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Toxicity of Carbon Tetrachloride in Japanese Quails: Evaluation the Effect of Artichoke (*Cynara scolymus*) Powder on Performance and Immune Response

Research Article

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ABSTRACT

This study was conducted to investigate the effects of artichoke powder on performance and immune function of Japanese quails poisoned with carbon tetrachloride (CCl_4). One hundred meat type (1-d-old) quails were assigned randomly to 4 treatment groups (n=25). Treatment groups included with the study were: 1) control group (without artichoke supplementation or CCl_4 administration, 2) group with artichoke supplementation and without CCl_4 administration, 3) group without artichoke supplementation and with CCl_4 administration (1 ml/kg of BW) and 4) group with artichoke supplementation and with CCl_4 administration (1 mL/kg of BW). CCl_4 was administered intraperitoneally on each 3 day for days 29 to 42. During the rearing period (0-42 days of age), quails had free access to feed and water. Performance parameters of daily gain, feed intake and feed conversion were determined on weekly basis. At the end of experimental period (day 42), blood samples were collected and then heterophils and lymphocytes were counted and their ratio (H/L ratio) was calculated. Results did not show any significant effect for artichoke powder and CCl_4 on body weight gain, feed intake and feed conversion ratio. Adding Artichoke to the diets at 2% level significantly increased the count of lymphocytes and decreased H/L ratio. Artichoke and CCl_4, each alone or both together, did not affect the relative weight of spleen, bursa of fabricius and liver.

KEY WORDS artichoke, immune system, Japanese quail, performance.

INTRODUCTION

The growth performance is influenced mainly by genetic characteristics, health, immune status and nutrition. In addition, birds encounter numerous stress-inducing factors during their lifespan and productive performance is lower under stress and those with immune suppression (Roth-Maier *et al.* 2005). Therefore, the application of immune-stimulating substances to enhance immune function can improve performance (Roth-Maier *et al.* 2005). Immune modulators such as medical plants activate the innate immune system. Medicinal plants contain a wide variety of active phytochemicals which stimulate the function of immune system. Many studies have shown that herbal feed additives act as natural growth promoter and as preventive of nutrition poisons (Johnson and Banerji, 2007; Sonkusale *et al.* 2011). Artichoke (*Cynara scolymus*) is a member of the compositae family that is widely grown in Mediterranean countries and is a rich source of natural antioxidants. The major bioactive components of artichoke leaves are flavonoids, cynarin, inulin, phenolic acids and caffeic acid (Joy and Haber, 2007). Artichoke has traditionally been used to treat jaundice and liver problems and research suggests that it can be applied as a harmless yet effective treatment for hypercholesterolaemia and also can positively effective on intestine micro flora, absorption of mineral and

blood lipid composition as well as in the prevention of colon cancer (Pittler *et al*. 2005; Hellwege *et al*. 2000). Research has shown that water extract of artichoke had some beneficial protective effects against the growth inhibiting effect of ochratoxin A and associated patho-morphological changes (Stove *et al.* 2004). Schutz *et al*. (2004) reported that, the total phenolic content of approximately 12 g/kg on a dry matter basis in artichoke pomace was a promising source of phenolic compounds that might be recovered and used as a natural antioxidant or as a feed ingredient.

Carbon tetrachloride (CCl_4), as a toxin, is an example of oxidative stressors that are used by researchers (Mujumddar *et al.* 1998). CCl_4 is an aliphatic hydrocarbon that has wide spread industrial application, for example in the production of chlorofluorocarbon refrigerants, foamblowing agents, cleaning compounds and organic solvents (Boger *et al.* 1987).

CCl_4 in the body converts to trichloromethyl (CCl_3^-). This very reactive free radical reacts with oxygen and converts to proxy trichloromethyl (CCl_3OO) which initiates the chain reaction of lipid peroxidation of endoplasmic reticulum membranes in liver that lead to liver damage and subsequently its destruction. This status is promoted by immune system (kupffer cells and neutrophilic granulocytes) activation by releasing cytokine and oxygen reactive species (Palmes and Spiegel, 2004). Although, the protection mechanisms within cells interact with the damages caused by oxygen reactive species, but due to the production of abnormally high levels of free radicals in stress conditions, the protection mechanisms are unable to provide the adequate protection. This makes the use of other ways of protection necessary (Ulicna *et al*. 2003). Since, there is little information on the effects of artichoke (a plant with antioxidative activity) on the productive performance of livestock, the objective of this study was to determine the effect of this medical plant as a feed additive on the growth performance and immune system function of Japanese quails poisoned with carbon tetrachloride.

