Global Veterinaria 6 (4): 389-398, 2011
ISSN 1992-6197

Growth Performance and Humoral Immune Response of Broiler Chicks Fed Diets Containing Graded Levels of Ground Date Pits with a Mixture of Dried Garlic and Thyme

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Abstract: This experiment was carried out to determine effects of dietary inclusion of ground pits of palm dates (*Phoenix dactylifera* L.) with or without medicinal plants (MP: a ground mixture of dried garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) on productive performance, antibody (Ab) response against New Castle Disease Virus (NDV), serum biochemical parameters and differentiable counts of white blood cells in broiler chicks. A total number of 240 day old male Ross chicks were distributed in 24 battery pens (n=10). Birds in four pens were assigned to feed each of six iso-caloric and iso-nitrogenous experimental diets including grounds date pits (0, 50 and 100 g/Kg diet) and medicinal plants (MP, 0 and 2 g/Kg diet). Body weight (BW) and feed intake (FI) was measured on days 21, 42 and 49 of age. Antibody response to inactivated NDV vaccine was used to evaluate the humoral immunity of chicks. At 14 and 28 days of age, all 240 chicks were vaccinated against NDV. Blood samples were withdrawn 0 and 7 d after first- and 14 and 21 d after second-vaccination for determination of primary and secondary Ab responses. Data in a 3 × 2 factorial arrangement was subjected to analysis of variance based on completely randomized design using the GLM procedure of SAS. Dietary treatment did have no significant effect on BW, BW gain, FI and FCR of birds (P>0.05). There was no interaction between dietary inclusion of date pits and MP inclusion on performance traits; however, a significant interaction was found on Ab titer 14 days after primary vaccination (P= 0.02). Ab titer of chicks fed the diet including 50 g DP without MP was the highest compared to birds fed other diets. Dietary DP inclusion significantly decreased the serum levels of HDL. In conclusion, including ground date pits to broiler diet up to 10% showed growth performance comparable to the corn-SBM diet. Further investigations on beneficial effects of dietary DP inclusion on blood levels of HDL should be appreciated.

Key words: Date pits · Garlic · Thyme · Growth performance · Humoral immunity · Broilers

INTRODUCTION

It is known that the cost of feed in broiler production could reach 75% of the total operational cost. Nutritionists have attempted to reduce feed costs by using locally available cheaper unconventional feedstuffs. The date palm (*Phoenix dactylifera* L.) is well adapted to the dry and semi-dry regions of the world. Iran is considered to be the 4th date producer country with an annual production of approximately 880,000 metric tons [1]. Production of dates in this region leaves behind a sizable amount of date by-products as well as low quality discarded dates. The date pits (DP) which produced, especially from the industry of date confectionery could be partly substituted imported corn or other cereals in poultry feed. Dates are composed of a fleshy pericarp and seed which constitutes between 10 and 15% of date fruit weight [2]. Kamel *et al.* [3] found that date pits up to 150 g kg^{-1} added to chicks' diets supported growth similar to birds fed control diets. The addition of date pits and date fruits to the diets significantly improved the body weight gain (BWG) of chicks as compared to chicks fed the control diet, after the first 2 weeks of the trial. In addition, including date pits at a 100 g kg^{-1} level to the finisher diets improved BWG and feed conversion ratio (FCR) of broiler chicks [2]. Vandepopuliere *et al.* [4] suggested that date pits at levels ranging from 50 to 270 g kg^{-1} could be included in broiler diets with no deleterious effect on growth performance. By investigating dietary inclusion of date fibre, a by-product of date syrup, El-Hag and

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Al-Shargi [5] and Al-Marzooqi *et al.* [6] concluded that date fibre could be used up to 150 g kg^{-1} in broiler and layer diets with no significant reduction in performance. Weight gains of broilers receiving diets containing 100 g kg^{-1} uncooked date pits were increased as compared to birds fed the control diet [7]. Tabook *et al.* [8] showed that substitution of maize by 100 and 150 g kg^{-1} date fibre in broiler diet significantly decreased nitrogen corrected apparent metabolisable energy (AMEn). They also reported that the inclusion of date fibre in the broiler diets except at 50 g kg^{-1} depressed average BWG, feed intake (FI) and FCR. Jumah *et al.* [9] found a gradual reduction in BWG of broiler diets included varying levels (0, 50 and 150 g kg^{-1}) of date pits. The extracts of date palm flesh and pits have been reported to have free radical scavenging activity [10].

