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The effects of artichoke aqueous extract on the growth performance, intestinal morphology, and blood metabolites in broiler chickens

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ABSTRACT

An experiment was conducted to study the efficacy of artichoke aqueous extract (AAE) on the performance, carcass characteristics, clinical blood chemistry values, and the gut morphology of female broiler chickens poisoned with carbon tetra chloride (CCl_4). Broiler chicks were categorized into four experimental groups each of which contained 10 chicks; control group, artichoke group (0.5g/kg/bwt of artichoke aqueous extract), CCl_4 group (1ml/kg/bwt of CCl_4), and artichoke+ CCl_4 group (0.5g/kg/bwt of artichoke extract plus 1ml/kg/bwt of CCl_4). Blood samples were analyzed for the clinical blood chemistry values, cholesterol, triglyceride, albumin, total protein, alanine aminotransferase (ALT), aspartate amino transferase (AST), and alkaline phosphates (ALP). Intestinal samples were processed for histological assessment. Results showed that chicks fed with artichoke supplemented diet had an improvement in the FCR during the 21-35 days of the experiment period compared with other groups. The significant increase of the albumin level was observed in artichoke group compared with CCl_4 group. There was a significant difference in the total protein level in the artichoke group in comparison with CCl_4 group and artichoke + CCl_4 group. Concentration of AST enzyme was determined significantly lower in the artichoke group compared with other groups. The artichoke group showed a significant increase in the villus length compared with the control and CCl_4 groups. However, the artichoke + CCl_4 group did not show a significant change ($P< 0.05$) in the villus length compared with the control group. In conclusion, our study showed that the use of artichoke extract improves morphological status in the small intestine in broiler chickens and subsequently improves the digestibility.

In conclusion, our study provides evidence that the use of artichoke extract improves morphological status in small intestine in broiler chickens and subsequently improves the digestibility.

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Introduction

Antibiotics as growth promoters in food animal production have been used since 1946 throughout the world (Humphrey *et al*. 2002). They are used in poultry production to improve therapeutic and prophylactic performance to stabilize intestinal microbial flora and to prevent some specific intestinal pathogen (Hughes *et al*. 2005). The elimination of antibiotic growth promoters from animal feed owing to world prohibition was expected to result in the increase of 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 herbal plants has drawn more attention (Liu *et al.* 2011; Papadopoulou *et al*. 2005). Researchers have reported the positive effects of extracts of herbal medicines on the function and growth of broilers (Germano *et al.* 1999). Cynara scolymus L. is the oldest known herbal medicine, which has been raised for thousands of years. Artichoke is full of Phenolic compounds such as mono-,di-caffeoylquinic acid and Flavonoid (Wang *et al.* 2003). Researchers have reported the positive effects of extracts of herbal medicines on the function and growth of broilers (Germano *et al.* 1999). Cynara scolymus L. is the oldest known herbal medicine, which has been raised for thousands of years. Artichoke is full of Phenolic compounds such as mono-,di-caffeoylquinic acid and Flavonoid (Wang *et al.* 2003). The antioxidant function of phenolic compounds is resulted from its radical-scavenging properties (Wang and Huang, 2004). The curative properties of the leaves of artichoke on dyspepsia have been verified (Blumenthal. 1998). Dyspepsia can be a result of insufficient secretion of bile by the liver, but using the leaves of artichoke makes digestion of food easier by stimulating the secretion of bile (Matuschowski *et al.* 1996). Also, it is argued that intestine function is very important in digestion. Therefore, in the present research, the effect of liquid extract of artichoke on the function and morphology of intestine tissue of broilers was tested.

