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Effects of thyme (*Thymus vulgaris*), organic acid, probiotic and prebiotic on carcass characteristics and intestinal microbial population in broiler chickens fed with normal and low protein diets

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ABSTRACT: The experiment was carried out to evaluate the effects of thyme (*Thymus vulgaris*), organic acid, probiotic and prebiotic on carcass characteristics and intestinal microbial microflora in broiler chicks in normal and low protein diets. A total of 388 Ross 308 broilers were equally assigned to 5 treatments with 4 replicates in a completely randomized design. The experimental treatments were; 1: control, 2: thyme extract (2 kg/ton), 3: organic acid (3 kg/ton), 4: probiotics (50 g/ton) and 5: prebiotics (2 kg/ton) with normal and reduced protein levels (10% lower than usual). At d 42, two chicks per replicate were randomly chosen, slaughtered and carcass percentage and carcass components percentage including a thigh, breast, liver, heart, gizzard, spleen, bursa of Fabricius, abdominal fat, and intestine were determined for live weight. The results showed that using a diet with a normal level of crude protein compared to a diet with a level of crude protein lower than usual, increased the percentage of spleen and heart of chickens ($P<0.05$). It seems that there is a close relationship between the percentage of fat in the abdominal cavity and the ratio of energy to protein in the diet. Also, there were no significant differences between treatments and their interactions about pH content of ileum of broilers ($P > 0.05$). Besides, the bacterial population of ileum of treated broilers had different behavior. The ileum bacteria improved with probiotic addition and it was predictable because of beneficial bacteria inclusion to diet. Comparing the means for litter traits, no significant differences were observed between the experimental treatments with each other and with the control group ($P>0.05$). Totally, before the common use of additives in different types of diets of poultry nutrition, thorough investigations should be carried out on mechanisms, compatibility with other components of the diet and safety evaluation.

***Keywords*:** Carcass characteristics; low protein diets; Organic acid; Probiotic; Prebiotic; Thyme;

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INTRODUCTION

Nowadays, intensive breeding of animals, especially poultry, has increased their sensitivity to intestinal diseases. Poultry are sensitive due to colonization with potentially harmful microorganisms such as *Salmonella* and *Escherichia coli* species, rotavirus and *Clostridium perfringens* (*C. perfringens*). In order to control some of these problems, the use of antibiotics has been expanded (Zhang et al. 2021).

Despite the favorable results of using antibiotics in livestock and poultry feed, there is an increasing pressure to eliminate their use in animal diets, and this is due to the sense of danger that consumers of livestock products have in relation to antibiotic-resistant microbes have been seen. Up to now, there have been reports about unaffected *Salmonella* (Li et al. 2022), unaffected *Campylobacter* (Wang et al. 2022), and unaffected enterococci (Cho et al. 2022) to several types of antibiotics, which are mostly attributed to the uncontrolled use of antibiotics, especially as food additives in animal feed. Recently, the risk of antibiotic-resistant microbes has received more attention and many researches have been conducted in order to find suitable alternatives for antibiotics (Zhang et al. 2021). On the other hand, it has been found that the normal microbial flora of the digestive system has positive effects on the body's immune system and can also prevent the colonization of the digestive system by pathogenic microorganisms (Shi et al. 2022). The use of oral antibiotics by disrupting the microbial balance in the digestive system removes its benefits for the host animal. Prebiotics, medicinal plants and probiotics can be mentioned as additives that are suitable alternatives to antibiotics (Pliego et al. 2022).

Firstly, volatile oils extracted from medicinal plants and spices are a mixture of aromatic compounds and various volatile substances, many of which have antimicrobial properties (Raza et al. 2022). The main and the active components of these compounds are terpenes and phenols (Raza et al. 2022). The action mechanism of these compounds is to damaging the lipoprotein wall of bacterial cells, which leads to leakage and reduction of cytoplasmic compounds (Bouyahya et al. 2022).