Nowadays, food safety is more seriously considered than before and many countries have tended to prohibit antibiotics because of their side effects on both bird and human. In feeding farm animals the actual research is aimed at finding natural feed additives that can replace antibiotics with natural occurring promoters (probiotics, prebiotics, feed enzymes, organic acids, herbs) to achieve the production goal [11-19]. Phytogenic feed additives are plant-derived products used in animal feeding to improve the performance of agricultural livestock. This class of feed additives has recently gained increasing interest, especially for use in poultry. Photogenic feed additives are discussed possibly to add to the set of nonantibiotic growth promoters, such as organic acids and probiotics, which are already well established in animal nutrition. Phytogenics, however, are a relatively new class of feed additives and our knowledge is still rather limited regarding aspects of their application. Further complications arise because phytogenic feed additives may vary widely with respect to botanical origin, processing and composition [20].

Thyme *(Thymus vulgaris* L.) is an herbaceous perennial plant belonging to the Lamiaceae family. Thymol, a major component of thyme-essential oils, has been widely studied for its antimicrobial properties [21]. Carvacrol, an isomer of thymol, is found in essential oils isolated from oregano and thyme. Like thymol, carvacrol also displays antimicrobial activity [22]. Given their antimicrobial activity, it would be expected that thymol and carvacrol could have positive effects on growth performance in broilers. Such studies showed that thyme plant could be considered as an alternative natural growth promoter for poultry instead of antibiotics [23].

Garlic (*Allium sativum* Linn) the spices of life is unique among the members of plant kingdom. Several clinical reports, including meta-analyses, have revealed a cholesterol lowering effect of garlic in humans [24]. Allicin (the active compound produced by garlic) may reduce the levels of serum cholesterol, triglyceride and LDL [25]. Mottaghitalab and Taraz [26] concluded that diets containing garlic powder has potential as feed additives, which may be beneficial in reducing serum and egg cholesterol in hens. Khan *et al.* [27] also reported that feed consumption, feed efficiency and egg mass were not affected when 0, 2, 6 and 8% dietary garlic powder was fed to the laying hens.

The objective of this study was to investigate effects of diet inclusion ground powder of palm DP on broilers' performance and immune response. In addition, we wondered whether supplementing corn-soybean meal- or corn-soybean meal-DP-based diet with a commercial powder of MP including thyme and garlic (1: 1) would have beneficial effects on broilers' performance and immune response.

MATERIALS AND METHODS

Birds and Diets: All procedures were approved by the Institutional Animal Care and Use Committee of Razi University. A total number of 240 male one-day old Ross 308 broiler chicks were distributed between 24 cage pens (battery) in a completely randomized experimental design with eight treatments and six replications of ten chicks each. The temperature was maintained at 32±1°C in the 1 st week and reduced by 2.5°C week." to 21°C. From day 1 until day 4 the lighting schedule was 24 h light. During days 5-49, the dark periods were increased to 1 h. Birds were fed diets (starter: 1-21, grower: 22-42 and finisher: 43-49 days) based on NRC. The height of used nipple drinkers were adjusted twice weekly by visual inspection. Water was offered on an *ad libitum* basis for all birds. The ingredients and composition of experimental diets is shown in tables 1, 2 and 3. A factorial 3×2 arrangement of treatments was used as three levels of ground pits of palm dates (0, 50 and 100 g/kg) and two levels of a 1:1 ground mixture of dried garlic and thyme (medicinal plants: MP 0 or 2 g/kg). Body weight (BW) ad FI was measured on 21, 42 and 49d and FCR was calculated for each rearing period.

