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Effects of Artichoke (*Cynara scolymus* L.) Leaf Meal and Vitamin E on Productive Performance, Intestinal Microflora and Morphology in Japanese Quail

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Abstract

A total of 240, one-day-old quail chicks (*Coturnix coturnix japonica*) were used to study the effects of Artichoke leaf meal (ALM) and vitamin E in diet on growth performance, ileal microbial populations and intestinal morphology in a 42-d trial. This experiment was performed as a completely randomized design with 4 replicates of 15 quails each, using a 4 × 2 factorial arrangement with diet and gender as the main effects. Four dietary treatments were formulated by addition of 2 levels (1.5 and 3 percent) of ALM and 300 mg/Kg vitamin E to the basal diet. Supplementing basal diet with ALM did not improve growth performance at different rearing periods, whereas dietary vitamin E increased feed intake and body weight gain at day 21 of age ($P<0.05$). The ileal populations of *lactobacillus* and coliforms was not affected by dietary ALM treatments, whereas vitamin E increased the population of total aerobic bacteria ($P\leq0.04$). The ileal villus height ($P\leq0.01$) and crypt depth ($P\leq0.008$) were reduced in quails fed on diets with ALM and vitamin E. The quails fed diets containing 3 percent ALM and 300 mg/Kg vitamin E had higher villus height:crypt depth ratio. The thickness of muscularies ($P\leq0.04$) and mucosa ($P\leq0.0007$) layers were decreased in birds fed diets containing ALM than control birds. Birds treated with ALM and vitamin E showed a shorter intestinal length ($P\leq0.02$) and a lower pancereas relative weight. The results of this study showed that supplementing diet by ALM (1.5 and 3%) and 300 mg/Kg vitamin E did not improve growth performance, ileal microbiota pupolations and intestinal morphometric indices in Japanese quail.

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Introduction

The modification of gut microbial population is used to improve growth performance and feed efficiency. The global ban on application of antibiotic growth promoters in poultry diets as a result of their potential side effects was led to identification and evaluation of alternative feed additive options to satisfy consumer's demands (Hertrampf, 2001; Humphrey *et al.*, 2002). The removal of antibiotic growth promoters from animal feed was expected to result in increased intestinal disorders as a result of gut pathogens proliferation and consequently reduced feed efficiency and animal performance (Hughes *et al.*, 2005). Hence, the possibility of growth-promoting and antioxidative properties for certain medicinal plants has recieved more attention (Papadopoulou *et al.*, 2005; Liu *et al.*, 2006). Most of these plants contain phenolic compounds which frequently documented as antimicrobial (Zhu *et al.*, 2004) and antioxidant agents (Gronbaek *et al.*, 1995; Knekt *et al.*, 2002) benefiting animals through modulation of gut function.

The importance of Artichoke as a medicinal plant is related to its phenolic compounds such as caffeoylquinic acid derivatives and flavonoids (Llorach *et al.*, 2002; Wang *et al.*, 2003; Joy and Haber, 2007) mainly accumulated in the leaves (Sanchez-Rabaneda *et al.*, 2003). The antioxidant function of phenolic compounds is resulted from its radical-scavenging properties (Wang and Huang, 2004). It has been reported that, Artichoke extract exert beneficial effects in birds challenged with mycotoxins (Stoev *et al.*, 2004). Moreover, Azcona *et al.* (2005) demonstrated a higher metabolizable energy during the first 21 days in chicks fed on diets with Artichoke extract. Advanteges were also reported for the use of Artichoke extract during the first weeks of life chicks (Mariani, 1998).

Few experimental studies have been published on the effects of ALM in Japanese quail. Therefore, this study was carried out to investigate the effects dietary ALM and Vitamin E on growth performance, intestinal morphology and microbial population in Japanese quail.