Secondly, thyme (*Thymus vulgaris*) is one of the medicinal aromatic plants of the mint family and is important due to its antimicrobial and antioxidant properties. The antioxidant ability of thyme essential oil is attributed to the phenolic compounds of thymol, carvacrol and thymohydroquinone (Belali et al. 2022).

Thirdly, prebiotics are non-digestible carbohydrates that stimulate growth and have a favorable effect on the beneficial bacteria of the microbial flora (Abd El-Hack et al. 2021). Prebiotics include different types such as fructo-oligosaccharides, gluco-oligosaccharides and mannan-oligosaccharides. Such oligosaccharides are isolated from the outer wall of the yeast *Saccharomyces cerevisiae* increasing immunity by binding to the bacterial cell wall, by preventing the damage of bacteria to the intestinal epithelial cells and stimulating the production of antibodies (Abd El-Hack et al. 2021).

Finally, probiotics are microorganisms used in poultry feed for the purpose of maintaining the beneficial microbial population and combating the pathogenic microbes of the digestive tract. The beneficial effects of probiotics on the host animal affect in two ways including firstly: the direct effect on the number of bacteria population: through, a: production of antibacterial compounds such as organic acids and volatile fatty acids; b: neutralization of toxins from pathogenic microbes; c: competition for nutrients; d: Competition for connection positions; and secondly: stimulating the immune system through a: increasing antibody levels; b: Increasing the activity of macrophages (Bhogoju and Nahashon, 2022). The goal of this study was to investigate the effects of prebiotics, probiotics, and thyme essential oil on intestinal microflora and carcass traits in aged broiler chickens fed with in normal and low protein diets.

MATERIALS AND METHODS

Birds and housing

The procedure with no. 1399-IAU. 09.20.2020, was permitted by the Experimental Animal Ethics Committee of the Maragheh Islamic Azad University, Iran. A total of one-day-old 388 Ross-308 broiler chickens were divided into 5 duplicated groups and weighed before starting trial. The experimental treated groups were; 1) the control group without any treatment, 2) the thyme extract treated group (2 kg/ton); 3) the organic acid treated group (GLOBACID, 3 kg/ton); 4) the probiotic treated group (Protoxin,50 g/ton) (Protoxin containing *Lactobacillus acidophilus* and *Bifidobacterium* each gram of Protoxin contained 0.1×10 CFU) (50 g/ton) and 5) the prebiotic treated group (*Mannan-oligosaccharide*) (2 kg/ton). All 5 duplicated groups received normal and 10% reduced protein level diets each.

All trial diets were formulated using the User-Friendly Feed Formulation (UFFDA) software program (Pesti and Miller, 1993) to provide the advised levels of nutrients specified for the Ross-308 broiler chickens (Table 1). *Ad libitum* feed and sanitized water were provided in all experimental periods [starter (1 to 10 days), grower (11 to 24 days) and finisher (25 to 42 days)]. The lighting program during the experimental periods consisted of 23 hours of light and 1 hour of darkness, and the environmental temperature was gradually decreasing from 33°C to 25°C on day 21 and kept constant until the end of this trial.

Carcass Characteristics

At day 42 of the experimental trials, two chickens per replicate were randomly chosen, humanely euthanized and carcass percentage and carcass components percentage including a thigh muscle, breast muscle, liver, heart, gizzard, spleen, bursa of Fabricius, abdominal fat, and intestine were determined for live weight.

Acidity and microbial population of ileum

To determine the pH and microbial population of the ileum of evaluated broiler chickens, the lower ileum, from Meckel's diverticulum to the branching point of the ceca, and its contents were removed with sterilized scissors into sterilized cans, were unloaded and transported to the laboratory and homogenized. To determine the pH, the sample container was taken to the pH meter device and the recorded number was registered as an intestinal pH.

For counting of bacteria in ileal digesta, one gram of sample digestion contents available in this area was added to a test tube containing 9 ml of physiologic serum and was thoroughly mixed and homogenized. Afterwards, the sample was plated on 2 different media: blue agar (EMB; Merck 1.03858) and Levine eosin methylene blue agar (L-EMB; Merck 1.03857).