Material and Methods

Providing liquid extract of artichoke

To provide the liquid extract, the leaves were picked and collected from the farm of the Agricultural Sciences and Natural Resources Univ. (ASNRU) of Gorgan, and after systemic verification, they were dried in shadow and ground with a laboratory mill to pass through a 3-mm screen (Iran khodsaz gristmill, ELS 300C, Iran). 100 grams of the powder was mixed with distilled water in proportion 1:10. Then, the mixture was put in water bath and kept in temperature of 50˚ C, for 48 hours. Finally, obtained extract was filtered, then freeze-drying process done and it was kept for the future use, under the temperature of -20˚ C.

Chemical analysis of artichoke

The compound of artichoke leaf was clarified through some experiments in the laboratory of domestic animals of ASNRU of Gorgan, by using Folin- Ciocalteu method (Guo *et al.* 2000) (Table 1).

Experimental treatments

The tests were performed in a completely randomized design recruiting 4 groups and 10 times repetitions. All replicates were housed in a poultry experimental facility under standard conditions of ventilation, heating and lighting. During the first week room temperature was set at 37 ± 1°C and then gradually decreased to 24 ± 1°C by d 28 and kept at 24 ± 1°C through experiment (Nazar *et al*., 2012). The broilers, which were 550-600 grams at their 21st day, were divided randomly into 4 groups, and each group was placed in a separate cage. Two basic rations (of the beginning and the growth) were used for all four groups. The beginning ration (from the birth until 21st day) contained 2900 kilo calories of metabolic energy and 20.84% raw protein, and the growth ration (from 22nd to the last day of the maintenance) contained 3000 metabolic energy and 18.75% raw protein (Table 2; NRC, 1994). The first group was selected as the control group. The second group received daily 0.5 g liquid extract of artichoke for each kilograms of the chickens' weight. The third group received 1 ml carbon tetra chloride for each Kg of the chicks through peritoneal injection. Experimental treatments continued for two weeks, from 21st to 35th days of chickens' life span. Chickens had free access to food and fresh water. Body weight gains (BWG), food intake (FI) and food conversion ratio (FCR) were measured at the end of the fourth and fifth weeks for each treatment.

Gavage feeding of liquid extract

0.5 g of the extract for each kilogram of a chicken's weight was solved in 2 cc distilled water every day,

and then by a special gavage syringe and through the mouth, were given directly to the 2nd and 3rd care groups for 14 days (from 22nd to 35th day).

Injection of carbon tetra chloride

Given the non-polar structure of the carbon tetra chloride, and because of its solubility in all kinds of mineral and herbal oil solvent, the olive oil was used as the solvent during the experiment. Two hours after prescription of the last dose of the extract, in 35th day, 1 cc of carbon tetra chloride was solved in 1 cc olive oil, and was injected in peritoneum. 28 hours after injection of carbon tetra chloride, the birds were sacrificed by cervical dislocation.

Determination of performance and carcass characteristics of broiler chicken

Body weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR) were recorded at the end third and fifth week. After slaughter, the abdominal cavity was opened to expose the visceral organs, and the carcass characteristics were evaluated

Measurement of blood biochemical parameters

Serum cholesterol, triglyceride, albumin, total protein and serum hepatic enzymes ;including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphate (ALP) were determined calorimetrically by using an especial kit (Pars Azmoon company, Iran).

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

Composition	Percentage
Protein	11.70
Crude fat	4.49
Moisture	7.70
Crude fiber	23.90
Ash	9.60
NFE	42.61
Total polyphenols	7.70
Antioxidant	6.92
NA	0.22
CA	0.45
Flavonoid	1.61
(Gross energy(Kcal/KG))	3713

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

Ingredinet	Starter phase (d 1 to 21)%	Finisher phase (d 22 to 35)%
Corn	57.25	63.3
Soybean meal	37.37	31.49
Sunflower Oil	1.65	1.97
Dicalcium carbonate	1.41	1.04
Calcium carbonate	1.26	1.33
Sodium chloride	0.42	0.32
Vitamin-Mineral premix[2]	0.5	0.5
Dl- Methionine	0.14	0.05
Calculated Composition		
Metabolized energy (Kcal/Kg)	2900	3000
Crude Protein (%)	20.84	18.75
Calcium (%)	0.91	0.84
Total phosphorus (%)	0.41	0.33
Sodium (%)	0.18	0.14
Lysine (%)	1.154	1
Methionine (%)	0.47	0.36
Methionine + cystine (%)	0.84	0.68

