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NONRUMINANT NUTRITION

# Appraisal of purple coneflower *(Echinacea purpurea)* extract on production performance, internal organs, and gut microflora of japanese quail

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**ABSTRACT.** An experiment was carried out to evaluate the effects of *Echinacea purpurea* (EP) extract at 5 levels (0, 0.25, 0.5, 1 or 2 mL $L^{-1}$ of drinking water) on performance, internal organs and gut microflora of Japanese quail (*Coturnix coturnix Japonica*). The results showed that EP extract decreased feed intake and body weight gain of quails. The feed conversion ratio of quails fed with EP extract at the level of 0.5 ml $L^{-1}$ drinking water was significantly decreased compared to the control group. Adding EP extract at the levels of 0.25, 0.5 or 1 mL $L^{-1}$ drinking water decreased carcass yield of the birds ($p < 0.05$). The relative weight of internal organs of the birds fed different levels of EP extract was not significantly different. Birds consumed EP extract at the levels of 1 or 2 mL $L^{-1}$ drinking water had lower ileal aerobic bacteria population compared to the control group; however, the ileal *Coliform* population increased by EP extract supplementation ($p < 0.05$). Results of the present study indicated that using EP extract at the level of 0.5 mL $L^{-1}$ drinking water could improve feed conversion ratio of quails; however, it decreased the carcass yield of the birds.

**Keywords:** *Echinacea purpurea*, japanese quail, performance, gut microflora.

## Avaliação do extrato de equinácea (*Echinacea purpurea*) no desempenho de produção, órgãos internos e microflora intestinal de codornas japonesas

**RESUMO.** Um experimento foi realizado para avaliar os efeitos do extrato de *Echinacea purpurea* (EP) em 5 concentrações (0, 0,25, 0,5, 1 ou 2 mL $L^{-1}$ de água potável) sobre o desempenho, órgãos internos e microflora intestinal de codornas japonesas (*Coturnix coturnix japonica*). Os resultados mostraram que o extrato diminuiu a ingestão alimentar e o ganho de peso corporal de codornas durante todo o período de estudo. A taxa de conversão alimentar de codornas alimentadas com extrato EP ao nível de 0,5 mL $L^{-1}$ foi significativamente menor em relação ao grupo controle. A adição de extrato EP aos níveis de 0,25, 0,5 ou 1 mL $L^{-1}$ diminuiu o rendimento de carcaça das aves ($p < 0,05$). O peso relativo dos órgãos internos das aves alimentadas com diferentes níveis de extrato EP não diferiu significativamente. As aves que consumiram o extrato EP nas concentrações de 1 ou 2 mL $L^{-1}$ apresentaram uma menor população de bactérias aeróbicas no íleo em comparação com o grupo controle; no entanto, a população ideal de *Coliform* aumentou nas aves suplementadas com extrato EP ($p < 0,05$). Nossos resultados indicaram que o uso de extrato EP no nível de 0,5 mL $L^{-1}$ de água potável melhora a taxa de conversão alimentar das codornas, no entanto, diminui o rendimento de carcaça dos animais.

**Palavras-chave:** *Echinacea purpurea*, codorna japonesa, desempenho, microflora intestinal.

## Introduction

Feed additives such as botanical extracts may promote the growth performance or carcass yield in farm animals by improving the utilization and efficiency of feedstuffs (Windisch et al., 2008). Herbal additives are very interesting in modern poultry production particularly in organic farms. They are applied as a growth promoter and might also prevent several diseases in birds, so they can be considered as safe antibiotic replacements without residual harmful chemicals in the meat. These additives may have positive impacts on the microecology of alimentary tract (Nasiroleslami & Torki, 2011). The content of effective materials in dietary herbal additives may be different, due to the differences in plant sections such as seed, leaf, root, bark, or harvesting time, and plantation origin. Moreover, the processing procedures such as extraction with non-aqueous solvents and other distillation methods can dramatically change the active materials and their levels in the end product (Windisch et al., 2008). Phytobiotics naturally prevent the incidence of especial disease in birds when used throughout the entire production period.

Herbal additives do not have withdrawal time and their residue in poultry meat or egg does not have adverse impact on human health condition (Windisch et al., 2008).