After counting the number of colonies per plate, the obtained number was multiplied by contrast dilution factor and the result was recorded as the number of colony forming units (CFU) per gram of sample

Litter moisture content

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

	Starter period (1 to 10 days)		Growing period (11 to 24 days)		Finishing period (25 to 42 days)	
Ingredients	normal protein	10% lower than normal protein	normal protein	10% lower than normal protein	normal protein	10% lower than normal protein
Corn	580	633	617	660	655	690
Soybean meal	371	318	238	280	280	240
Oil	14	14	21	25	30	35
Di-calcium phosphate	23	23	23	23	23	23
Salt	3	3	3	3	3	3
Vitamin [1]	2.5	2.5	2	2.5	2.5	2.5
Mineral [2]	2.5	2.5	2	2.5	2.5	2.5
DL-Methionine	3	3	3	3	3	3
L-Lysine	1	1	1	1	1	1
Chemical composition of basal diet						
Metabolisible energy, kcal/kg	3000	3000	3100	3100	3200	3200
Crude protein, %	23.2	20.7	21.5	19.35	19.5	17.55
Calcium, %	0.81	0.81	0.45	0.55	0.56	0.54
Available phosphorus, %	0.5	0.5	0.54	0.54	0.53	0.63
Lysine, %	1.42	1.24	1.29	1.29	0.93	1.01
Methionine + Cysteine, %	1.04	1.04	0.99	0.93	0.93	0.88

[1*]Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D_3 (cholecalciferol), 0.05 mg; vitamin E (tocopherol acetate), 18 mg; vitamin K_3, 2 mg; thiamine, 1.8 mg; riboflavin, 6.6 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; Antioxidant 100 mg. [2*]Mineral premix per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu ($CuSO_4.5H_2O$), 10 mg; I (K_1, 58% I), 1mg; Se ($NaSeO_3$, 45.56% Se), 0.2 mg.

Individual litter samples were separated and weighed (15 g) before drying each sample for 24 h at 105°C (Barker et al., 2011). To determine the dry weight of each sample, samples were weighed again after drying. Moisture content (MC) was determined using the wet and dry weights (W) (American Society of Agricultural and Biological Engineers, 2007):

$$MC\ (\%) = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100$$

Litter microbial population

To check microbial population of broiler litter under test, at the 35 days of rearing, using clean and sterilized containers, a sample composed of one gram of litter sample and mixed with 9 ml of physiologic serum was taken and transported to the laboratory in sterile conditions and homogenized. The process was carried out similarly with the process of determining microbial population of ileum.

Statistical Analysis

The study had a completely randomized design. Data normality was tested using Statistical Analysis System (SAS). Carcass characteristics, pH and microbial population of the contents of the ileum and litter also the moisture of litter of broiler chickens data were subjected to PROC GLM procedure of SAS software. Data were log-transformed before analyzing to compensate for any unequal variances. Tukey's test at the 5% level of probability was used to compare the means (Palangi, 2021).

RESULTS AND DISCUSSION

In our experimental trials, the effect of supplementation of thyme essential oils, organic acid, probiotic and prebiotic on carcass components percentage in broiler chickens fed with normal and low crude protein diets is mentioned in Table 2. Experimental treatments had significant effects on chicken carcass traits ($P<0.05$). Using a diet with a normal level of crude protein compared to a diet with a level of crude protein lower than usual, we noted an increased in the percentage of weight of spleen and heart of chickens. We also noted the significant effects of additives on carcass traits ($P<0.05$).

In our study, the use of organic acids reduced the carcass percentage compared to the control group without treatment. In addition, the percentage of visceral fat and gizzard increased in the treatments containing additives compared to the treated groups without additives. In comparison with the diet without additives, organic acids increased the size of the spleen, but the rest of the additives decreased it. The percentage of hearts in treated groups containing additives was higher compared to the treated groups without additives. Also, the percentage of breast in the treated groups containing additives decreased in comparison to the control groups. Considering the interaction effects between additives and crude protein level of diets, it was also significant differences on carcass traits ($P<0.05$).

