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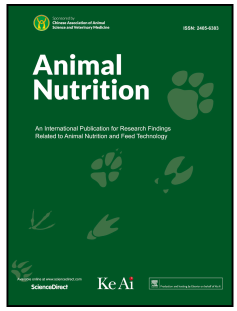

Effects of stinging nettle (*Urtica dioica*) powder on laying performance, egg quality, and serum biochemical parameters of Japanese quails

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Effects of stinging nettle (*Urtica dioica*) powder on laying performance, egg quality, and serum biochemical parameters of Japanese quails

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Abstract

The aim of this experiment was to evaluate the effects of dietary supplementation of stinging nettle powder (SNP) on laying performance, egg quality, and some selected serum biochemical parameters of quails. One hundred and forty-four 10-wk-old Japanese quails (initial body weight = 199 ± 18 g) were divided into 3 dietary treatment groups (basic diet without SNP [SNP0], SNP0 with 3% SNP [SNP3], SNP0 with 6% SNP [SNP6]) with 4 replicates of 12 quails for a rearing period of 12 wk. At 22 wk of age, the final body weights of the SNP3 and SNP6 groups were significantly ($P = 0.001$) reduced compared to that of the SNP0 group. Daily feed intake was not statistically different among the groups. The mean number of eggs laid ranged from 65 to 69 with laying rates from 76.8% to 82.1%. The percentage of cracked eggs was not significantly different among the groups and ranged from 1.6% to 1.9%. The egg weight was similar and the feed conversion ratio was closer among the groups. The egg yolk cholesterol, serum cholesterol and serum triglyceride levels in the SNP6 group were significantly reduced ($P < 0.001$) compared to those of the SNP0 group. Serum Ca, P and Mg were not significantly influenced by the supplementation. In conclusion, the results demonstrated that the supplementation of SNP to the quail diet at the level of 6% reduced quail egg yolk cholesterol, serum total cholesterol and serum triglyceride levels and did not negatively influence quail performance.

Keywords: Stinging nettle; Japanese quail; Performance; Egg quality; Serum parameters

1. Introduction

Antibiotic growth promoters were added in poultry diets to promote growth rate, feed conversion and reduce mortality through improved intestinal microbiota (Miles et al., 2006; Pfaller, 2006). The continuous use of antibiotics in diets could possibly lead to cross-resistance and development of antibiotic resistant bacteria in humans (Goodarzi et al., 2014; Nanekarani et al., 2012). This has brought the ban of antibiotics in poultry diets and exposed poultry producers to decrease the use of antibiotics and find potential alternatives in the poultry diet (Kadykalo et al., 2018). In food industry, phytogenic feed additives, most of which are medicinal plants in origin have been recognized by consumers as safe, non-antibiotic substances with potential alternatives to the antibiotics in poultry diets (Brenes and Roura, 2010; Castanon, 2007; Landy et al., 2011a, b; Toghyani et al., 2015; Varel, 2002). The phytogenic feed additives possess active components, the secondary metabolites of the medicinal plants, known as phytobiotics or botanicals in such a way that they have positive effects on animal performance and health (Ghazaghi et al., 2014; Windisch et al., 2008). Traditional medicinal herbs were the most acceptable as possible alternatives to antibiotic growth promoters because of their natural property, growth-promoting, and anti-oxidative effects (Liu et al., 2006; Park and Yoo, 1999). Thus, research efforts continued on a number of medicinal herbs to evaluate their potential alternatives to antibiotic growth promoters in poultry industry (e.g., Mehri et al., 2015; Kheiri et al., 2018; Yan et al., 2011; Mousavi et al., 2017). One of such medicinal herbs is a stinging nettle (*Urtica diocia*; Sharma et al., 2018), which to the knowledge of the authors, was evaluated in limited studies as phytogenic feed additive in poultry diets.