Antibody Response to NDV and Serum Biochemical Parameters: Antibody response to inactivated Newcastle disease virus (NDV) vaccine was used to examine the humoral immunity of chicks. At 14 and 28 days of age,

Table 1: Composition and ingredients of experimental diets during starting period (0-21 d)

Days of age	Starter (0 -21 d)					
Date pits	0.00	0.00	5.00	5.00	10.00	10.00
Medicinal Plant (MP)	0.00	0.20	0.00	0.20	0.00	0.20
Feed ingredients	g / 100 g diet					
Corn	53.77	53.84	51.84	51.91	49.91	49.98
Soybean meal	31.95	32.08	32.95	33.08	33.95	34.08
Date pits	-	-	5.00	5.00	10.00	10.00
Wheat bran	8.32	7.92	4.36	3.96	0.40	-
Soybean oil	2.15	2.15	2.15	2.15	2.15	2.15
Dicalcium phosphate	1.33	1.33	1.35	1.36	1.38	1.38
Lime stone	1.23	1.23	1.11	1.11	0.98	0.98
Common salt	0.50	0.50	0.50	0.50	0.50	0.50
Medicinal plant	-	0.20	-	0.20	-	0.20
Vit. and Min. Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Lysine-HCL	0.12	0.12	0.10	0.10	0.09	0.08
DL-Methionine	0.13	0.13	0.14	0.14	0.14	0.14
Calculated analyses						
ME (Kcal/kg)	2800	2800	2800	2800	2800	2800
Crude protein (%)	20.12	20.12	20.12	20.12	20.12	20.12
Ether extract (%)	4.48	4.48	4.48	4.48	4.48	4.48
Crude fiber (%)	4.33	4.33	4.33	4.33	4.33	4.33
Calcium (%)	0.87	0.87	0.87	0.87	0.87	0.87
Available P (%)	0.39	0.39	0.39	0.39	0.39	0.39
Lys (%)	1.13	1.13	1.13	1.13	1.13	1.13
Met (%)	0.43	0.43	0.43	0.43	0.43	0.43

[1]The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000 IU (*all-trans*-retinal); cholecalciferol, 2,000 IU; vitamin E, 20 IU (*á*-tocopheryl); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg; and antioxidant, 250 mg.

Table 2: Composition and ingredients of experimental diets during growing period (22-42 d)

Days of age	Grower (22 -42)					
Date pits	0.00	0.00	5.00	5.00	10.00	10.00
Medicinal Plant (MP)	0.00	0.20	0.00	0.20	0.00	0.20
Feed ingredients	g / 100 g diet					
Corn	64.06	64.13	62.13	62.20	60.20	60.27
Soybean meal	23.85	23.98	24.85	24.98	25.85	25.98
Date pits	-	-	5.00	5.00	10.00	10.00
Wheat bran	8.66	8.26	4.70	4.30	0.74	0.33
Dicalcium phosphate	0.93	0.93	0.95	0.96	0.98	0.99
Lime stone	1.28	1.28	1.16	1.16	1.04	1.04
Common salt	0.50	0.50	0.50	0.50	0.50	0.50
Medicinal plant	-	0.20	-	0.20	-	0.20
Vit. and Min.Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Lysine-HCL	0.16	0.16	0.15	0.15	0.13	0.13
DL-Methionine	0.05	0.05	0.06	0.06	0.07	0.07
Calculated analyses						
ME (Kcal/kg)	2800	2800	2800	2800	2800	2800
Crude protein (%)	17.5	17.5	17.5	17.5	17.5	17.5
Ether extract (%)	2.88	2.88	2.88	2.88	2.88	2.88
Crude fiber (%)	4.03	4.03	4.03	4.03	4.03	4.03
Calcium (%)	0.79	0.79	0.79	0.79	0.79	0.79
Available P (%)	0.31	0.31	0.31	0.31	0.31	0.31
Arg (%)	1.08	1.08	1.08	1.08	1.08	1.08
Lys (%)	0.98	0.98	0.98	0.98	0.98	0.98
Met (%)	0.33	0.33	0.33	0.33	0.33	0.33
Thr (%)	0.64	0.64	0.64	0.64	0.64	0.64

[1]The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000 IU (*all-trans*-retinal); cholecalciferol, 2,000 IU; vitamin E, 20 IU (*á*-tocopheryl); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg; and antioxidant, 250 mg

Table 3: Composition and ingredients of experimental diets during finishing period (43-49)