Materials and Methods

Plant meal preparation

Artichoke leaves were collected at vegetative stage from the research farm (latitiude: 37°00' - 37°30'N; longitude: 54°00' - 54°30'E; altitude: 155 m) of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Golestan province, Iran. Collected leaves were dried in shadow and ground with a laboratory mill to pass through a 3-mm screen (Iran khodsaz gristmill, ELS 300C, Iran). Nutrient compositions of Artichoke leaves were determined proximate analysis (AOAC, 1999). The total values of phenolic compounds were measured by colorimeter, using Folin- Ciocalteu method (Guo *et al.*, 2000) (Table 1).

Table 1. Proximate composition and phenolic compounds of Artichoke leaf powder (As-fed)

Item	Percentage
Moisture	7.70
Protein	11.70
Crude fat	4.49
Crude fiber	23.90
Ash	9.60
NFE	42.61
Ca	0.45
Na	0.22
Total P	0.33
Total polyphenols	7.70
Flavonoids	1.61
Antioxidants	6.92
Gross energy (Kcal/Kg)	3713

Birds and experimental design

A total of 240, one-day-old Japanese quail chicks were used in this study. The chicks were individually weighted and assigned randomly to 4 experimental diets with 4 replicates of 15 birds each. All 16 replicates were housed in a poultry experimental facility under standard conditions of ventilation, heating and lighting. Room temperature was set at 37 ± 1°C during the first week and then gradually decreased to 24 ± 1°C by d 28 and kept at 24 ± 1°C through day 42 of experiment (Nazar *et al.*, 2012). Quails were subjected to a continuous artificial lighting througthout the experiment. The 4 × 2 factorial experiment was performed with a completely randomized design, considering diet and gender as main fixed effects. Four experimerntal diets were formulated by inclusion of 2 levels (1.5 and 3 percent) of ALM with and without addition of 300 mg/Kg vitamin E to the basal diet. A standard corn-soybean meal based diet (basal diet) was formulated to meet or exceed the nutrient recommendations for quails (Table 2; NRC, 1994) and fed from d 1 until the end of experiment. Quails had free access to feed and fresh water throughout the trial.

Collected data

Body weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured at the end of wk 3 and 6 for each cage. On d 42, two randomly choosen birds from each pen were killed by severing the neck following the cervical dislocation. The digestive tract with contents was removed aseptically and the proventriculus, gizzard, and small intestine segments were excised, cleaned, dried with desiccant paper, and wigthed. The weight of the empty organs was expressed relative to live BW.

Table 2. Compositions and calculated analyses of the basal diet[1]

Ingredients	% (unless noted)
Corn	48.96
Soybean meal	45.10
Soybean oil	2.89
Dicalcium phosphate	0.75
Calcium carbonate	1.30
Common salt	0.35
Mineral premix[2]	0.25
Vitamin premix[3]	0.25
DL-Methionine	0.15
Calculated analysis	
Metabolizable Energy (Kcal/Kg)	2900
Crude protein	24.00
Calcium	0.80
Available phosphorous	0.30
Sodium	0.15
Lysine	1.39
Methionine	0.50
Methionine + Cystine	0.88

[1]Calculated composition was according to NRC (1994).
[2]Mineral premix (each Kg contained): Mn, 50000 mg; Fe, 25000 mg; Zn, 50000 mg; Cu, 5000 mg; Iodine, 500 mg; Choline chloride, 134000 mg.
[3]Vitamin Premix (each Kg contained): Vitamin A, 3600000 IU; Vitamin D_3, 800000 IU; Vitamin K_3, 1600 mg; Vitamin B_1, 720 mg; Vitamin B_2, 3300 mg; Vitamin B_3, 4000 mg; Vitamin B_5, 15000 mg; Vitamin B_6, 150 mg; Vitamin B_9, 500 mg; Vitamin B_{12}, 600 mg; Biotin, 2000 mg.