Stinging nettle is a wild, herbaceous, perennial flowering plant whose leaves and stems are covered by stinging hairs (trichomes) containing a fluid of formic acid and histamine, which causes blisters when entering the skin (Adhikari et al., 2016; Bisht et al., 2012; Bourgeois et al, 2016). Nettle is used in several fields such as agriculture, cosmetics, textiles, medicine and food (Adhikari et al., 2016; Bacci et al., 2009; Bourgeois et al., 2016; Łuczaj et al., 2012; Said et al., 2015; Uprety et al., 2012). Nettle was reported as a food with high nutritional and functional values (Rutto et al., 2013); nettle leaves are rich in proteins, fats, carbohydrates, vitamins, minerals and trace elements (Said et al., 2015). On dry matter basis, nettle contains 30% protein with a much better amino acid profile than other leafy plants. According to Rutto et al. (2013), 8 essential amino acids are necessary for the human body, including 6 largely available in stinging nettle (isoleucine, leucine, lysine, phenylalanine, threonine and valine), methionine and tryptophan. Nettle is also a good source of polyunsaturated fatty acids (60%) whose 50% corresponds to linoleic acid (C18:2), an omega-6 and it can strengthen the immune system, body resistance against bacterial or viral infections and antioxidant activity (Rutto et al., 2013). This is because nettle possesses antioxidant, antimicrobial, antifungal and antiviral properties (Rutto et al., 2013; Upton, 2013).

According to Hashemi et al. (2018), stinging nettle had a strong growth promoting ability and potential to impact the hemoglobin and hematocrit in broiler chickens. This indicates the functional and nutritional roles of the stinging nettle could be an alternative potential to evaluate its effects as supplementation in the poultry diet. The aim of this experiment was to evaluate the effects of feeding stinging nettle powder (SNP) supplemented diet on laying performance, egg quality, and some selected serum biochemical parameters of quails.

2. Materials and methods

2.1 Ethical considerations

The ethical procedures, experimentation and housing were carried out according to guidelines of the Ethics Committee of Liege University (Belgium) for the humane care and use of animals in research. There is no need for explicit approval of the Ethical Committee for such an experiment according to the Decree of 29 May 2013. The quails were fed 3 different (non-toxic) diets, subjected to no invasive procedures and there was no induction of pain or suffering during the trial or sampling.

2.2 Animals and experimental set up

This experiment was carried out for a period of 12 wk in a private farm located in Chemini, Bejaia, Algeria. One hundred and forty-four 10-wk-old female Japanese quails (*Coturnix coturnix japonica*) with an average body weight of (199 ± 18 g) were divided into 3 treatments (4 replicates per treatment, 12 birds per replicate) in a complete randomized design. The quails were housed in floor pens (60 cm × 60 cm × 50 cm), enriched with wood shavings. The building was ventilated and temperatures ranged from 16 to 28 °C and the mean was between 18 and 22 °C. The quails were housed under identical conditions and provided lighting 16 h per day using fluorescent lights controlled by timers until the end of the experiment. Quails are important species for the production of meat and eggs due to their rapid growth, early sexual maturity (start laying eggs at 6 to 8 wk), production rate (180 to

300 eggs per year) and their short incubation period (Moula et al., 2014; Reddish et al., 2003).

2.3 Experimental diets and treatments

The stinging nettle leaves were collected from field, near the private farm located in Chemini, Bejaia (Algeria). The collected samples were air-dried under shade, crushed to coarse using mortar and pestle and transformed to a powder using a small feed mill grinder. The basic control diet was analyzed for nutrient composition at Laboratory of Forage Analysis (Department of Agricultural and Environmental Analyses, Province of Liege, Belgium). The parameters measured were dry matter (DM), crude protein (CP), crude fiber (CF), ash, Ca and P (Table 1). The diet was analyzed according to AOAC Official Methods (Horwitz, 2006): DM, by drying the samples at 105 °C for 16 h (AOAC 930.15); ash, by combustion in a muffle furnace at 550 °C for 6 h (AOAC 990.03); CP, as total nitrogen (N) using the Kjeldahl method (N × 6.25) (AOAC 990.03); and CF was determined using the AOAC Methods (Method 962.09 and 985.29, 1995). There were 3 dietary treatments: 1) basic diet without SNP (SNP0), 2) basic diet with 3% SNP (SNP3), and 3) basic diet with 6% SNP (SNP6) (Table 1). The formulation of the basic diet was adopted according to the nutrient requirements of poultry (NRC, 1994). The diets contained no growth promoters or antibiotics. Diet and water were provided *ad libitum* throughout the 12 wk of the experiment.