[1] *Calculated composition was in compliance whit according to NRC (1994).*

[2] *Vitamin Premix (each Kg contained): Vitamin A, 3600000 IU; Vitamin D3, 800000 IU; Vitamin K3, 1600 mg; Vitamin B1, 720 mg; Vitamin B2, 3300 mg; Vitamin B3, 4000 mg; Vitamin B5, 15000 mg; Vitamin B6, 150 mg; Vitamin B9, 500 mg; Vitamin B12, 600 mg; Biotin, 2000 mg. Mineral premix (each Kg contained): Mn, 50000 mg; Fe, 25000 mg; Zn, 50000 mg; Cu, 5000 mg; Iodine, 500 mg; Choline chloride, 134000 mg.*

Histological assessment

The tissue samples from the duodenum were fixed overnight (10% formalin), embedded in paraffin, sectioned (5 µm), and mounted for Hematoxylin and eosin (H&E) staining. Slides were observed with a light microscope (Olympus BX51, Olympus Corp., Tokyo, Japan), and images were recorded by using an attached digital camera (1300R, Qimaging Corp., Burnaby, BC, Canada). Villus length, crypt depth and muscularis mucosa thickness were acquired and measured by using image analysis software (Olympus Soft Imaging Solutions, version 3.2, Germany). The villi length was measured from the tip to the villi base, and then the crypt depth was measured from the base of the crypt (Figure-1). The mean value of each replicate was obtained and used for statistical analysis.

Statistical analysis

Data were analyzed in compliance with the mixed procedure of SAS software, and least square means were compared using Tukey-Kramer test. The results were significant when $P < 0.05$.

Result

The effects of supplementation of artichoke extract on broiler chickens performance shown in Table 3. There were no significant differences in body weight gain throughout the experimental period among different groups (Table 3). Chicks fed the diets supplemented with artichoke extract showed an improvement in the FCR during 21-35 days of experimental period compared to other groups (Table 3). The effects of supplementation of artichoke extract on the carcass characteristics of broiler chickens at 35 d of age shown in Table 4.

Table 3: *The effect of supplementation of artichoke extract on growth performance of female broiler chickens in different rearing period*

Treatments	21 to 28 d		29 to 35 d		21 to 35 d	
	BWG[1]	FCR[2]	BWG	FCR	BWG	FCR
Control	427.36	2.08	516.43	2.19	943.79	2.08[b]
Artichoke	434.17	2.12	519.61	1.94	952.78	2.02[ab]
CCl_4	375.16	2.63	507.06	2.8	808.1	2.65[a]
Artichoke+ CCl_4	430.44	2.16	433.49	2.25	950.51	2.23[ab]
P-value	0.57	0.19	0.32	0.15	0.34	0.05
SEM	32.07	0.17	32.03	0.31	56.28	0.15