There are many medicinal plants with considerable effects on chickens such as EP. The EP belongs to the family of Asteraceae whose products are among the most widely used phytogenics. The active materials in EP have positive effect on immunological parameters (Nasiroleslami & Torki, 2011). All varieties of EP contain caffeic acid derivatives, alkamides, flavonoids, essential oils, and polyacetylenes, whose medical effectiveness have been already proved in special illnesses (Thygesen, Thulin, Mortensen, Skibsted, & Molgaard, 2007). Previous studies stated that EP has immunoregulation, antiinflammation and antioxidant characteristics (Zhai et al., 2007; Lee, Chen, Shieh, Lin, & Yu, 2009), with no hypersensitivity or other side effects during usage period (Saunders, Smith, & Schusky, 2007). Nasiroleslami and Torki (2011) reported that the FCR in laying hens fed diets containing EP was positively improved. Ghalamkari et al. (2011) reported that using of 10 g EP $kg^{-1}$ diet improved total antioxidant activity in serum of broiler chicks. Landy, Ghalamkari, Toghyani, & Moattar (2011) found that EP had positive effect on growth performance and humoral immune responses in broiler chicks. Nasir and Grashorn (2010a) found that broilers given diets in which EP was supplied at (inform the concentration) had better average daily weight gain in comparison with control group. Many experiments have studied the antibiotic activity of EP and it was totally indicated that EP has antibiotic and immunostimulant properties (Matthias, Banbury, Bone, Leach, & Lehmann, 2008). The ethanolic juice of EP increased the number of lymphocytes and total leukocytes in hens and pigs (Bohmer, Salisch, Paulicks, & Roth, 2009). Jamroz, Wertelecki, Houszka and Kamel (2006) found that herbal feed additives stimulated the gut mucosal secretion in broilers. It is interesting to note that mucus secretion can weaken the adhesion of pathogens and it may help to stabilizing the healthy balance of the microflora in the gastrointestinal tract of the animals (Jamroz et al., 2006). Thus, the objective of this study was to evaluate the effect of EP on performance, internal organs and gut microflora of Japanese quail (*Coturnix coturnix Japonica*).

## Material and methods

### Birds, diets, and management

The experiment was conducted according to the protocol approved by Animal Care Committee of Amol University of Special Modern Technologies, Mazandaran, Iran. A total of 200 one-day old Japanese quail chicks were obtained from a commercial quail farm and were divided into 20 groups of 10 birds each. The experimental diets were formulated according to National Research Council [NRC] (1994) recommendations. The ingredient content and composition of the basal diet are shown in Table 1. Hydroalcoholic extract of EP was prepared from Zardband Company. Experimental treatments consisted of five levels of EP: 0, 0.25, 0.5, 1 and 2 mL $L^{-1}$ alcoholic EP (Zardband Company, Iran, pH = 5.7, density = 1.07, caffeic acid = 2.99 mg $mL^{-1}$). The basal diet was fed as mash and prepared with the same batch of ingredients for starter (1-21 d) and grower (22-42 d) periods. All birds had free access to feed and water. The ingredients and chemical composition of the basal diets are shown in Table 1. Experiment data was recorded 15 to 42 d of rearing period. Temperature was initially set at 37°C on d 1 and then was linearly decreased by 0.5°C per day until reaching 21°C. During the study, the birds received a lighting program of 24 L : 0 D from 1 to d 7, and afterward 23 L : 1 D until d 42.

**Table 1.** The ingredients and nutrient composition of diets.

| Ingredients (%) | Starter (1-21 d) | Grower (22-42 d) |
|---|---|---|
| Corn | 47.00 | 60.00 |
| Soybean meal | 43.5 | 33.40 |
| Corn gluten germ | 3.00 | 1.00 |
| Vegetable oil | 2.7 | 2.00 |
| Oyster shell | 1.2 | 1.10 |
| Dicalcium phosphate | 1.5 | 1.45 |
| Common salt | 0.30 | 0.32 |
| L-Threonine | 0.05 | 0.04 |
| DL-Methionine | 0.25 | 0.20 |
| Vitamin and mineral premix [1] | 0.50 | 0.50 |
| Calculated contents (%) | | |
| ME (kcal $kg^{-1}$) | 2925 | 3020 |
| Crude protein | 24 | 20.20 |
| Calcium | 0.95 | 0.86 |
| Available phosphorus | 0.44 | 0.41 |
| Sodium | 0.19 | 0.18 |
| Lysine | 1.31 | 1.06 |
| Methionine+Cystine | 0.68 | 0.60 |
| Threonine | | |

1vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D3, 9800 IU; vitamin E, 121 IU; B12, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

### Measuring birds performane

The experimental period lasted 42 d. On d 14, 21, 28, 35 and 42, birds were weighed by pen, and feed consumption was recorded. Then, feed conversion ratio was calculated for each phase. European production efficiency factor (EPEF) was calculated according to the following formula:

EPEF = [body weight (kg) × survival rate (%) / FCR × age (days)] × 100

### Carcass characteristics

Two birds per pen around the average weight of the pen were selected and slaughtered through cervical dislocation to determine the carcass traits at 42 d of age. The edible carcass (without viscera or feet and skin), breast, thigh, liver, spleen, and empty gizzard and were weighed and their percentage was expressed as a ratio to live body weight.