2.4 Laying performance and egg quality

The quails were weighed at the beginning the end of the experiment. Feed intake was recorded per pen on a weekly basis, corrected for mortality that was recorded on a daily basis. The quails were daily monitored throughout the experiment: eggs were collected, counted, weighed and checked for integrity. A total of 288 eggs (8 eggs per test period and per pen; 32 eggs per experimental group) were harvested from 14 to 22 wk of age with an interval of 4 wk in between and evaluated for physical quality parameters as previously described (Moula et al., 2010, 2014; Zita et al., 2013). For the determination of egg yolk cholesterol level, 4 eggs were collected per pen, hard-boiled for 15 min, cooled and stored at 2 to 8 °C. For the egg yolk cholesterol analysis, the yolks were separated and yolk lipids were extracted from 0.1 g yolk with 4 mL of isopropanol, vortexed, centrifuged at 1,300 × *g* for 10 min and filtered. The filtered samples were spectrophotometrically (Schimadzu model

1208 spectrophotometer) analyzed using a commercial kit (Biosystems, Spain) and egg yolk cholesterol was determined. Finally, the cholesterol content was calculated according to Boehringer (1989).

2.5 Serum biochemical parameters

At the end of the 12-wk period of the experiment, the quails were starved overnight, and blood samples were taken from 48 quails (16 per treatment or 4 per replicate). Serum was separated by centrifugation at 1,300 $\times$ g for 15 min, incubated for 1 h at room temperature, stored at –20 °C and analyzed in less than a week. Serum levels of total cholesterol, triglycerides (Biosystems, Spain), Ca, P and Mg (Chema Diagnostica, Italy) concentrations were measured by a spectrophotometer (Schimadzu, model 1208) using commercial colorimetric diagnostic kits.

2.6 Statistical analysis

Statistical analysis was performed using SAS for Windows (version 9.4; SAS Institute). The pen was the experimental unit for growth and laying performances. For egg quality parameters and serum biochemical parameters, the corresponding average values were used as experimental units. All data were checked for normality and outliers of the residuals. Normality of sample distribution was assessed and confirmed with the Kolmogorov-Smirnov test. Effects of age (14, 18 and 22 wk), diet (SNP0, SNP3 and SNP6) and age-diet interactions on egg quality parameters were statistically analyzed as a 2 × 2 factorial design using the General Linear Model (GLM) procedure of SAS, as indicated below:

$$Y_{ijk} = \mu + A_i + D_j + (AD)_{ij} + e_{ijk},$$

where μ is the overall mean; A_i is the *i*th main effect of age (*i* = 14, 18, 22); D_j is the *j*th main effect of diet (*j* = SNP0, SNP3, SNP6); $(AD)_{ij}$ is the *ij*th interaction effect between age and diet; and e_{ijk} is the random error. Effects of diets on growth performance, egg production and serum biochemical parameters were subjected to GLM Univariate ANOVA. When there was a statistically significant effect (an interaction for egg quality parameters) between treatments, a Tukey post-hoc test was used to determine the significantly differed treatments. The chi-square test was used to compare the percentage of broken eggs between different groups. Results are reported as means and standard error of the means (SEM). Statistical significance was accepted at $P < 0.05$.