Days of age	Finisher (43 – 49 d)					
Date pits	0.00	0.00	5.00	5.00	10.00	10.00
Medicinal Plant (MP)	0.00	0.20	0.00	0.20	0.00	0.20
Feed ingredients	g / 100 g diet					
Corn	68.28	68.36	66.36	66.43	64.42	64.49
Soybean meal	19.93	20.07	20.93	21.06	21.92	22.05
Date pits	8.70	8.30	4.77	4.34	0.78	0.38
Wheat bran	0.76	0.76	0.78	0.79	0.81	0.81
Dicalcium phosphate	1.21	1.21	1.09	1.09	0.97	0.97
Lime stone	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	-	0.20	-	0.20	-	0.20
Medicinal plant	0.25	0.25	0.25	0.25	0.25	0.25
Vit. and Min.Premix[1]	0.08	0.08	0.07	0.07	0.05	0.05
Lysine-HCL	0.02	0.02	0.03	0.03	0.04	0.04
DL-Methionine	0.02	0.02	0.03	0.03	0.04	0.04
Calculated analyses						
ME (Kcal/kg)	2850	2850	2850	2850	2850	2850
Crude protein (%)	16.02	16.02	16.02	16.02	16.02	16.02
Ether extract (%)	3.01	3.01	3.01	3.01	3.01	3.01
Crude fiber (%)	3.82	3.82	3.82	3.82	3.82	3.82
Calcium (%)	0.71	0.71	0.71	0.71	0.71	0.71
Available P (%)	0.26	0.26	0.26	0.26	0.26	0.26
Arg (%)	0.97	0.97	0.97	0.97	0.97	0.97
Lys (%)	0.83	0.83	0.83	0.83	0.83	0.83
Met (%)	0.29	0.29	0.29	0.29	0.29	0.29
Thr (%)	0.58	0.58	0.58	0.58	0.58	0.58

[1]The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000 IU (*all-trans*-retinal); cholecalciferol, 2,000 IU; vitamin E, 20 IU (*á*-tocopheryl); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg; and antioxidant, 250 mg.

all 240 chicks were vaccinated against NDV. Blood samples were withdrawn 0 and 7 d after first- and 14 and 21 d after second-vaccination for determination of primary and secondary antibody (Ab) responses. The non-heparinized blood samples (1.5 mL/ chicken, one bird per pen) were placed at 37°C for 2 h, centrifuged (3,000×g for 15 min) to separate sera and stored at 20°C until analysis. The sera were applied to hemagglutination inhibition (HI) test to determine Ab to NDV, expressed as reciprocal log2 values for the highest dilution that displayed HI test. Blood biochemical parameters (HDL, LDL, TG and glucose) were measured for blood samples taken on day 49 of age. For differentiable count of white blood cell, blood samples were collected in K3EDTA tubes. Each sample was coded and offered for manual microscopic and automated count.

Statistical Analysis: To statistical analysis of data a two-way factorial ANOVA was applied for all measured parameters. The used model was: $Yijk = \mu + Aj + Bj + (A.B)ij + eijk$, Where Yijk = tested parameter of a broiler chick fed diet containing graded levels of DP (0, 50 and 100 g/kg) and MP (0 and 2 g/kg), Ai=diet type DP, Bj=MP (A.B)ij = interaction between DP and MP addition; eijkl = error term. All experimental data were subjected to the GLM procedure of SAS as a complete randomized design. All statements of significance are based a probability of less than 0.05. The mean values were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Productive Performance: Approximate analyses of the DP sample used in the present experiment was DM= 95.52%, ME= 2000 kcal/kg, CP= 7.3%, EE= 7.1%, CF= 48.2%, Ca= 0.865% and available P= 0.03%). Effects of diet inclusion of date pits and ground powder of thyme and garlic on FI, FCR, BW and BWG of broiler chicks are presented in tables 4-7, respectively. There was no significant effect of dietary treatment on productive performance of chicks in the present experiment. Including ground DP in diet up to 10% did have no deleterious effect on broiler performance. In addition, no beneficial effect of adding thyme and garlic to diet was seen on chicks' productive performance. After substituting corn by date pits at levels of 50, 100 and 150 g kg^{-1} in broiler starter diets, Kamel *et al.* [3] showed that the inclusion of date pits in the diets supported chick growth as efficiently as the control diet at all levels.