On d 42, two quails of each replicate were killed following a 12-h fasting to limit intestinal throughput. Quails were immediately processed and dissected. The digestive tract with contents was removed and the ileum was aseptically separated from the Meckel's diverticulum up to 10 cm proximal to the ileocecal junction and then dried with desiccant paper. The specimens (2 cm) taken from the middle part of the segment were gently rinsed twice in 0.9% saline to remove digesta and fixed in 10% neutral buffered formalin. The samples were processed for 24 h in a tissue processor with ethanol as dehydrant and embedded in paraffin. Embeded tissue samples were sectioned to a thickness of 5 µm by microtome (Slee mainz, Cut 4055), stained with hematoxylin and eosin, then examined with an optical microscope (Olympus, BX60) equipped with a digital camera (Olympus, DP70). The images were analyzed for villus height, crypt depth, villus height:crypt depth ratio, and thickness of the muscularis and mucosa layers using image software. A total of 10 intact, well-oriented villus–crypt units were selected for each intestinal cross-section. All measurements were done only where the villus-crypt unit was perpendicular to the muscularis mucosa. The villus height was measured from the tip of the villus to the villus-crypt junction. The total mucosal thickness was measured from the top of the villus to the border over the muscularis mucosa. The depth of crypt was then defined as the difference between the total mucosal thickness and the villus height. The thickness of the muscularis externa was measured.

Microbial assesments

On d 42, two birds from each replicate were killed by cervical dislocation and processed properly for collection of their intestinal tract. Samples of ileal fresh digesta (0.2 g) were diluted in physiological serum (saline) to an initial 10^{-1} dilution. The ileum contents of each bird were pooled for serial dilution. Microbial populations were determined by serial dilution (10^{-4} to 10^{-6}) of ileal samples in anaerobic diluents before inoculation onto petridishes of sterile agar. *Lactobacilli,* aerobic bacteria and coliforms were grown on culture media of Rogosa SL agar, Plate Count Agar and Violet Red Bile Agar (VRBA), respectively. Plates were incubated anaerobically (73% N, 20% CO_2, 7% H_2) at 37°C. Plates were counted between 24 and 48 hrs after inoculation. Colony forming units were defined as being distinct colonies measuring at least 1 mm in diameter.

Statistical analysis

Data collected were subjected to ANOVA using GLM procedure of SAS software (SAS, 2003). The pen mean was an experimental unit for productive triats (BWG, FI and FCR). Main effect means and the interactions were reported. Microbiological concentrations were subject to $\log_{10}$ transformation before analysis. When significant effects were found, comparisons among multiple means were made by Duncan's multiple range tests. Differences were considered statistical significance when $P<0.05$.

Results

Supplementation of basal diet with ALM and vitamin E did not significantly affect BWG during 22-42 and 1-42 days experimental periods. However, birds fed diets containing 3 percent ALM and vitamin E showed a decreased and increased BWG at 21 d of age ($P<0.05$), respectively, when compared with the birds fed on the basal diet. In addition, supplementing basal diet with ALM did not affect FI at different rearing experimental periods, whereas vitamin E significantly increased FI at 21 d of age when compared to birds fed basal and Artichoke supplemented diets ($P<0.05$). Feed conversion ratio values were similar in quails treated with vitamin E at different experimatal periods compared to control, whereas it was increased ($P<0.05$) in the birds fed on diet containing 3 percent ALM at 21 d of age (Table 3).