3. Results

3.1 Laying performance

The mean initial body weights of the 10-wk-old quails were similar among the 3 dietary treatment groups at the beginning of the experiment (Table 2). However, the mean final body weights of the quails fed the SNP3 and SNP6 were significantly (*P* = 0.001) reduced, compared to that of the SNP0 group at the end of the rearing period of 12 wk. Daily feed intake was not statistically different among the 3 groups, but in total each quail consumed an average of 3,600, 3,598 and 3,573 g for SNP0, SNP3 and SNP6 groups, respectively. The mortality was 6.25% for the SNP0 group, 8.33% for the SNP3 group and 4.17% for the SNP6 group, but it was not significantly affected by the SNP supplementation. Laying performances were not significantly different among the 3 groups. The mean number of eggs laid during the experimental period ranged from 65 to 69 eggs with laying rates from 76.8% to 82.1%. The percentage of cracked eggs was not significantly different and ranged from 1.6% to 1.9% depending on the group. The mean egg weight was similar among the 3 groups and the overall mean was 12.4 g. The feed conversion ratio was 3.43, 3.47 and 3.48 for the SNP0, SNP3 and SNP6 groups, respectively.

3.2 Egg quality

The effects of SNP on egg quality parameters of Japanese quail (14, 18 and 22 wk of age) are reported in Table 3. Remarkably, egg yolk cholesterol of quails was significantly affected by age (*P* < 0.001), diet (*P* < 0.001) and the age-diet interaction (*P* < 0.001). The eggs in the SNP6 group had less cholesterol levels than those of the SNP0 and SNP3 groups by 5.40% and 2.63%, respectively.

The effects of SNP on some selected serum biochemical parameters of 22-wk-old Japanese quails are shown in Table 4. The SNP supplementation significantly (*P* < 0.001) reduced serum concentrations of total cholesterol and triglycerides. The supplementation of SNP at 6% in diet decreased (*P* < 0.001) both total cholesterol and triglycerides, but SNP at 3% reduced (*P* < 0.001) total triglyceride without affecting cholesterol. Serum Ca, P and Mg were not significantly influenced by the diet.

4. Discussion

In the fastest and intensively growing global poultry industry, layers experience reduced performance and egg quality, possibly due to increasing nutrient metabolism during egg formation or weakened immune systems, shifting the function of dietary nutrients to health-maintenance. For instance, egg laying birds have chronic hyperglycemic and they require high amounts of lipid synthesis during egg formation (Pál et al., 2002), suggesting evaluation of potential feed ingredients and development of target functional nutrients.

In this experiment of the 12-wk period, a quail was laying a mean of 67 eggs, which corresponds to a mean laying rate of 79.7%. This rate was higher than previously reported (Garwood and Diehl, 1987; Moula et al., 2014; Schwartz and Allen, 1981), in which the annual laying rate of a quail ranged from 49% to 74.4%. In this study, only the egg weight but no other parameters of the egg physical quality were significantly affected by age of the quails. In consistent with this, Moula et al. (2014) and Zita et al. (2013) studied in quails and reported a significant effect of age on the weight of the whole egg. The present and previous studies confirm the linear relationship between the weight of whole egg and age of quails. Although the stinging nettle had a growth promoting ability (e.g., Hashemi et al., 2018), the reduction in the final body weights of quails observed in the present study could attribute to other factors. Chowdhury et al. (2002) and Reddy et al. (1991) reported that egg production, egg weight, feed intake and feed conversion ratio were not affected by diets. Mortality was not different between the 3 groups and the rates obtained were lower than reported in other studies (Moula et al., 2014; Yalçin et al., 2007) ranging from 10% to 16%. The reasons for high mortality remain unclear and further analysis including post mortem examinations will be necessary.

In the literatures, egg quality parameters can be classified into 2 main groups: external (egg weight and shell thickness) and internal (albumin index, yolk index and Haugh unit) traits (Song et al., 2000; Toussant and Latshaw, 1999; Wolanski et al., 2007). The external egg qualities are important for acceptability by consumers while the interior qualities are basic parameters for the egg production industry. The present study showed that the effect of SNP was limited on the egg quality parameters, but remarkably and significantly reduced the concentration of egg yolk cholesterol. The present finding is in agreement with nettle supplementation of layer diets that showed no considerable effect on general egg quality (Loetscher et al., 2013).