Table 4: Effects of dietary inclusion of date pits (0, 50 and 100 g/kg) and medicinal plants (0 and 2 g/kg) on feed intake (FI, g/ chick/ day) of broiler chicks

	Feed intake (g/ chick/ day)			
	0-21	22-42	43-49	0-49
Treatments Date pits				
0.0 (g/kg diet)	39.11± 1.90	121.71±10.55	127.06± 9.25	95.96±6.83
50 (g/kg diet)	40.70± 2.71	126.17± 8.04	129.31±12.23	98.72± 7.40
100 (g/kg diet)	41.19± 1.72	116.82±8.38	123.11± 10.05	93.71± 6.33
Medicinal plant				
0.0 (g/kg diet)	40.64±2.48	122.79±11.20	126.12±12.05	96.51±8.11
2 (g/kg diet)	40.03±2.06	120.34±7.73	126.86±9.08	95.75±5.75
SEM	0.460	1.938	2.132	1.406
CV	5.40	7.17	7.89	6.92
	P values			
Date pits (DP)	0.16	0.13	0.47	0.34
Medicinal Plant (MP)	0.51	0.50	0.86	0.78
DP × MP	0.37	0.15	0.09	0.15

Values were calculated from seven individual birds per group and expressed as means ± SD. SEM= standard error of means

Table 5: Effects of dietary inclusion of date pits (0, 50 and 100 g/kg) and medicinal plants (0 and 2 g/kg) on feed conversion ratio (FCR, g feed: g gain) of broiler chicks

	Feed conversion ratio (g feed: g gain)			
	0-21	22-42	43-49	0-49
Treatments Date pits				
0.0 (g/kg diet)	1.56±0.52	2.19±0.28	1.92±0.33	2.17±0.17
50 (g/kg diet)	1.62±0.11	2.16±0.22	2.12±0.43	2.19±0.11
100 (g/kg diet)	1.59±0.14	2.09±0.32	2.14±0.45	2.14±0.11
Medicinal plant				
0.0 (g/kg diet)	1.60±0.12	2.21±0.33	2.08±0.41	2.20±0.14
2 (g/kg diet)	1.59±0.09	2.08±0.18	2.04±0.45	2.14±0.12
SEM	0.021	0.055	0.085	0.026
CV	6.43	13.35	20.95	5.66
	P values			
Date pits (DP)	0.53	0.78	0.56	0.69
Medicinal Plant (MP)	0.81	0.26	0.84	0.33
DP × MP	0.15	0.73	0.33	0.07

Values were calculated from seven individual birds per group and expressed as means ± SD. SEM= standard error of means

Table 6: Effects of dietary inclusion of date pits (0, 50 and 100 g/kg) and medicinal plants (0 and 2 g/kg) on body weight (BW, g/chick) of broiler chicks

	Body weight (g/chick)			
	0	21	42	49
Treatments Date pits				
0.0 (g/kg diet)	45.25±1.58	530.09±33.68	1710.61±117.57	2160.24±107.15
50 (g/kg diet)	44.00±2.20	533.31±53.58	1775.44±219.06	2203.18±225.32
100 (g/kg diet)	45.12±1.25	555.93±61.50	1760.38±252.95	2153.30±226.32
Medicinal plant				
0.0 (g/kg diet)	45.08±1.31	541.77±53.82	1732.37±237.91	2160.78±217.12
2 (g/kg diet)	44.50±2.11	537.78±48.36	1765.26±156.64	2183.69±160.75
SEM	0.356	10.222	40.356	38.211
CV	3.89	9.03	11.79	8.53
	P values			
Date pits (DP)	0.31	0.52	0.81	0.84
Medicinal Plant (MP)	0.42	0.84	0.70	0.76
DP × MP	0.42	0.11	0.30	0.11

Values were calculated from seven individual birds per group and expressed as means ± SD. SEM= standard error of means

Table 7: Effect of dietary inclusion of date pits (0, 50 and 100 g/kg) and medicinal plants (0 and 2 g/kg) on body weight gain (BWG, g/ chick/ day) of broiler chicks