MATERIALS AND METHODS

Plant material

Fresh artichoke leaves were obtained from the research farm of Gorgan University, Gorgan, Golestan, Iran. The artichoke leaves were prepared according to the following procedure. Leaves were cut in pieces and dried in shade for 10-14 days and then heated in an oven at a temperature below 50 ˚C for 48 hours. The dried leaves were then pulverized to form coarse powder in a grinding machine. The active constituents of artichoke leaves were determined by the procedure introduced by Constantinescue *et al.* (1967).

Birds, experimental design and diets

In total, 100 one-day-old Japanese quail chicks were individually weighed (8±2 g) and randomly distributed into four treatment groups. Treatment groups included with the study were: 1) control group (without artichoke supplementation or CCl_4 administration, 2) group with artichoke supplementation and without CCl_4 administration, 3) group without artichoke supplementation and with CCl_4 administration (1 mL/kg of BW) and 4) group with artichoke supplementation and with CCl_4 administration (1 mL/kg of BW).

CCl_4 was administered intraperitoneally on each 3 day for days 29 to 42 (Sonkusale *et al.* 2011). Chicks had *ad libitum* access to feed and water and diet was formulated to meet or exceed the nutrient recommendations by NRC (1994) (Table 1).

Table 1 Ingredients and chemical composition of the basal diet

Ingredients (%)	0 to 42 days
Corn grain	48.96
Soybean meal	45.1
Oil	2.89
Di calcium phosphate	0.75
Carbonate calcium	1.3
Vitamin premixes[1]	0.25
Mineral premixes[2]	0.25
Methionine	0.15
Salt	0.35
Chemical composition	
Metabolizable energy (kcal/kg)	2900
Crude protein (%)	24
Calcium (%)	0.8
Available p (%)	0.3
Sodium (%)	0.15
Lysine (%)	1.39
Methionine (%)	0.5
Methionine + cysteine (%)	0.88

[1] Vitamin premixes: 2/5 kg of vitamin supplements includes: vitamin A: 9000000 IU; vitamin D_3: 2000000 IU; vitamin K_3: 4000 mg; vitamin B_1: 1800 mg; vitamin B_2: 8250 mg; vitamin B_3: 10000 mg; vitamin B_5: 30000 mg; vitamin B_6: 3000 mg; vitamin B_9: 1250 mg; vitamin B_{12}: 1500 mg and Biotin: 5000 mg.
[2] Mineral premixes: 2/5 kg mineral supplement containing: Mn: 165350 mg; Iron 250000 mg; Zn: 249000 mg; Cu: 40000 mg; Iodine: 1600 mg and Choline chloride: 335350 mg.

Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured weekly. At 42 d of age, a total of 12 male birds from each treatment were weighed and killed by cervical dislocation. The spleen, bursa and liver were removed from each bird.

The organ weights were immediately measured following dissection and were expressed relative to body weight (g of organ/kg of BW). Blood samples for smear preparation obtained by cardiac puncture using ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes. Leukocyte counts were determined by analyzing blood smears stained with May-Grunwald-Giemsa.

Differentials counts of 100 white blood cells per blood smear were made by Fair *et al*. (1999) procedure. The heterophil:lymphocyte ratio was calculated using the following formula (Gross and Siegel, 1983):

Heterophil:lymphocyte ratio= number of heterophils / number of lymphocytes

Bird housing and management

All chicks were reared on floor under similar standard environmental and management conditions from hatch to 42 days of age.

Room temperature was 38 ˚C on the first day and every day 0.5 ˚C was reduced until day 28, afterward the temperature was fixed at 24 ˚C. A continuous lighting program was provided during the experiment. All experimental protocols were approved by the Animal Care and Use Committee of the College of Animal Science of Gorgan University (Golestan, Iran).

Statistical analysis

A completely randomized design was performed. Data were analyzed using GLM procedure of SAS software (SAS, 2003) and when results of analysis of variance revealed an existence of significant difference among treatment means, the Duncan multiple range test was used ($P<0.05$).