### Ileal microflora

The ileums (defined as the region between Meckel's diverticulum and the ileocecal junction) were excised and contents were collected by gently pressing the fingers to move the content into tubes at 28 and 42 d of age. Digesta of birds within a replicate were pooled, put on ice until they were transported to the laboratory for enumeration of microbial population. One g of ileal content was homogenized in 9 ml sterile water. Each sample was serially diluted. Using these diluted sub samples, Lactobacillus was enumerated on De Man-Rogosa-Sharpe (MRS) agar after incubation at 37°C in an anaerobic chamber for 48 h (Guban, Korver, Allison, & Tannock, 2006). Coliforms was counted on CHROM agar ECC (EF322- Paris France) after incubation at 37°C in an aerobic chamber for 48 h (Sallam, 2007). The total count of aerobic bacteria was determined on plate count agar (PCA) for 24 h at 37°C. Results were expressed as the log10 of colony forming units (CFU) per gram of ileal digesta. Tryptose sulfite-cycloserine (TSC) agar media was used for *C. perfringens* enumeration (Oxoid CM587 with the addition of SR88 and SR47). Colonies on TSC agar that were suspected to be *C. perfringens* were plated secondarily on blood agar (Garridol, Skjervheim, Oppegaard, & Sørum, 2004).

## Statistical analysis

All percentage data were subjected to arcsine transformation prior to analysis. While conclusions were drawn from the transformed data, only untransformed data are presented for relevance. Statistical analysis was conducted using the GLM procedure of SAS software. Data of the experiment were statistically analyzed using a completely randomized design (CRD) (Statistical Analysis Systems [SAS], 2002). Means were compared using Duncan's new multiple range test (Duncan, 1955). The level of significance was reported at $p < 0.05$.

## Results and discussion

The inclusion of various levels of EP extract to drinking water on feed intake is indicated in Table 2. At present study, adding EP extract to drinking water decreased feed intake of birds during the last weeks and also whole period of the trial. Researchers found that plant extracts had wide range of activities on digestive tract, immune and endocrine systems of birds, and some especial plants have physio-pathological (anti-inflammation, anti-oxidant effect) and anti-microbial activities (Nasir & Grashorn, 2010a). The present results showed that adding EP extract to drinking water at the level of 1 or 2 mL $L^{-1}$ decreased BWG of quail as compared with the control group ($p < 0.05$) (Table 3). Landy et al. (2011) showed that broilers fed diet containing 5 g coneflower per kg diet continuously had higher BWG compared with all other treatments in the 14 and 28 days. Results of the present study indicated that adding EP extract to drinking water at the level of 0.5 mL $L^{-1}$ decreased FCR in quail (Table 4).

**Table 2.** Evaluation of *Echinacea purpurea* extract on feed intake (g) of Japanese quails.

| Treatments | 15-21 d | 22-28 d | 29-35 d | 36-42 | overall |
|---|---|---|---|---|---|
| 1 | $120.9^{a}$ | 125.5 | $130.1^{a}$ | $224.0^{a}$ | $600.6^{a}$ |
| 2 | $111.6^{ab}$ | 123.6 | $103.2^{b}$ | $182.5^{b}$ | $521.0^{b}$ |
| 3 | $113.3^{ab}$ | 125.4 | $102.4^{b}$ | $184.5^{b}$ | $525.7^{b}$ |
| 4 | $119.5^{a}$ | 111.9 | $98.4^{b}$ | $152.7^{c}$ | $482.5^{b}$ |
| 5 | $107.4^{b}$ | 117.5 | $92.0^{b}$ | $164.5^{bc}$ | $481.6^{b}$ |
| SEM | 3.010 | 6.31 | 5.00 | 9.34 | 17.88 |
| p-value | 0.033 | 0.491 | 0.0008 | 0.0009 | 0.0017 |

[a, b, c] Means within each column with no common superscript are significantly different ($p < 0.05$).