The results of our work were consistent with previously reported studies describing the beneficial effects of organic acids such as fumaric versus lactic acid as a substitute for some feed additives such antibiotics on carcass quality of broiler chickens (Brzoska and Stecka, 2007; Viola and Vieira, 2007).

In our trials, the effects of additives were not significant on spleen percentage in diets with lower protein percentage, while in diets with normal protein percentage, the highest percentage of spleen was observed in the treated groups containing organic acids in feed. In low-protein diets with the usual crude protein containing additives, the highest percentage of breast muscles was obtained in the treatments containing less crude protein and without additives, while the use of additives significantly reduced the percentage of breast muscles with both crude protein levels. In the present study the dietary protein level was not adjusted according to the energy level (decreasing the level of protein intake for constant levels of energy), and the amount of energy intake increased in the group containing low level of dietary protein. Other researchers stated that the cost of feed can be reduced by lowering the protein of broiler chicken feed (Kamran et al. 2008), on the other hand, some other researchers stated that the reduction of dietary protein has no effect on the amount of carcass fat (Sahraei and Sharjatmadarj, 2007).

The increase of fat in the abdominal cavity in broilers is a negative factor, and so far many efforts have been made to reduce this stored fat in the body (Kobayashi et al., 2013). The increased price of the edible components of the broiler diets has led broiler breeders of this industry to push nutrition and management towards increasing the consumable components of the carcass (Sharifi et al., 2015).

Also, the effect of supplementation of thyme es-

Table 2: Effect of supplementation of thyme (*Thymus vulgaris*) essential oils, organic acid, probiotic and prebiotic on carcass components percentage in broiler chicks fed with normal and low crude protein diets.

Effect	Carcass weight	Abdominal fat	Intestine	Gizzard	Liver	Spleen	Bursa of Fabricius	Heart	Thigh muscle	Breast muscle
Protein										
Low	72.8	3.239	6.848	2.648	2.789	0.139[b]	0.272	0.891[b]	27.39	33.11
Normal	72.06	3.304	6.804	2.722	2.842	0.162[a]	0.236	0.961[a]	27.648	32.558
SEM	0.3562	0.093	0.1083	0.0459	0.0377	0.0037	0.0143	0.0202	0.231	0.256
P value	0.1463	0.6269	0.7749	0.2587	0.3193	< 0.0001	0.0841	0.0177	0.4342	0.131
Additive										
Thyme extract	72.83[ab]	3.392[a]	6.7977	2.664[ab]	2.879	0.14[b]	0.246	1.003[a]	27.18	33.109[b]
No additive	73.07[a]	2.651[b]	6.556	2.398[b]	2.725	0.155[ab]	0.271	0.772[b]	27.1014	35.011a
Organic Acid	70.389[b]	3.701[a]	6.88	2.84[a]	2.764	0.181[a]	0.255	1.03[a]	28.279	30.737[c]
Prebiotic	72.930[ab]	3.116[ab]	7.155	2.665[ab]	2.805	0.142[b]	0.227	0.881[ab]	27.335	32.572[bc]
Probiotic	72.928[ab]	3.497[a]	6.743	2.857[a]	2.906	0.135[b]	0.271	0.994[a]	27.7	32.744[b]
SEM	0.563	0.1477	0.171	0.0727	0.0596	0.0059	0.0227	0.0319	0.365	0.4051
P value	0.0046	<0.0001	0.1761	0.0002	0.1758	<0.0001	0.6207	<0.0001	0.15	<0.0001
Protein*Additive										
Low* thyme extract	73.177	3.1675	6.485	2.57	2.705	0.1275[b]	0.255	0.902	26.675	34.128[abc]
Low*No additive	73.932	2.577	6.482	2.38	2.682	0.14[b]	0.28	0.735	27.185	35.440[a]
Low*Organic Acid	71.04	3.845	7.24	2.85	2.857	0.1525[b]	0.302	1.022	28.33	29.873[d]
Low*Prebiotic	73.272	2.945	7.41	2.502	2.822	0.1425[b]	0.22	0.9	26.767	33.688[abc]
Low*Probiotic	72.577	3.662	6.625	2.937	2.877	0.1325[b]	0.302	0.897	27.997	32.428[bcd]
Normal* thyme extract	72.482	3.617	7.11	2.757	3.052	15.25[b]	0.2375	1.105	27.685	33.090[bcd]
Normal*No additive	72.215	2.725	6.63	2.417	2.767	0.1700[b]	0.2625	0.81	27.022	34.583[ab]
Normal*Organic Acid	69.737	3.557	6.52	2.83	2.67	0.2100[a]	0.2075	1.037	28.227	31.603[cd]
Normal*Prebiotic	72.587	3.287	6.9	2.827	2.787	0.1425[b]	0.235	0.8625	27.9	31.458[cd]
Normal*Probiotic	73.277	3.332	6.862	2.777	2.935	0.1375[b]	0.24	0.99	27.4	33.060[abc]
SEM	0.7964	0.2089	0.2423	0.1027	0.0843	0.0083	0.032	0.0452	0.5166	0.5729
P value	0.6217	0.2212	0.6054	0.1674	0.5596	0.008	0.4633	0.1044	0.359	0.0028