RESULTS AND DISCUSSION

Chemical composition of artichoke leaves meal

The chemical analyses of artichoke leaf powder are shown in Table 2. Artichoke contents of total phenols, flavonoid and antioxidant were 2.387, 1.614 and 6.920 percent, respectively.

Table 2 Chemical composition of artichoke leaves meal on air dry

Items	Percentage
Moisture	7.7
Dry matter	92.30
Crude protein	11.69
Crude oil	4.49
Crude fiber	23.87
Ash	9.6
Gross energy (kcal/kg)	3712.61
Ca	0.45
P	0.22
Na	0.33

Performance

The main and interaction effects of artichoke powder and CCl_4 on growth performance of Japanese quails are shown in Table 3. Results showed that artichoke powder and CCl_4 did not affect on body weight gain, feed intake and feed conversion ratio throughout the study (0 to 42 days).

Table 3 Main and interaction effects of artichoke and carbon tetrachloride on the performance of Japanese quails

Dietary artichoke level	BWG (g)	FI (g)	FCR (g/g)
	0 to 42 days	0 to 42 days	0 to 42 days
0 %	32.70	154.00	4.70
2 %	30.48	152.50	5.05
SEM	5.22	46.58	1.47
P-value	0.67	0.97	0.81
CCl_4 administration			
0 (control)	33.62	155.81	4.41
1 mL/kg of BW	29.55	150.68	5.34
SEM	5.22	46.58	1.47
P-value	0.44	0.91	0.53
Artichoke (Ar.) × CCl_4			
0 % Ar. × 0 mL CCl_4	35.96	161.74	4.06
2 % Ar. × 0 mL CCl_4	31.27	149.88	4.77
0 % Ar. × 1 mL CCl_4	29.42	146.26	5.34
2 % Ar. × 1 mL CCl_4	29.68	155.10	5.34
SEM	5.22	46.58	1.47
P-value	0.64	0.82	0.81

BWG: body weight gain; FI: feed intake and FCR: feed conversion ratio.
SEM: standard error of the means.

Immune organs and liver weight

The main and interaction effects of artichoke powder and CCl_4 on the relative weight of lymphoid organs and liver are indicated in Table 4. Results showed that artichoke powder and CCl_4 did not affect the relative weight of spleen, bursa and liver.

White blood cell

The main and interaction effects of artichoke powder and CCl_4 on the percentage of white blood cells (lymphocyte and heterophil) and also the heterophil to lymphocyte ratio (H/L) are shown in Table 5. Results showed that the level of 2% of artichoke powder significantly increased the count of lymphocytes and consequently decreased H/L ratio.

Oxidative stress is one of the main problems faced by poultry farmers.

Previous studies demonstrated the negative effects of oxidative stresses like poisonous and stress conditions on birds (Ulicna *et al.* 2003; Roth-Maier *et al.* 2005). Therefore, the application of immune-stimulating substances like medical plants to reduce the side effects of oxidative stresses is increasingly interested. Reported effects of dietary supplementation with medical plants on growth performance are variable. Many of researchers reported positive effect of medicinal plants on broiler performance (Ponte and Rosado, 2008; Germano *et al*. 2005; Hassan *et al*. 2004).

Table 4 Main and interaction effects of artichoke and carbon tetrachloride on the relative weight of immune organs and liver

Dietary artichoke level	Spleen (%)	Bursa of fabricius (%)	Liver (%)
0 %	0.08	0.09	2.99
2 %	0.06	0.08	2.69
SEM	0.02	0.01	0.25
P-value	0.37	0.43	0.24
CCl_4 administration			
0 (control)	0.07	0.09	2.99
1 mL/kg of BW	0.07	0.08	2.69
SEM	0.02	0.01	0.25
P-value	0.93	0.30	0.24
Artichoke (Ar.) × CCl_4			
0 % Ar. × 0 mL CCl_4	0.07	0.09	3.11
2 % Ar. × 0 mL CCl_4	0.07	0.09	2.88
0 % Ar. × 1 mL CCl_4	0.09	0.08	2.88
2 % Ar. × 1 mL CCl_4	0.05	0.07	2.50
SEM	0.02	0.01	0.25
P-value	0.20	0.71	0.76

SEM: standard error of the means.