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

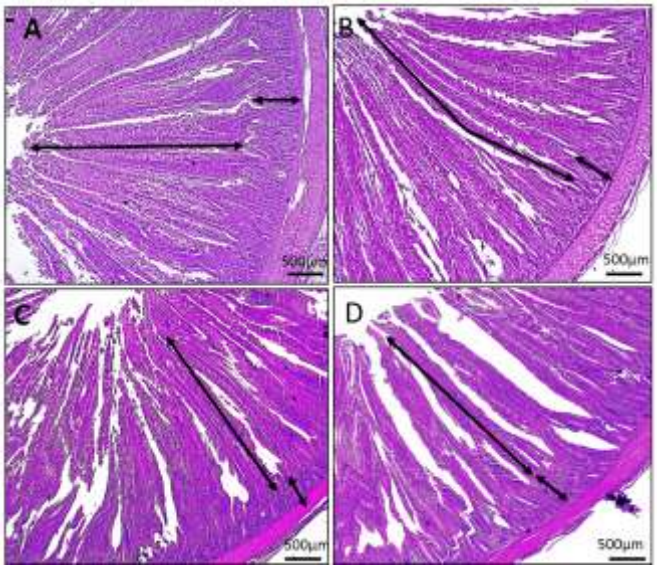

Fig. 1. *Effect of artichoke aqueous extract on intestinal histology of broiler Chickens. Control group (A), artichoke group (B), CCl_4 group (C), and the group received both artichoke extract and CCl_4 (D). Villus length (large arrows), crypt depth small arrows. Magnification of 4X.*

Table 4: *The effect of supplementation of artichoke extract on the carcass characteristics of female broiler chickens at 35 d of age*

Treatments	Body weight (g)	Thigh weight (g)	Breast weight (g)	Heart weight (g)	Gizzard weight (g)	Spleen weight (g)	Pancreas weight (g)
Control	1531.87	265.17^{a}	311.22	11.94^{a}	40.84	1.57	4.91
Artichoke	1543.22	272.41^{a}	300.84	11.73^{a}	45.55	1.52	4.53
CCl_4	1521.26	215.23^{b}	292.4	9.10^{b}	46.48	1.69	4.78
Artichoke+ CCl_4	1530.87	257.14^{ab}	309.49	10.38^{ab}	42.56	1.63	4.71
P-value	0.18	0.028	0.69	0.014	0.18	0.93	0.58
SEM	55.44	13.27	15.55	0.53	1.77	0.19	0.28

There were no significant differences in body weight at the moment of slaughtering. The effects of artichoke extract on clinical blood chemistry values of broiler chickens are presented in Table 5. Blood serum cholesterol and triglyceride levels were not influenced by dietary treatments on d 21 and 35 ($P < 0.05$). The significant increase was detected in the albumin level in artichoke group compared to CCl_4 group. There was significant difference in the total protein level in artichoke group compared to CCl_4 group and artichoke extract+ CCl_4 group. Concentrations of AST enzyme were detected substantially lower in the artichoke group compared to those of the other groups. A significant increase in serum AST concentration was observed in the CCl_4 group compared to those of the control.

Artichoke aqueous extract reduced AST blood level in the group receiving CCl_4 plus artichoke extract compared to CCl_4 group. A significant increase in serum AST concentration was observed in the CCl_4 group compared to those of the control. Artichoke aqueous extract reduced AST blood level in the group receiving CCl_4 plus artichoke extract compared to CCl_4 group. ALT and ALP serum levels did not differ statistically among treatments. Histological examinations of the small intestine from birds fed the dietary treatments are shown in Table 6 and Figure 1. No significant differences in crypt depth, muscularis mucosae thickness and villus/crypt ration were observed between treatments.

Table 5: *The effect of supplementation of artichoke extract on clinical blood chemistry values of female broiler chickens at 35 d of age*

Treatments	Cholesterol	Triglyceride	Albumin	Total protein	ALT[1] (IU/L)	AST[2] (IU/L)	ALP[3] (IU/L)
Control	121.9	94.69	1.62^{a}	5.34^{a}	5.94	141.04^{b}	931.11
Artichoke	110.67	90.72	1.56^{ab}	5.32^{a}	4.28	72.41^{c}	491.85
CCl_4	122.75	95.57	1.11^{c}	2.95^{c}	11.23	248.06^{a}	1937.66
Artichoke+ CCl_4	111.4	93.44	1.33^{b}	4.02^{b}	7.9	207.79^{a}	1601
P-value	0.99	0.67	0.01	0.0001	0.3	0.047	0.7
SEM	7.2	3.3	0.11	0.16	2.32	17.46	268.15