[a, b, c] Means within each column with different superscripts are significantly different (P<0.05)

sential oils, organic acid, probiotic and prebiotic on carcass components weight of broiler chickens fed with normal and low crude protein diets is mentioned in Table 3. It seems that there is a close relationship between the percentage of abdominal fat and the ratio of energy to protein in the diet. The smaller this ratio, the less fat will be stored in the abdominal (Rezaei et al., 2004). By reducing the level of crude protein in the diet, the ratio of energy to protein is changed and more energy is available, so the fat content of the carcass will increase (Rezaei et al., 2004).

In the present study, there was no significant difference between the average fat around the ventriculus between the treated groups, but numerically, the treated groups that received a lower level of protein than the standard had more fat in the area around ventriculus. The weight of lymphoid organs, such as spleen is one of the indicators of the immune status in birds (Li et al., 2022). The liver weight of the treated groups receiving standard protein level was statistically greater than that the one of the treated groups with low protein levels. It is possible that in the treatments with higher protein levels, the level of arginine is higher and has led to an increase in the weight of the liver, or it can be attributed to the induction effect of arginine on the endocrine glands, which leads to the stimulation of the release of pituitary hormone and pancreatic hormones such as glucagon, insulin and growth hormone. This hormonal induction can stimulate protein biosynthesis and increase liver activity and liver weight (Lillehoj et al., 1996). Among the different nutrients in the diet, proteins and amino acids are the most important raw materials for the synthesis of structural tissues of the abdominal. It has been shown that in growing animals, the storage of aminoacids in the muscle represents the absorption of 65% of the total daily protein intake (Siyadati et al., 2011).

It can be concluded that since the liver is considered the main organ of lipid synthesis or lipogenesis in poultry (Nukreaw et al., 2011), the increase in liver weight in diets with a low protein level or with a high ratio of energy to protein is probable. It has been previouslly reported that the increase in body fat percentage with low protein diets may be due to increased feed consumption or less use of energy for protein synthesis and increased fat synthesis from excess energy (Kobayashi et al., 2013). Another interpretation of this may be related to the fact that birds fed with a low protein diet need less energy to excrete uric acid from the urine and therefore spend more energy on fat synthesis in the abdominal, especially the liver (Kamran et al., 2010).