Table 3. Influence of supplementation of Artichoke leaf meal and vitamin E in diet on growth performance of Japanese quail in different rearing periods

Treatments	1 to 21 d			22 to 42 d			1 to 42 d		
	BWG[1]	FI[2]	FCR[3]	BWG[1]	FI[2]	FCR[3]	BWG[1]	FI[2]	FCR[3]
Control	99.30[b]	243.85[b]	2.45[b]	133.15	669.43	5.02	232.45[ab]	913.28	3.93
1.5% Artichoke	96.19[b]	241.04[b]	2.50[b]	128.81	658.06	5.11	225.00[b]	899.10	3.99
3% Artichoke	87.37[c]	238.26[b]	2.72[a]	137.11	686.52	5.00	224.48[b]	924.78	4.12
300 mg vitamin E	110.87[a]	277.45[a]	2.50[b]	130.90	698.77	5.33	241.77[a]	976.27	4.03
SEM	1.96	25.4	0.20	5.76	26.15	0.25	3.46	25.4	0.04
P-value	0.01	0.04	0.01	0.45	0.07	0.06	0.02	0.34	0.45

[1]Body weight gain (g); [2]Feed intake (g); [3]Feed conversion ratio (g of feed/g of BWG).
[a-c] Means within a column without a common superscript differ significantly ($P<0.05$).

Results for ileal bacterial counts are shown in Table 4. No significant interaction was found between diet and gender on ileal bacterial populations. The coliform population of ileum did not differ by the diet and gender. Supplementing basal diet with ALM (1.5 and 3%) did not affect total aerobic bacteria, whereas diet containing 300 mg/Kg vitamin E increased ileal populations of total aerobic bacteria ($P\leq0.04$). The ileal population of *lactobacillus* was increased numerically by supplementing of basal diet with ALM.

Table 4. Influence of supplementation of Artichoke leaf meal and vitamin E in diet on illeal microbial population of 42-d-old Japanese quail

Treatment	*Lactobacillus* (log_{10} cfu/g of DM)	Coliforms (log_{10} cfu/g of DM)	Total aerobic bacteria (log_{10} cfu/g of DM)
Diet:			
Control	8.18	6.24	6.41[b]
1.5% Artichoke	8.55	6.37	6.56[b]
3% Artichoke	8.28	6.10	7.02[b]
300 mg Vitamin E	8.15	6.16	7.40[a]
SEM	0.28	0.33	0.29
Gender:			
Male	8.50	6.41	6.60
Female	8.22	6.03	6.86
SEM	0.15	0.23	0.20
Significance:			
Diet	0.12	0.15	0.04
Gender	0.22	0.31	0.11
Diet × Gender	0.75	0.55	0.45

[a,b] Means within a column without a common superscript differ significantly ($P<0.05$).

Results on ileal morphology in quails are presented in Table 5. No significant interaction between diet and gender and gender effects were found on morphometrical indices. The ileal villus height ($P\leq0.01$) and crypt depth ($P\leq0.008$) were reduced in quails treated with ALM and vitamin E, when compared with control birds. The villus height to crypt depth ratio was significantly increased in quails fed diets containing 3 percent ALM and 300 mg/Kg vitamin E compared with those fed other diets. Moreover, the thickness of muscule ($P\leq0.04$) and mucosa (*P≤0.0007*) layers in birds fed diet containing ALM (1.5 and 3%) decreased when compred with the control birds.

Table 5. Influence of supplementation of Artichoke leaf meal and vitamin E in diet on ileum mucosa morphometry of Japanese quail

Treatment	Villus height (µm)	Villus widthness (µm)	Crypt depth (µm)	Villus height:crypt depth	Muscule thickness (µm)	Mucosa thickness (µm)
Diet:						
Control	855.67[a]	104.80	143.40[a]	5.58[b]	176.01[a]	29.85[a]
1.5% Artichoke	556.63[b]	138.69	97.98[b]	5.72[b]	105.40[c]	15.85[b]
3% Artichoke	707.63[b]	109.78	89.87[b]	8.16[a]	120.44[cb]	14.44[b]
300 mg Vitamin E	728.80[b]	112.37	109.40[b]	6.78[a]	159.84[ab]	34.33[a]
SEM	4.97	2.85	2.03	0.63	2.80	1.09
Gender:						
Male	716.00	106.02	109.51	6.36	127.29	23.73
Female	708.38	126.80	110.80	6.74	153.54	23.50
SEM	3.5	2.01	1.4	0.44	1.98	0.77
Significance:						
Diet	0.01	0.49	0.008	0.16	0.04	0.0007
Gender	0.88	0.23	0.87	0.65	0.13	0.92
Diet × Gender	0.72	0.65	0.45	0.63	0.18	0.29

[a,b] Means within a column without a common superscript differ significantly (*P<0.05*).