[1] *Alanine aminotransferase (ALT);* [2] *Aspartate aminotransferase (AST);* [3] *Alkaline phosphate (ALP)*

Table 6: *The effect of supplementation of artichoke extract on gut morphology of female broiler chickens at 35 d of age*

Villus length/crypt depth	Muscularis mucosa thickness (µm)	Crypt depth (µm)	Villus length (µm)	Treatments
May-61	20/55	296/85	1571.43 ^{c}	Control
21-Jun	22/10	286/88	2025.05 ^{a}	Artichoke
May-98	18/86	270/86	1565.77^{c}	CCl_4
Jun-48	23/26	288/41	1870.71 ^{ab}	Artichoke + CCl_4
0.22	0.34	0.6	0.0001	P-value
1.69	1.57	19.49	42.85	SEM

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Discussion

Holtman *et al.* (2003) indicated that artichoke extract, can help to relieve symptoms of patients with indigestion. It is reported that the chlorogenic acid through durability of the intestinal juice, help to improve digestive performance (Olthof, *et al.* 2001). In this study, it is reported that the effects of artichoke extract on gut morphology used broiler chickens as the animal model. Our study clearly demonstrate the artichoke extract has significant effects on the small intestine and subsequently promotes their digestion and absorption functions. The villus height to crypt ratio is a benefit parameter to measure the absorption capacity of the small intestine (montagne *et al.*, 2004). In this experiment villus height to crypt depth ratio in duodenum increased in broiler supplement with artichoke leaves extract compared with control group. Samanta *et al.* (2010) showed that adding probiotics to broilers feed for 7 days increases villous height compared to control group. Moreover, Garcia *et al.* (2007) showed that using medicinal plants in feed causes a higher villous in chickens. Changes in the intestinal morphology, villus height and crypt depth could justify the presence of toxins (xu *et al.*, 2003).

Thus, reduced villus height, crypt depth and muscularis thickness in tetrachloride carbon group might be associated with tetrachloride carbon injection to chickens. It is concluded that Cynara scolymus L. extract can lead to the rise of food absorption as well as digestion improvement process. The results of the present research contrast with this point that herbal plants do not have significant and outsized influence on the amount intake food (Hernandez *et al.* 2004; Lee *et al.* 2013). The researchers showed that addition of mint leaves to food intake had no significant effect on poultry food intake. In contrast to their findings, Cross *et al.* (2007) reported that herbal medicine and their extracts can result in weight gain, rise of food intake and improvement of conversion ratio. In the experiment conducted by Ocak *et al.* (2008), no significant difference was observed in relative gain weight by adding mint to the food intake, also weight of digestive organs such as periventricular, gizzard, pancreas, intestine did not undergo significant change by taking mint related extracts. The research revealed that, phenol compositions existing in thyme extract strongly links to proteins and as a result prevent tryptophan an of amino

acids, lysine and cysteine it is argued that this phenomen induces the reduction of protein biological value Previous study results have indicated that the intake of 200 ppm of combined essence extracted from Cinnamomum verum pepper as well as 8000 ppm mixed mint essence species (Rosemary, Thyme and Dianthus) can improve the digestion of dry substance and raw fat in the food intake. Thyme oil extract effect on the digestive system, especially colorectal broilers, and cause the secretion of digestive enzymes and androgen. Thyme oil extract at a dose of 20% increase in weight and also Consumption of food (Papadopoulou *et al.* 2005). In a study conducted on broiler chikens it was found that use of garlic extract for three weeks can due to inhibition of liver enzymes HMGcoA- reductase (LDL-C) and reduce serum total cholesterol and cholesterol but had no effect on HDL-C (Qureshi *et al.* 1983).

In conclusion, our study provides evidence that the use of artichoke extract improves morphological status in small intestine in broiler chickens and subsequently improves the digestibility. Thus, with artichoke extract, the intestinal structure of duodenum is more favorable for bird and may have possible benefit for some pathological condition such as dyspepsia.

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