	Body weight gain (g/ chick/ day)			
	0-21	22-42	43-49	0-49
Treatments Date pits				
0.0 (g/kg diet)	24.26±2.02	55.76±5.43	35.91±11.88	38.87±1.80
50 (g/kg diet)	25.36±2.52	59.15±8.70	29.53±16.02	39.53±3.88
100 (g/kg diet)	26.04±2.94	56.88±9.09	24.14±12.97	37.84±2.93
Medicinal plant				
0.0 (g/kg diet)	25.12±2.91	56.39±9.62	31.10±15.19	38.69±3.75
2 (g/kg diet)	25.32±2.20	58.13±5.44	28.62±13.29	38.80±2.03
SEM	0.516	1.571	2.861	0.602
CV	9.51	14.27	47.66	7.41
	P values			
Date pits (DP)	0.35	0.70	0.28	0.50
Medicinal Plant (MP)	0.84	0.61	0.67	0.93
DP × MP	0.10	0.51	0.51	0.12

Values were calculated from seven individual birds per group and expressed as means ± SD. SEM= standard error of means

Table 8: Effect of dietary inclusion of date pits (0, 50 and 100 g/kg) and medicinal plants (0 and 2 g/kg) on white blood cell counts (heterophil, lymphocyte, monocyte, eosinophil and basophil as well as heterophil to lymphocyte ratio) – (on day 28 of the experiment)

	White blood cell counts (%) – 28 d					
Item[1]	H[2]	L	M	E	B	H:L
Treatments Date pits						
0.0 (g/kg diet)	30.87±5.54	63.37±6.50	2.37±2.82	1.75±1.39	1.75±1.67	0.50±0.13
50 (g/kg diet)	34.00±5.24	60.87±4.70	1.25±1.28	2.00±1.07	1.87±1.88	0.57±0.13
100 (g/kg diet)	33.50±7.65	61.75±8.05	1.25±2.81	2.25±1.16	1.37±1.06	0.56±0.21
Medicinal plant						
0.0 (g/kg diet)	33.17±6.48	60.92±6.51	2.08±3.03	1.92±1.16	1.92±1.50	0.56±0.17
2 (g/kg diet)	32.42±6.01	63.08±6.29	1.17±1.47	2.08±1.24	1.42±1.24	0.53±0.15
SEM	1.250	1.298	0.484	0.241	0.280	0.032
CV	19.80	11.12	148.81	62.91	90.00	30.85
	P values					
Date pits (DP)	0.59	0.76	0.57	0.73	0.79	0.65
Medicinal Plant (MP)	0.78	0.45	0.36	0.75	0.42	0.66
DP × MP	0.54	0.81	0.36	0.49	0.95	0.61

[ab]Means within column with different superscripts are significantly different (P < 0.05).

1. Heterophil, Lymphocyte, Monocyte, Eosinophil, Basophil, heterophil to lymphocyte ratio

2. Values were calculated from seven individual birds per group and expressed as means ± SD. SEM= standard error of means

Table 9: Effect of dietary inclusion of date pits (0, 50 and 100 g/kg) and medicinal plants (0 and 2 g/kg) on white blood cell counts (heterophil, lymphocyte, monocyte, eosinophil and basophil as well as heterophil to lymphocyte ratio) – (on day 42 of the experiment)

	White blood cell counts (%) – 42 d					
Item[1]	H	L	M	E	B	H:L
Treatments Date pits						
0.0 (g/kg diet)	26.12±5.44	67.37±6.88	2.00±0.92	2.62±1.50	1.62±1.99	0.40±0.12
50 (g/kg diet)	32.37±7.52	60.50±6.99	1.50±1.19	3.63±2.44	2.00±1.19	0.55±0.20
100 (g/kg diet)	27.37±5.58	67.37±6.86	1.12±1.25	2.87±1.64	1.25±1.28	0.42±0.12

Table 9: Continued

		White blood cell counts (%) – 42 d					
Item[1]		H	L	M	E	B	H:L
Medicinal plant							
0.0 (g/kg diet)		29.75±6.12	64.08±6.75	1.42±1.08	2.83±1.11	1.75±1.71	0.48±0.16
2 (g/kg diet)		27.50±7.09	66.08±8.15	1.67±1.23	3.25±2.45	1.50±1.31	0.43±0.16
SEM		1.343	1.508	0.233	0.383	0.306	0.033
CV		22.73	11.20	76.06	54.38	94.28	34.43
		P values					
Date pits (DP)		0.16	0.12	0.35	0.47	0.63	0.12
Medicinal Plant		0.41	0.51	0.61	0.54	0.69	0.47
DP × MP		0.72	0.81	0.54	0.02	0.26	0.76
DP	MP						
0.0	0.0	27.75±2.63	65.00±5.71	1.50±0.58	2.75±0.96	2.50±2.52	0.43±0.08
0.0	2	24.50±7.42	69.75±7.93	2.50±1.00	2.50±2.08	0.75±0.96	0.36±0.15
50	0.0	34.50±7.94	60.25±9.18	1.50±1.29	2.00±0.82	1.75±0.96	0.60±0.23
50	2	30.25±7.54	60.75±5.44	1.50±1.29	5.25±2.50	2.25±1.50	0.51±0.18
100	0.0	27.00±4.69	67.00±4.24	1.25±1.50	3.75±0.96	1.00±1.41	0.41±0.09
100	2	31.00±3.46	63.67±6.11	0.67±1.15	2.67±1.53	2.00±1.00	0.49±0.10
CV				54.38			
P value				0.09			

