2], but fat digestibility result was similar to the report of Cross *et al*. [22]. Khaksar *et al*. [2] found that the addition of Fermacto to broiler diets decreased GTT. Although in previous research [20] mixture of plant essential oils or plant extracts improved digestibility of fat and dry matter, Cross *et al*. [22] found that the supplementation of broiler diets with some medicinal herbs (Marjoram, Oregano, Rosemary, Thyme and Marjoram) and their essential oils did not have any effect on AMEn and digestibility of organic and dry matter.

Cumin essential oil showed increase to bile acids and bile salts synthesis and secretion [7]. In addition the use of CEO provided higher concentration and secretion of digestive enzyme in pancreatic and small intestine [8, 10, 11]. Simultaneous with these actions, GTT is reduced [20] and subsequently improved digestibility [26, 27] and performance. In current study, the fat digestibility was not influenced by the addition of CEO to diets which may be related to sex, strains and age of birds or concentration and method of CEO consumption.

Table 6: Effect of Fermacto and cumin essential oil (g kg^{-1}) on transit time and fat digestibility in broiler chickens fed %100 recommended crude protein and digestible amino acids (RDAA) at d 16[1]

	control	Fermacto	Cumin essential oil			
Starter[2]	0	2	0.2	0.4	0.8	SEM
			Digestibility (%)			
Fat	86.51	87.63	87.28	88.62	87.22	1.21
			Transit time (Minute)			
16 day	166.8	161.3	157	164.4	155.6	6.36

[1]Each un-supplemented finisher diet was fed to the corresponding birds.

[2]The Fermacto and CEO content in starter diets (g kg^{-1}), whereas the grower diets contained half of the same supplementation of the starter diets

Table 7: Effect of cumin essential oil (CEO) and Fermacto (g kg^{-1}) on blood chemistry and cell differentiation in broiler chickens fed 100% recommended crude protein and digestible amino acids (RDAA) at d 28[1]

Starter[2]												
Supplementation (g kg^{-1})		Glucose	Cholesterol	Triglyceride	HDL	LDL	VLDL	WBC (10^{-6})	RBC (10^{-9})	Hetrophyle	Monocyte	Lymphocyte
				(mg/dl)				(ml)		(proportion of WBC)		
Control	0	171	131.66	30.33^{b}	79	43	6.33 ^{b}	24.3	2,486	0.21	0.023	0.767
Fermacto	2	195.66	131.66	40.66^{ab}	81.33	45	8^{ab}	23.8	2,408	0.22	0.017	0.763
CEO	0.2	197.66	151.66	36.66^{ab}	87.66	54.66	7.33^{ab}	24.6	2,435	0.22	0.02	0.76
CEO	0.4	209	120.33	55 ^{a}	78	37	11^{a}	23.6	2,441	0.28	0.023	0.696
CEO	0.4	169.7	131.66	39^{ab}	87.33	45.66	7.66^{ab}	22.3	2,350	0.18	0.02	0.8
MSE		12.97	12.47	7.06	5.24	5.86	1.34	0.814	680	0.064	0.007	0.063

[a, b] Means within each row with uncommon superscript are significantly different (P< 0.05).

[1]Each un-supplemented finisher diet was fed to the corresponding birds.

[2]The Fermacto and CEO content in starter diets (g kg^{-1}), whereas the grower diets contained half of the same supplementation of the starter diets

Table 8: Effect of Fermacto and cumin essential oil (g kg^{-1}) on Anti-SRBC, IgM and IgG titres in broiler fed %100 recommended crude protein and digestible amino acids (RDAA) [1]

	Control	Fermacto	Cumin essential oil (g kg^{-1})			
Starter [2,3]	0	2	0.2	0.4	0.8	SEM
			Day 7 after injection (35 d of age)			
SRBC	4.98	5.25	5.18	4.8	5.15	0.226
IgG	3.97	4.25	4.18	4	4.434	0.313
IgM	1.01	1	1	0.8	0.716	0.323
			Day 14 after injection (42 d of age)			
SRBC	3.6	3.5	3	3.5	3.2	0.173
IgG	3	3	2.9	3.2	2.9	0.148
IgM	0.6	0.5	0.4	0.1	0.3	0.221

[1]Five birds per pen were injected by SRBC (5% suspension in PBS, 1 ml/chick) at 28 day of age.

[2]The Fermacto and CEO content in starter diets (g kgG), whereas the grower diets contained half of the same supplementation of the starter diets.

[3]Each un-supplemented finisher diet was fed to the corresponding birds.

Blood Chemistry and Cell Differentiation: Effect of Fermacto and different level of CEO on blood chemistry and cell differentiation of birds fed diet with 100% RDAA are shown in Table 7. The inclusion of Fermacto and/or various levels of CEO in diets did not have an influence on cholesterol, glucose, HDL and LDL concentration in serum (mg/dl) at d 28. The serum triglyceride and VLDL concentrations were lower (P<0.05) in chicks fed diet contained 0.4 g kg^{-1} CEO (in starter) as compared to those fed control diet which is in contrary to the reports by Lee *et al*. [21] and Yasni *et al*. [28]. They found dietary Carvacrol (a component of Thyme essential oil) and α-curcumene (a portion of essential oil from Curcuma xanthorrhiza) lowered serum triglyceride. It has been reported that dietary cumin seed or CEO lowered serum cholesterol concentration [29]. The hypocholesterolemic

effect of CEO has been ascribed to inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [29], the rate controlling enzyme of the cholesterol synthetic pathway. However, our results did not show the hypocholesterolemic effect of CEO.

The RBC and WBC count as Hetrophyle, Monocyte and Lymphocyte cell enumeration were not significantly influenced when birds fed 100% RDAA diet with Fermacto and/or CEO at d 28. The Hetrophyle cell count tended to be higher in birds fed starter diet contained 0.4 g kg^{-1} CEO. Antibody response values against SRBC as measured by total, IgM and IgG levels are shown in Table 8. Birds total antibody, IgM and IgG titers did not respond to the addition of Fermacto and/or various levels of CEO in diets. These results were in contrary to reported by Kong *et al*. [30], whom showed that 4 Chinese herbal ingredients may be applied as immune stimulators for an active vaccine in chickens at both in vitro and in vivo assays. Almost all of the Chinese herbal ingredients used in that study substantially enhanced in vitro chick embryo fibroblast proliferation and promoted the humoral immunity in response to Newcastle disease virus infection in vivo. In general, there were not any changes in humoral immune response in the present study, which might be associated with an age of birds or concentration and method of CEO consumption.

In conclusion, this study revealed that the supplementation of diets with CEO and Prebiotic Fermacto may have some beneficial effects on broiler chickens performance. The Fermacto and various level of cumin essential oil did not influence the relative organ weights, carcass yields, fat digestibility, gastrointestinal transit time, humoral immune response and blood cell enumeration, but increased (P<0.05) serum triglyceride and VLDL concentrations. Formulating diets based on 95% RDAA requirements did not have any adverse effects on performance in starting and growing periods, which may be used to decrease nitrogen excretion and lessen the expenditure of broiler chicken production.

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