Regarding the addition of thyme, Sarica et al. (2005) found that feeding broilers with different levels of thyme powder (1 and 2%) did not change the relative weight of thigh, heart, liver and gizzard. Another group of researchers supported that finding describing the use of 200 mg of Shirazi thyme oil, cinnamon, pepper, as well as 5 grams of mint plant extracts (including sage, thyme, and rosemary) per kilogram of broilers' diet without significant effect on the weight of the gizzard, liver, proventriculus, pancreas, large intestine, and small intestine (Hernandez et al., 2004). On the other hand, Ocak et al. (2008) reported that adding 2% of thyme powder to the diet of broilers of the commercial strain of Ross improved the percentage of carcass and fat in the abdominal cavity, but had no effect on the weight of the heart, liver, and gizzard. Lihua et al. (2007) reported that 150 mg of thyme extract per kg of broiler diet increased the percentage of carcass and muscle of breast and thigh, but decreased the percentage of abdominal fat. It has been suggested that the increase in carcass and breast percentage in chickens fed with thyme powder can be related to the antimicrobial effects of chemical compounds of this

Table 3: Effect of supplementation of thyme (*Thymus vulgaris*) essential oils, organic acid, probiotic and prebiotic on carcass components weight in broiler chicks fed with normal and low crude protein diets.

Effect	Live weight	Carcass weight	Abdominal fat	Intestinal	Gizzard	Liver	Spleen
Protein							
Low	2305.65	1682.725	54.588	115.559	44.234	47.102	2.405
Normal	2292.625	1650.85	54.904	112.256	44.992	47.065	2.761
SEM	27.3626	20.061	1.91	2.387	0.956	0.9497	0.0797
P value	0.7374	0.265	0.9072	0.3312	0.5766	0.9781	0.0023
Additive							
thyme extract	2353.125	1714.375^{a}	57.875^{a}	116.25	45.5^{ab}	49.375^{a}	2.461^{ab}
No additive	2283.188	1669.427^{ab}	45.355^{b}	109.226	40.216^{b}	45.67^{ab}	2.681^{ab}
Organic Acid	2231.25	1576^{b}	58.625^{a}	108.5	44.75^{ab}	43.625^{b}	2.911^{a}
Prebiotic	2315.625	1688^{ab}	52.625^{ab}	121.5	44.875^{ab}	47.5^{ab}	2.482^{ab}
Probiotic	2312	1686.125^{ab}	59.25^{a}	114.062	47.625^{a}	49.25^{a}	2.38^{b}
SEM	43.264	31.719	3.021	3.774	1.512	1.502	0.1259
P value	0.364	0.031	0.0077	0.1031	0.0206	0.0392	0.0283
Protein*Additive							
Low* thyme extract	2443.75	1788.75	56.5	116.375	46	48.5	2.342
Low*No additive	2284.5	1690.12	44.69	109.297	40.42	45.51	2.43
Low*Organic Acid	2225	1597.25	61.5	115.75	45.25	45.75	2.517
Low*Prebiotic	2331.25	1708.75	50.25	128	42.75	48.5	2.512
Low*Probiotic	2243.75	1628.75	60	108.37	46.75	47.25	2.222
Normal* thyme extract	2262.5	1640	59.25	116.125	45	50.25	2.58
Normal*No additive	2281.875	1648.75	46.02	109.155	40.212	45.827	2.93
Normal*Organic Acid	2237.5	1554.75	55.75	101.25	44.25	41.5	3.305
Normal*Prebiotic	2300	1667.25	55	115	47	46.5	2.3452
Normal*Probiotic	2381.25	1743.5	58.5	119.75	48.5	51.25	2.537
SEM	61.1847	44.857	4.272	5.33	2.138	2.124	0.178
P value	0.1514	0.0775	0.7671	0.1051	0.6961	0.3439	0.1943

[a, b, c] Means within each column with different superscripts are significantly different ($P<0.05$)

medicinal and aromatic plant (Lihua et al., 2007). The plant compounds reduce the deamination of protein and amino acids in the contents of the digestive system, and reduce the speed of their decomposition by some microbial enzymes such as urease. As a result, an increased level of protein absorption can be seen leading to a higher weight of the carcass (Lee et al., 2003).