Table 5 Main and interaction effects of artichoke and carbon tetrachloride on the count of white blood cells

Dietary artichoke level	Heterophile	Lymphocytes	H/L
0 %	8.54	90.21[b]	0.09[a]
2 %	7.75	91.46[a]	0.08[b]
SEM	0.40	0.42	0.005
P-value	0.06	0.004	0.007
CCl_4 administration			
0 (control)	8.12	91.12	0.08
1 mL/kg of BW	8.16	90.54	0.08
SEM	0.40	0.42	0.005
P-value	0.92	0.17	0.60
Artichoke (Ar.) × CCl_4			
0 % Ar. × 0 mL CCl_4	8.66	90.33	0.09
2 % Ar. × 0 mL CCl_4	7.58	91.91	0.07
0 % Ar. × 1 mL CCl_4	8.41	91.08	0.09
2 % Ar. × 1 mL CCl_4	7.91	91.00	0.08
SEM	0.40	0.42	0.005
P-value	0.48	0.43	0.39

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

However, the results of this study are in agreement with some previous studies which did not indicate any significant effect for herbs, plant extracts, essential oil and / or the main components of essential oil on body weight, feed intake and feed conversion in broilers (Cross *et al*. 2002; Hernandez *et al*. 2004; Bampidis *et al*. 2005). In contrast to our results, there was a reduction in body weight gain and feed intake with the first 4 weeks in broilers fed diets supplemented with artichoke powder (Abdo *et al*. (2007). Reduced body weight gain was also reported by Tajodini *et al*. (2014) in broilers receiving 3% of artichoke at the end of experiment. It seems that artichoke due to its anti-nutritional compounds has a negative effect on feed intake, feed conversion ratio and consequently on body weight gain. Consistent with this, Abdo *et al*. (2007) reported that withdrawal of artichoke powder from 28 to 42 days increased feed intake, and improved feed conversion ratio and body weight gain. The complexity of organismal responses and limited understanding of pathways involved in dietary immunomodulation has been observed in different species (Clarke and Mullin, 2008). Reported effects of dietary immunomodulators on immune function generally vary by the treatment and type of the experiment. In the current study, artichoke powder did not affect the liver and lymphatic relative weights, but increased the number of lymphatic cells. The latter result is similar to the findings of Tajodini *et al*. (2014) and Alime *et al*. (2009), who reported increase in the total count of white blood cells in broilers in response to dietary artichoke supplementation. Similarly, Fallah *et al*. (2013) reported that the use of artichoke powder and its extract can lead to an increase in percentage of lymphocytes. The elevation of the heterophil:lymphocyte ratio is a hematological indicator that animals are coping with chronic stress (Gross and Siegel, 1983). Schranner *et al*. (1989) reported the effect of a complex drug on the humoral immune response of broilers. Administration of this complex drug enhanced humoral immune response in chickens. Herbs that are rich in flavonoids such as thyme (Thymus vulgaris) extend the activity of vitamin C and act as antioxidants that may serve to enhance immune function (Rahimi *et al*. 2011).

Zulkifli *et al*. (2003) reported that birds exposed to stress or illness will increase the count of heterophil in their blood. Cosentino *et al*. (1999) reported that polysaccharides extracted from some plants are involved in stimulating the growth of many organs such as the spleen, thymus and bursa of fabricius, which are responsible for producing T cells, macrophages and lymphocytes. Khaligh *et al*. (2011) did not show significant differences in liver weight of chicks fed of the herb mixture. In contrast, Guo *et al*. (2000) reported that use of the medicinal plants increased weight of the lymphoid organs such as the thymus, spleen and bursa of fabricius in broilers. Stoev *et al*. (2000) showed a combination use of ochratoxin A and artichoke extract significantly decreased the relative weight of lym-

phoid organs and increased the relative weight of the liver in broiler chicks. Results of this study are also supported by the findings of Jung *et al*. (2010) who reported increase in activity of the immune system in response to medical herbal plant.

However, immune-stimulation may have adverse effects on growth performance by forcing nutrients toward immune organs, thereby decreasing the amount of nutrients available for growth (Hevener *et al*. 1999; Takahashi *et al*. 2000).

CONCLUSION

The results of this study showed that adding artichoke powder to the diet of Japanese quails can be useful in preventing the harmful effect of oxidative stressors by enhancing their immune system responses. However, further studies on the effects of artichoke powder or its extract are needed.

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