[ab]Means within rows with different superscripts are significantly different (P < 0.05).

1. Heterophil, Lymphocyte, Monocyte, Eosinophil, Basophil, heterophil to lymphocyte ratio

Values were calculated from seven individual birds per group and expressed as means ± SD. SEM= standard error of means

Growth performance of chicks fed diets containing date pits similar to or better than chicks fed control diets has been also seen in other studies [28]. Javandel *et al.* [29] reported daily FI per chicks influences by dietary garlic meal level so that the birds fed diets with 0.125 and 0.25% garlic meal had a significantly higher daily FI than birds fed 0.5% dietary garlic meal at 0-21. Cross *et al.* [30] observed no significant difference in BWG between broilers fed a wheat-soyabean meal based diet with or without the thyme herb. Also, the inclusion of thyme oil did not affect BWG of broilers over a 42-d growth period [31]. Lim *et al.* [32] observed no difference in the FI and FCR of laying hens fed 0, 1, 3 and 5% garlic powder, 200 ppm of Cu, or 3% garlic powder- 200 ppm of Cu. Lewis *et al.* [33] and Demir *et al.* [34] reported a trend to improved BWG in broiler chickens fed low concentrations of commercial garlic products.

Differential Counts of White Blood Cells: Effects of diet inclusion of date pits and ground powder of thyme and garlic on differential counts of white blood cells of broiler chicks on days 28 and 49 of age (first and second blood sampling) are presented in tables 8-9, respectively. Differential counts of white blood cells of broiler chicks did not affected by dietary treatments.

Antibody Response to NDV: Effects of diet inclusion of date pits and ground powder of thyme and garlic on Ab titers against NDV in broiler chicks is presented in table 10. Ab titers against NDV in broiler chicks did not affected by dietary treatments; however, the interaction between dietary inclusion of DP and MP on Ab titer on day 28 of age (primary Ab response) was significant, so that the highest Ab titer was seen in the birds fed diet with 5% DP with no MP. Serum HI Ab titer is an indicator of the humoral immunity in chickens [35].

Serum Biochemical Parameters: Effects of diet inclusion of date pits and ground powder of thyme and garlic on serum biochemical parameters (including cholesterol, HDL, LDL, TG and glucose) in broiler chicks are presented in table 11. Although blood levels of cholesterol, LDL and glucose was not affected by dietary treatment; blood levels of HDL in birds fed on the DP-included diets was significantly lower than birds fed on the control diet. There was significant interaction between dietary inclusion of DP and MP on blood level of TG, so that the highest blood level of TG was seen in birds fed diet with 10% DP with no MP. Sengül *et al.* [36] shown that supplemented antibiotic, thyme oil extract and thyme water extract did not significantly affect concentration of

Table 10: Primary and secondary antibody response (Log_2) against Newcastle Disease Virus of broiler chicks fed diets including date pits (0, 50 and 100 g/kg) and medicinal plants (0 and 2 g/kg)