**Table 3.** Evaluation of *Echinacea purpurea* extract on body weight gain (g) of Japanese quails.

| Treatments | 15-21 d | 22-28 d | 29-35 d | 36-42 | overall |
|---|---|---|---|---|---|
| 1 | 47.9 | 56.9 | 31.1 | $26.3^{a}$ | $162.3^{a}$ |
| 2 | 46.5 | 52.6 | 32.2 | $22.1^{ab}$ | $153.5^{ab}$ |
| 3 | 45.2 | 57.6 | 26.9 | $31.3^{a}$ | $161.2^{a}$ |
| 4 | 47.5 | 55.8 | 26.1 | $14.3^{b}$ | $143.8^{b}$ |
| 5 | 45.5 | 51.2 | 24.7 | $23.3^{ab}$ | $144.9^{b}$ |
| SEM | 2.903 | 3.38 | 2.54 | 2.99 | 4.781 |
| p-value | 0.952 | 0.620 | 0.219 | 0.015 | 0.038 |

[a, b] Means within each column with no common superscript are significantly different ($p < 0.05$).

**Table 4.** Evaluation of *Echinacea purpurea* extract on feed conversion ratio (FCR) and European efficiency factor in Japanese quails.

| Treatments | 15-21 d | 22-28 d | 29-35 d | 36-42d | Overall (15-42 d) | European fficiency factor |
|---|---|---|---|---|---|---|
| 1 | 2.56 | 2.22 | 4.22 | $9.01^{ab}$ | $3.70^{a}$ | 200 |
| 2 | 2.42 | 2.34 | 3.32 | $9.07^{ab}$ | $3.39^{ab}$ | 207 |
| 3 | 2.53 | 2.20 | 3.94 | $5.91^{c}$ | $3.26^{b}$ | 228 |
| 4 | 2.52 | 2.06 | 3.78 | $10.75^{a}$ | $3.35^{ab}$ | 201 |
| 5 | 2.37 | 2.32 | 3.94 | $7.22b^{c}$ | $3.35^{ab}$ | 204 |
| SEM | 0.138 | 0.181 | 0.436 | 0.914 | 0.127 | 10 |
| p-value | 0.854 | 0.812 | 0.686 | 0.018 | 0.189 | 0.363 |

[a, b] Means within each column with no common superscript are significantly different ($p < 0.05$).

Contrary to our findings, Miraghaee et al. (2011) reported that there was no significant difference in feed intake, BWG, and feed conversion ratio of broiler chickens fed EP extract. Habibian Dehkordi et al. (2011) found that EP supplementation of broiler chicken diet reduced feed consumption and increased BWG of broilers, which indicated the beneficial effects of EP on feed intake and BWG of birds. European efficiency factor of quails consumed EP extract was not significantly different from the control group. The diversity in the effects of EP supplementation on birds' productive traits may be due to the procedure of extraction and conservation of EP product, which can affect the active compound in EP, as well as the different levels of EP in the water.

Plant materials are assumed to improve performance of the birds by stimulating the secretion of digestive enzymes which cause to better nutrient digestion and absorption. The presence of active ingredients and phenolic compounds can reduce numbers of intestinal pathogens, thus minimizing wasting the nutrients (Nasir & Grashorn, 2010b). The antibacterial, anti-oxidant, anti-inflammatory activity of herbal extract polyphenols have been reported previously ( Hajati, Hassanabadi, Golian, Nassiri-Moghaddam, & Nassiri, 2015a; 2015b; Gessner et al., 2013; Liu et al., 2016). Ebrahimi, Rahimi and Khaki (2015) reported that EP alcoholic extract in drinking water (1:1000 v $v^{-1}$) statistically increased the relative weight of the spleen and Bursa of Fabricius as lymphoid tissues. Nasir and Grashorn (2010b) examined the effects of EP in drinking water on growth performance, immunity and stress characteristics of broilers. Roth-Maier, Bohmer, Maaß, Damme and Paulicks (2005) found that supplementation with Echinacea cob, which has similar compounds including caffeic acid derivatives, decreased BW. Hassan, Askar and El-Shourbagy (2004) reported an improvement in FCR due to the dietary supplementation of herbal additives. Plants have a wide range of secondary metabolites, especially, isoprene derivatives and flavonoides with antioxidant characteristics, which have positive effect on gastrointestinal tract function (Shin, Lee, Hwang, Kim, & Suh, 1995).

The positive effect of growth promoting feed additives on livestock may due to stabilizing feed hygiene and beneficially modulating the gut ecosystem by controlling pathogens. Herbal derivatives have a number of active ingredients and pharmacologically active substances that are beneficial for maintaining health and enhancing performance of poultry. Previous studies found that phytobiotics may stimulate secretion of digestive enzymes (lipase and amylase) and intestinal mucous in broilers, feed digestion process, impair adhesion of pathogens and stabilize microbial balance in the gut (Lee et al., 2003). Adding EP extract to drinking water at the levels of 0.25, 0.5 or 1 mL $L^{-1}$ decreased carcass yield of quails compared to control group, but the relative weight of internal organs was not different (Table 5).