Furthermore, the effect of supplementation of thyme essential oils, organic acid, probiotic and prebiotic on pH and microbial population of the contents of the ileum of broiler chicks fed with normal and low crude protein diets is mentioned in Table 4. There were no significant differences between treatments and their interactions about pH content of ileum of broiler chickens (P > 0.05). Besides, the bacterial population of ileum of showed different behavior among broiler chickens from our treated groups. The ileum bacteria showed a predictable improvement with the probiotic addition to the respective low and normal protein level diets in our study.

Moreover, the bacteria population mass from ileum decreased with thyme addition and this may be due to the essential oils content. Harmful bacteria of the digestive produce toxic metabolites such as amines, phenols, and indoles having growth-reducing effects, and adversely affect on poultry growth performance (Van der Klis and Jansman, 2002), but thyme, due to its phenolic compounds, causes the growth and stimulation of beneficial bacteria such as *Lactobacillus* spp. in the intestine. These bacteria play an important role in improving poultry performance (Tschirch, 2000). Correspondingly, thyme and similar plant additives improve the accessibility of nutrients and their digestion through the increase of goblet cells and absorption (Pelicano et al., 2005). On the other

Table 4: Effect of supplementation of thyme (*Thymus vulgaris*) essential oils, organic acid, probiotic and prebiotic on pH and microbial population of the contents of the ileum of broiler.

Effect	pH of intestinal contents	Bacterial (billion per gram)	Gram-negative bacteria (million per gram)
Protein			
Low	5.59	15458.75	86.75
Normal	5.49	14870.25	105.00
SEM	0.0361	201.1742	25.7186
P value	0.197	0.155	0.752
Additive			
thyme extract	5.42	15065.75	114.50
No additive	5.52	15233.25	77.25
Organic Acid	5.49	16140.50	95.50
Prebiotic	5.43	15351.25	104.50
Probiotic	5.54	15004.25	70.50
SEM	0.0332	256.4107	12.9827
P value	0.843	0.681	0.843
Protein*Additive			
Low* thyme extract	5.34	15984.50[ab]	31.75
Low*No additive	5.48	15061.75[b]	66.25
Low*Organic Acid	5.51	15252.50[ab]	68.00
Low*Prebiotic	5.49	15171.25[ab]	46.75
Low*Probiotic	5.49	17338.50[a]	42.25
Normal* thyme extract	5.41	15141.75[ab]	53.00
Normal*No additive	5.35	15069.75[b]	41.25
Normal*Organic Acid	5.35	15462.25[ab]	33.25
Normal*Prebiotic	5.25	15673.50[ab]	49.00
Normal*Probiotic	5.39	15546.50[ab]	36.50
SEM	0.0283	166.6585	4.8751
P value	0.582	0.050	0.777

[a, b, c]Means within each column with different superscripts are significantly different (P<0.05)

hand, the carbohydrates in medicinal plants also decrease the pH in the lower part of the small intestine under anaerobic fermentation conditions, which leads to improved feed intake. These compounds have antimicrobial properties and by disinfecting the digestive system, they prevent the breakdown of amino acids by harmful microbes (Garcia et al., 2007). Correspondingly, thyme affects the digestive system, especially the intestines of broiler chickens, causing the secretion of digestive enzymes. Thyme also facilitate the length of intestinal villi, surface and number of villi epithelial cells, it increases the contact surface of the digested nutrients, and finally, the body weight and feed conversion ratio are improved (Hernandez et al., 2004).

On the other hand, medicinal plants decrease the activity of acetyl coenzyme carboxylase enzyme by increasing beneficial bacteria in the intestine (Jamroz et al., 2003). Also, these beneficial bacteria decrease the rate of protein and amino acid decomposition in the digestive system which results in less fat accumulation in the abdominal (Lee et al., 2003), but the abdominal fat increase in the antibiotic group can be attributed to a decrease in the immune response and an increase in fat retention (Humprey et al. 2002).