Eggs are a major source of dietary cholesterol, which may cause coronary heart disease (Bragagnolo and Rodriguez-Amaya, 2003), retinal abnormalities, and lipid peroxidation that improves lipid profile (Fernández-Robredo et al., 2008). To reduce the cholesterol and lipid profile and to enhance the nutritional value of egg, several studies have been conducted in the laying hens (Carrillo-Domìnguez et al., 2005; Chen et al., 2005; Milinsk et al., 2003; Raes et al., 2002; Zotte et al., 2006). With similar focus, in the present study, the reduced levels of the total cholesterol and triglycerides showed the potential effects of the stinging nettle to regulate these blood metabolites in quails. The present study is in consistent with the findings of Mansoub (2011) in which 2% nettle powder supplementation to the diet of laying hens reduced the total cholesterol and triglycerides, but in contrast to the limited effects reported by Keshavarz et al. (2014).

The present findings showed that the SNP in the quail diet improved egg quality and serum biochemical parameters without negative effect on laying performance. The SNP contains phytosterol (Di Virgilio et al., 2015), which has low density lipid cholesterol lowering effects. Therefore, the SNP could be a potential ingredient to the quail diet with its positive effect of reducing the concentration of egg yolk cholesterol, serum total cholesterol, and serum triglycerides.

5. Conclusions

The results demonstrated that the supplementation of SNP to the quail diet at the level of 6% reduced concentrations of quail egg yolk cholesterol, serum total cholesterol, and serum triglycerides and did not negatively influence quail performance. The results also suggest that the SNP could be added to the diets of quails to improve quail egg quality and serum biochemical profile.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1

Dietary ingredients, nutrient composition and metabolizable energy of the experimental diet (%)

Item	SNP0	SNP3	SNP6
Ingredients			
Corn	50.00	50.00	50.00
Wheat	12.00	12.00	12.00
Soybean meal	32.00	32.00	32.00
Vegetable oil	2.00	2.00	2.00
Limestone	1.55	1.55	1.55
Dicalciumphosphate	0.50	0.50	0.50
Salt	0.50	0.50	0.50
Methionine	0.50	0.50	0.50
Lysine	0.20	0.20	0.20
Mineral-vitamin premix[1]	0.75	0.75	0.75
Stinging nettle powder (SNP)	0	0.03	0.06
Calculated nutrient composition			
Dry matter	90.60	90.60	90.60
Crude protein	19.00	19.00	19.00
Crude fibre	3.90	3.90	3.90
Crude ash	2.35	2.35	2.35
Calcium	3.00	3.00	3.00
Phosphorus	0.6	0.6	0.6
Metabolizable energy, MJ/kg	11.3	11.3	11.3

SNP0 = basic diet without SNP; SNP3 = SNP0 with 3% SNP; SNP6 = SNP0 with 6% SNP.
[1] Provided per kg of diet: Mn from $MnSO_4.H_2O$ 80 mg; Fe from $FeSO_4.7H_2O$ 30 mg; Zn from ZnO 60 mg; Cu from $CuSO_4.5H_2O$ 5 mg; Co from Co_2O_3 0.5 mg; I from Ca $(IO_3)_2.H_2O$ 0.2 mg; vitamin A 6,000 IU; vitamin D3 600 IU; vitamin E 20 IU; vitamin K 2 mg; vitamin B1 2 mg; vitamin B2 2.4 mg; vitamin B6 2 mg; vitamin B12 1.2 mg; niacin 19 mg; folic acid 3 mg; pantothenic acid 14 mg; biotin 0.50 mg.