Table 6. Influence of supplementation of Artichoke leaf powder and vitamin E in diet on the intestinal length (cm) and relative weigth of digestive organs (g/100 g live body weight) in 42-d-old Japanese quail

Treatment	Intestinal length	Intestinal weight	Proventriculus weight	Gizzard weight	Pancrease weight	Digestive tract weight
Diet:						
Control	61.93[a]	7.69	0.55	2.87	0.89[a]	19.55[b]
1.5% Artichoke	56.75[b]	8.27	0.50	2.93	0.75[ab]	23.88[a]
3% Artichoke	56.93[b]	8.12	0.53	2.97	0.65[b]	20.58[b]
300 mg Vitamin E	57.06[b]	8.16	0.54	2.67	0.65[b]	20.75[b]
SEM	0.68	0.43	0.11	0.23	0.16	0.52
Gender:						
Male	58.62	7.59	0.50	2.94	0.60	17.81
Female	57.71	8.90	0.55	2.78	0.88	24.57
SEM	0.47	0.03	0.07	0.16	0.11	0.36
Significance:						
Diet	0.02	0.59	0.66	0.24	0.09	0.003
Gender	0.53	0.001	0.59	0.14	0.00	0.0001
Diet × Gender	0.75	0.54	0.79	0.24	0.47	0.18

[a,b] Means within a column without a common superscript differ significantly (*P<0.05*).

No differences were observed in intestinal, proventricular and gizzard relative weights between birds received dietary treatments (Table 6). Birds treated with ALM and vitamin E showed a shorter intestinal length than those fed with basal diet ($P<0.05$). The addition of 3 percent ALM and 300 mg/Kg vitamin E resulted in lower relative weight of pancreas than control diet with no supplmentation. The relative weight of digestive tract was increased by supplementing diet with 1.5 percent ALM compare with control diets.

Discussion

Results of the current study with respect to no change in growth performance (BWG, FI, FCR) by diets supplemanted with ALM are consistent with findings of Melo and Harkes (2007) who revealed no significant differences among broilers treated with two levels of Artichoke extract (300 and 600 g/ton) for BWG and FI at the end of 42-day trial. Nevertheless, our results on FCR disagree with the findings of Melo and Harkes (2007) and also Azcona *et al*. (2005). Melo and Harkes, (2007) reported that supplementing diet with Artichoke extract (600 g/ton) from 1-21 days of age improved feed efficiency in 21 and 42 day-old broilers. These authors concluded that improved feed conversion may be attributed to the reduced feed intake. Moreover, a better conversion in broilers treated with Artichoke extract can be attributed to an improved lipid digestibility due to an increased bile secretion and also increased metabolizable energy of birds during their first weeks of life (Azcona *et al*., 2005). In addition, positive results of Artichoke protection were reported by Stoev *et al.* (2004) and Mariani (1998). Differences between our results with others may be attributed to the variation of experimental conditions, differences in the type of Artichoke used (meal *vs.* extract) and probably environmental stresses including handling, vaccination, and various other instances that might negatively affect growth performance through activation of immune system (Hevener *et al*., 1999; Takahashi *et al*., 2000).