The presence of harmful microbial population in the digestive system stimulate the immunoglobulins production in the poultry body, as a result, protein storage in muscle tissue is reduced therefore weight gain and growth are also limited. In addition, the bacteria in the gut compete with the host for the use of amino acids so can reduce the efficiency of nitrogen utilization in the body (Furuse and Yokota, 1985). Also, medicinal plants such as thyme can stimulate the activity of digestive system in poultry, improve the function of the liver and increase the secretion of digestive enzymes of the pancreas, and finally can improve the growth and performance of poultry (Langhout, 2000). On the other hand, the antioxidant properties of thyme protect intestinal villi that cause

Table 5: Effect of supplementation of thyme (*Thymus vulgaris*) essential oils, organic acid, probiotic and prebiotic on percentage of moisture and microbial population of broiler litter.

Effect	Litter moister content (%)	Bacterial (billion per gram)	Gram-negative bacteria (million per gram)
Protein			
Low	51.87	1383.00	19.84
Normal	51.52	1624.25	18.61
SEM	0.3172	138.0871	1.2179
P value	0.620	0.424	0.653
Additive			
Thyme extract	51.59	1558.25	19.29
No additive	51.59	1672.75	1928
Organic Acid	51.83	1452.75	25.46
Prebiotic	53.48	1799.00	19.52
Probiotic	52.12	1368.25	34.36
SEM	0.3026	104.3643	3.7022
P value	0.309	0.750	0.685
Protein*Additive			
Low* thyme extract	51.30	1519.50	19.75
Low*No additive	52.63	3059.75	27.47
Low*Organic Acid	52.33	1351.00	24.53
Low*Prebiotic	52.36	2300.00	21.53
Low*Probiotic	51.04	1964.75	24.32
Normal* thyme extract	53.11	1003.25	15.645
Normal*No additive	52.47	2667.00	19.86
Normal*Organic Acid	51.61	1184.75	20.04
Normal*Prebiotic	50.12	1166.25	14.84
Normal*Probiotic	53.13	1185.00	12.37
SEM	0.2381	171.0255	1.2768
P value	0.066	0.042	0.178

[a, b, c]Means within each column with different superscripts are significantly different ($P<0.05$)

increasing the absorption of nutrients. Likewise, growth is also improved by increasing the efficiency of nitrogen retention by reducing the formation of active oxygen, protein oxidation and nitrogen excretion (Manzanilla et al., 2001).

Moreover, the effect of supplementation of thyme essential oils, organic acid, probiotic and prebiotic on percentage of moisture and microbial population of litter of broiler chickens fed with normal and low crude protein diets mentioned in Table 5.

Comparing the means for litter traits using Tukey's test, we found no significant differences between the experimental treatments with each other and with the control group (Table 5). After extensively exploring the data of previous related studies, we described for the first time the microbial load in broiler chickens litter with these diets. The effectiveness of additives especially herbal dietary additives might be affected by internal and external factors such as infections and environmental conditions (Giannenas et al., 2003; Samadian et al., 2013). Broiler chick quality, health status and environmental managing also play significant roles in the efficiency of herbal and other additives in poultry diets (Hashemi and Davoodi, 2010).

CONCLUSIONS

Since the knowledge is still limited in practical usages of additives in different formulations of broiler chicken diets, and since there are a great disparity in herbal compounds, process and geographical origin of the plants used as feed additives, the complexity of our studied subject multiplies. In summary, before using such additives in different diets for chickens, further investigations on mechanisms, compatibility with other components of the diet and safety evaluation are needed in order to improve chicken growth under commercial conditions.

CONFLICT OF INTEREST DECLARATION

The authors declare that they have no conflict of interest.

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COMPLIANCE WITH ETHICAL STANDARDS

Animal experiments were approved by the Animal Care Committee of the Islamic Azad University and all experiments were performed in accordance with the regulations and guidelines established by this committee.

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