		Antibody response (Log2) against Newcastle Disease Virus			
		7 days after 1st vaccination		2nd vaccination	
		Primary Ab response		Secondary Ab response	
Days of age		21	28	42	49
Treatments Date pits					
0.0 (g/kg diet)		1.12±1.12	2.37±2.13	5.25±0.46	4.50±1.60
50 (g/kg diet)		1.12±1.126	3.00±2.00	5.50±0.53	5.25±0.71
100 (g/kg diet)		1.25±1.49	2.25±1.98	5.50±0.53	4.75±0.89
Medicinal plant					
0.0 (g/kg diet)		1.17±1.19	3.08±1.62	5.25±0.45	4.50±1.31
2 (g/kg diet)		1.17±1.27	2.00±2.21	5.58±0.51	5.17±0.83
SEM		0.246	0.404	0.103	0.230
CV		98.97	66.39	9.23	21.81
		P values			
Date pits (DP)		0.97	0.64	0.52	0.37
Medicinal Plant (MP)		1.00	0.13	0.12	0.14
DP × MP		0.05	0.02	0.52	0.17
		Interactions			
DP	MP				
0.0	0.0	0.75±0.96	3.50±1.00^{ab}	5.00±0.00	3.75±1.89
0.0	2	1.50±1.29	1.25±2.50^{b}	5.50±0.58	5.25±0.96
50	0.0	2.00±0.82	4.50±0.58^{a}	5.25±0.50	5.50±0.58
50	2	0.25±0.50	1.50±1.73^{b}	5.75±0.50	5.00±0.82
100	0.0	0.75±1.50	1.25±0.96^{b}	5.50±0.58	4.25±0.50
100	2	1.75±1.50	3.25±2.36^{ab}	5.50±0.58	5.25±0.96
CV			66.39		
P value			0.05		

^{a,b}Means within the same row with common superscripts do not differ (P > 0.05)
Values were calculated from seven individual birds per group and expressed as means ± SD. SEM= standard error of means

Table 11: Effect of dietary inclusion of date pits (0, 50and 100 g/kg) and medicinal plants (0 and 2 g/kg) on serum biochemical parameters (cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and blood sugar) in broiler chicks (on day 49 of the experiment)

		Serum biochemical parameters (mg/dL)				
Parameters		CHOL[1]	HDL	LDL	TG	BS
Treatments Date pits						
0.0 (g/kg diet)		126.38±14.66	66.87± 8.08^{a}	47.00±7.44	113.38±12.69^{b}	193.13±65.13
50 (g/kg diet)		115.00±11.62	57.87±5.44^{b}	41.88±6.27	100.88±27.80^{b}	137.25±111.8
100 (g/kg diet)		119.00±9.59	58.25±6.47^{b}	44.38±5.15	142.25±36.81^{a}	176.00±107.3
Medicinal plant						
0.0 (g/kg diet)		118.25±10.94	59.58± 7.15	42.75±5.31	121.08± 38.16	176.08±81.74
2 (g/kg diet)		122.00±14.22	62.42± 8.32	46.08±7.24	116.58± 25.38	161.50±11.30
SEM		2.563	1.576	1.314	6.486	19.553
CV		10.33	11.12	14.26	20.94	54.00
		P values				
Date pits (DP)		0.20	0.02	0.29	0.01	0.47
Medicinal plant		0.47	0.32	0.21	0.66	0.70
DP × MP		0.48	0.43	0.49	0.04	0.08
DP	MP					
0.0	0.0				112.00±9.93^{b}	
0.0	2				114.75±16.50^{b}	
50	0.0				88.00±26.13^{b}	
50	2				113.75±26.04^{b}	
100	0.0				163.25±24.85^{a}	
100	2				121.25±36.98^{b}	
CV					20.94	
P value					0.01	

^{a,b}Means within the same row with common superscripts do not differ (P > 0.05).
1- Cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein, Blood Sugar
Values were calculated from seven individual birds per group and expressed as means ± SD. SEM= standard error of means

TG, total cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol in Japanese quails. Lee *et al.* [37] found that thymol and carvacrol did not affect plasma cholesterol levels in either high or low cholesterol broilers. Lim *et al.* [34] also reported that HDL-cholesterol was not influenced when the layers were fed 0, 1, 3 and 5% garlic powder for 5 weeks.

In conclusion, including ground date pits to broiler diet up to 10% showed growth performance comparable to the corn-SBM diet. The advantage in using date pits in broiler feed is that they are an inexpensive feed ingredient found in abundance in Iran and other countries in Persian Gulf region. The addition of dried powder of garlic and thyme to corn-SBM- or corn-SBM-DP diet did not have any beneficial effects on broiler growth performance and serum anti-NDV Ab titers. Dietary DP inclusion significantly decreased the serum levels of HDL.

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