**Table 5.** Evaluation of *Echinacea purpurea* extract on relative weight of carcass and internal organs in japanese quails.

| Treatments | Carcass | Thighs | Breast | Liver | Heart | Gizzard | Spleen |
|---|---|---|---|---|---|---|---|
| 1 | $66.0^{a}$ | 16.0 | 26.0 | 1.96 | 0.86 | 1.64 | 0.04 |
| 2 | $62.7^{b}$ | 15.5 | 25.5 | 1.95 | 0.85 | 1.69 | 0.04 |
| 3 | $62.7^{b}$ | 15.6 | 25.3 | 2.23 | 0.94 | 1.66 | 0.04 |
| 4 | $63.2^{b}$ | 15.6 | 25.3 | 1.94 | 1.13 | 1.63 | 0.04 |
| 5 | $64.8^{ab}$ | 16.4 | 25.1 | 1.85 | 1.58 | 1.67 | 0.06 |
| SEM | 0.745 | 0.362 | 0.544 | 0.181 | 0.367 | 0.117 | 0.007 |
| p-value | 0.771 | 0.414 | 0.771 | 0.647 | 0.617 | 0.996 | 0.209 |

[a, b] Means within each column with no common superscript are significantly different ($p < 0.05$).

This is in agreement with the findings of Nasir and Grashorn (2010a) who reported that broilers supplemented with EP juice significantly decreased carcass percentage as compared to control group. Also, they found that liver percent was significantly lower in EP treated birds as compared to control birds Nasir and Grashorn (2010a). Rininger, Kickner, Chigurupati, McLean and Franck (2000) stated that EP has an effect similar interferon (IFN), activating macrophages and inducing the production of interleukin (IL)-1 and IFN. Results of EP extract on gut microflora population (CFU $mg^{-1}$) of Japanese quails are shown in Table 6.

**Table 6**. Evaluation of *Echinacea purpurea* extract on gut microflora (CFU $mg^{-1}$) of japanese quails.

| Treatments | Total aerobic bacteria | Coliforms | Clostridia | Lactobacilli |
|---|---|---|---|---|
| 1 | $6.30^{a}$ | $3.23^{b}$ | $2.68^{ab}$ | 4.31 |
| 2 | $6.04^{ab}$ | $5.03^{a}$ | $0.97^{b}$ | 4.23 |
| 3 | $6.16^{ab}$ | $5.56^{a}$ | $4.27^{a}$ | 4.79 |
| 4 | $5.59^{b}$ | $5.26^{a}$ | $2.03^{ab}$ | 4.21 |
| 5 | $5.89^{b}$ | $5.29^{a}$ | $1.96^{ab}$ | 4.49 |
| SEM | 0.087 | 0.575 | 0.870 | 0.347 |
| p-value | 0.030 | 0.048 | 0.046 | 0.751 |

[a, b] Means within each column with no common superscript are significantly different ($p < 0.05$).

Adding EP extract to drinking water at the levels of 1 or 2 mL $L^{-1}$ decreased total aerobic bacteria (Table 6). Different levels of EP extract decreased ileal *Coliforms* population of quails, but ileal *Clostridia* population of birds consumed EP extract was not different from control group. Guo et al. (2004a, 2004b, 2004c) found that plants derivatives promoted the growth performance, decreased the populations of coliforms and *C. perfringens*, and boosted both cellular and humoral immune responses of chickens infected with avian *Mycoplasma gallisepticum* or *Eimeria tenella*. Jamroz and Kamel (2002) reported that the dietary plant derivatives diminished *Escherichia coli* population comparing to the control birds. Phytogenics have shown to modulate the intestinal microflora composition via the reduction of *Coliforms* at 14 day of age and the beneficial fortification of gut microflora with beneficial bacteria such as the *Lactobacillus* and *Bifidobacterium* at 42 day of age (Mountzouris et al., 2011). Also it was reported that mixture of thymol and carvacrol increases the population of Lactobacillus in ileum (Akyurek & Yel, 2011). In conclusion, adding EP extract to drinking water of Japanese quail may improve FCR of quail and have positive effect on gut microflora population. It seems that further research is needed to clear the mechanisms of EP extract effectiveness in quails.

## Conclusion

*Echinacea purpurea* extract supplementation could therefore be used in quails diet to improve feed conversion ratio of quails; however, it decreased the carcass yield of the birds.

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