We observed no significant effects of ALM and vitamin E on ileal coliform pathogenic population. However, populations of beneficial *Lactobacillus* bacteria increased numerically by supplementating basal diet with ALM. Moreover, ileal total aerobic bacteria count increased in birds treated by 300 mg/Kg vitamin E. These results suggest that the intake of polyphenols compounds such as ALM might favor the growth of beneficial bacteria. It seems that some of these microorganisms might be able to use these compounds as nutritional substrates. For example, lactobacilli bacteria are able to metabolize phenolic compounds as energy substrate (García-Ruíz *et al.*, 2008). In addition, many *in vitro* studies showing that many fungi, bacteria, and yeasts are able to grow and develop on polyphenolic compoumds (Arunachalam *et al*., 2003).

The functional status of the small intestine is characterized in part by villus height, villus widthness, crypt depth, villus height:crypt depth ratio and thickness of muscule and mucosa layers. It is assumed that an increased villus height or villus widthness along with a decreased in crypt depth support digestive function of the intestine as a result of increased absorptive surface area, expression of brush border enzymes and nutrient transport systems (Caspary, 1992; Zijlstra *et al.* 1996). In current study, the inclusion of ALM and vitamin E did not increase villus height, wherase decreased crypt depth. Liu *et al*. (2011) reported no changes in villus height or crypt depth in the jejnum of broilers treated with chicory forage diet (60 and 120 g/Kg). It has been documented that physiological effects of dietary fiber is also effective on intestinal development. In this context, Brunsgaard and Eggum (1995) reported that the physiological effect of dietary fiber is more dramatic in the distal gasterointestinal tract. Baurhoo *et al.* (2007) reported that increased villi length might be due to an increased colonization of beneficial bacteria such as lactobacilli and bifidobacteria. In contrast to the latter report, our findings showed a decreased villius height along with a slight increased *lactobacillus* population in quails fed diets supplemented with ALM. Regarding other parameters of gut integrity, crypt depth was reduced in birds treated with ALM and vitamin E compared with those fed basal diet. In contrast to our findings, Ferket *et al.* (2002) reported that crypt depth was not affected in turkeys treated with an antibiotic-free diet. It has been documented that a deeper crypt may show faster tissue turnover to permit renewal of the villus, which suggests that the host's intestinal response mechanism is trying to compensate for normal sloughing or atrophy of intestinal mucosal layer (Gao *et al.*, 2008). In the present study, an increase in ileal villus height:crypt depth ratio in quails fed diets supplemented with ALM (3%) and vitamin E was also found. Moreover, thicknes of muscule and mucosa layers were decreased in birds fed diets containing ALM (1.5 and 3%), which is consistent with reports showing decreased thickness of walls by the inclusion of feed antibiotics in diets of chicken (Miles *et al.*, 2006). Thinner muscule and mucosa layers of quails treated with ALM can be partly attributed to the distension of intestine due to increased bulk-forming capacities of fiber diet (Kalmendal *et al.*, 2011). In the current study, birds treated with dietary treatmants (1.5 and 3% ALM and 300 mg/Kg vitamin E) had a shorter intestinal length than control birds. In addition, quails feds diet containing 3 percent ALM and 300 mg/Kg vitamin E had a lower pancerease relative weight compared to control quails. This study showed that dietary treatments of ALM and vitamin E did not affect intestinal length and relative weights of intestine, proventriculous and gizzard which was consistent with Liu *et al.* (2011), who studied the effects of chicory (*Cichorium intybus* L.) forage on broilers. However, the relative weight of digestive tract was increased by dietary treatment of 1.5 percent ALM; this could be attributed in part due to increased intestinal weight.

Conclusion

It can be concluded that polyphenol-rich ALM (1.5 and 3%) did not improve growth performance; whereas dietary vitamin E increased feed intake and body weight gain at day 21 of age. The ileal populations of *lactobacillus* and coliforms did not affect by dietary ALM treatments, whereas vitamin E increased the population of total aerobic bacteria. The ileal villus height and crypt depth were reduced by ALM and vitamin E dietary treatments. The ALM (3%) and 300 mg/Kg vitamin increased villus height:crypt depth ratio. The thickness of muscularies and mucosa layers were decreased by ALM.

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