Table 2

Effect of stinging nettle powder on laying performance of Japanese quails from 10 to 22 wk of age

Item	SNP0	SNP3	SNP6	SEM	*P*-value
Initial body weight, g	195.90	199.35	202.31	1.54	0.234
Final body weight, g	245.29[a]	235.77[b]	231.31[b]	1.61	0.001
Feed intake, g/d	42.86	42.83	42.54	0.49	0.964
Egg production, n	67.00	69.00	65.00	1.12	0.275
Egg production, %	80.06	82.14	76.79	1.33	0.275
Cracked eggs, %	1.85	1.82	1.57	0.31	0.933
Egg weight, g	12.49	12.34	12.23	0.11	0.653
FCR, g diet/g egg	3.43	3.47	3.48	0.04	0.870

SNP0 = basic diet without SNP; SNP3 = SNP0 with 3% SNP; SNP6 = SNP0 with 6% SNP; FCR = feed conversion ratio; [a, b] Within a row, mean values with different letters were significantly different ($P<0.05$).

Table 3

Effect of age (A), stinging nettle powder (diet, D), and age-diet interaction (A×D) on egg quality parameters in Japanese quails

Item	Age (wk)	Diet			SEM	*P*-value		
		SNP0	SNP3	SNP6		A	D	A×D
Egg weight, g	14	12.25	12.37	12.19	0.09	<0.001	0.372	0.450
	18	12.50	12.81	12.50	0.10			
	22	12.97	12.78	12.78	0.06			
	Mean	12.67	12.69	12.56	0.05			
Shell thickness (0.01 mm)	14	18.90	19.21	19.24	0.11	0.636	0.037	1.000
	18	18.75	19.06	19.09	0.11			
	22	18.82	19.13	19.17	0.08			
	Mean	18.83^{a}	19.13^{b}	19.17^{ab}	0.10			
Albumen index, %	14	5.44	5.63	6.00	0.11	0.523	0.783	0.092
	18	6.06	5.75	5.69	0.11			
	22	5.72	5.94	5.81	0.08			
	Mean	5.73	5.81	5.83	0.05			
Yolk index, %	14	43.68	43.13	43.29	0.50	0.789	0.883	0.990
	18	43.15	43.08	43.26	0.30			
	22	43.47	43.41	43.51	0.20			
	Mean	43.44	43.26	43.39	0.18			
Haugh, unit	14	83.00	82.56	82.56	1.30	0.140	0.826	0.967
	18	80.50	80.75	82.12	1.02			
	22	80.47	78.75	80.19	0.85			
	Mean	81.11	80.20	81.27	0.60			
EYC, mg/g	14	14.67^{a}	14.90^{a}	13.21^{b}	0.12	<0.001	<0.001	<0.001
	18	14.42^{a}	12.61^{b}	14.47^{c}	0.14			
	22	12.86^{a}	12.86^{a}	12.07^{b}	0.07			
	Mean	13.70^{a}	13.31^{b}	12.96^{c}	0.08			

SNP0 = basic diet without SNP; SNP3 = SNP0 with 3% SNP; SNP6 = SNP0 with 6% SNP; Albumen index = albumen height/ [albumen length + albumen width] × 100; Yolk index = yolk height/ yolk diameter × 100; Haugh unit = log (albumen height + 7.57-1.7 × egg weight$^{0.37}$) × 100; EYC = egg yolk cholesterol; $^{a, b, c}$ Within a row, mean values with different letters were significantly different (P<0.05).

Table 4

Effect of stinging nettle powder on some selected serum biochemical parameters of Japanese quails at 22-wk of age (mg/dL)

Item	SNP0	SNP3	SNP6	SEM	*P*-value
Calcium	23.64	23.52	23.33	0.21	0.829
Phosphorus	13.45	13.52	13.33	0.20	0.927
Magnesium	3.45	3.52	3.33	0.20	0.927
Total cholesterol	108.44[a]	105.50[a]	95.13[b]	1.35	<0.001
Triglycerides	867.5[a]	738.5[b]	380.5[c]	30.89	<0.001

SNP0 = basic diet without SNP; SNP3 = SNP0 with 3% SNP; SNP6 = SNP0 with 6% SNP; [a, b, c] Within a row, mean values with different letters were significantly different (*P*<0.05).

Conflict of interest

The authors declare that they have no conflict of interest.

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