



Effect of Adding Broccoli Leaves (*Brassica oleracea* L. var. *italica*) Extract to Drinking Water on Eggs Production and Intestinal Microflora of Japanese Quail *Coturnix japonica* Temmink & Schlegel, 1849

Duha S.A. Al-Ashoor & Khalid C.K. Al-Salhie*

Department of Animal Production, College of Agriculture, University of Basrah, Iraq

*Corresponding author: Knnz1977@yahoo.com

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Abstract: This study was carried out to determine the effects of adding broccoli leaves extract to drinking water on eggs production and intestinal microflora (total bacteria, lactobacilli and *Escherichia coli*) of breeder Japanese quails. One hundred and thirty-two 45 days-old of Japanese quails (males and females) were randomly distributed into four groups. Each group has three replicates. The groups were included: The first group was kept without any addition as the control. The extraction of broccoli leaves as 100, 200 and 300 mg. l⁻¹ were added to bird's drinking water of the second, third and fourth groups respectively. The results showed a significant improvement in eggs production (HD %), accumulative eggs per 30 days and eggs mass in the third and fourth groups compared to the other groups. While, no significant differences were shown in the feed consumption, feed conversion ratio and eggs weight among groups. Caecal and duodenum *E. coli* showed a significant decrease in third and fourth groups compared to the others. However, duodenum lactobacilli increased significantly in the fourth group compared to other groups. It was also showed no significant differences in the total duodenum bacteria among studied groups. While, the total caecal bacteria decrease significantly in the third group compared to other groups. Based on the presented results can be concluded that alcoholic extract of broccoli leaves at levels 200 and 300 mg. l⁻¹ could enhance productive characteristics (eggs production HD %, accumulative eggs per 30 days and eggs mass). On the other hand, it increased duodenum lactobacilli and decreased caecal and duodenal *E. coli* population of breeder Japanese quails.

Keywords: Broccoli leaves, Eggs production, Intestinal microflora, Quail.

Introduction

During the past few years, Broccoli (*Brassica oleracea* L. var. *italica*) has gained a great deal of attention with the aid of healthier consumers. It is a highly nutritious herb, rich in vitamins and minerals, bioactive phytochemicals (glucosinolates, phenolic compounds and

flavonoids) and antioxidants (Dominguez-Perles *et al.*, 2010). Broccoli is also a rich source of indol-3-carbinol (Li *et al.*, 2017). These constituents presented in broccoli are considered to be very common because they have many antioxidants properties and benefits

(Ravikumar, 2015). Oleic and linoleic acids prevailed in the sprouts of broccoli, while the flowers contained caproic, stearic and oleic acids (Paško *et al.*, 2018). Broccoli leaves are also considered as a good source of calcium and manganese compared to other tissues and it has the highest myrosinase activity (Liu *et al.*, 2018a). Broccoli leaves are contained beta-carotene and other healthy phytonutrients (Farahmandi *et al.*, 2013). Fractional and chemical analysis of different broccoli parts showed that florets recorded the highest levels of crude protein (22.4%) and amino acid (18.6%) but the lowest levels of crude fibre (11.7%) compared to broccoli leaves and stem (Campas-Baypoli *et al.*, 2009). A previous study on the layer hens, the use of dried broccoli leaves and stems up to 9 % of the diet did not affect eggs production, but improved eggs quality with increased xanthophyll yolk and reduced yolk cholesterol levels (Hu *et al.*, 2011). Mueller *et al.* (2012) suggested an extract of broccoli sprouts containing sulforaphane (SFN) as a phytochemical feed additive in animal nutrition and it did not appear a negative effect on performance parameters of broiler chickens. Liu *et al.* (2018b) studied the effect of fermented broccoli on harmful bacteria in broiler meat, and they found that fermented broccoli decreased *Salmonella*, *Campylobacter*, *Clostridium perfringens*, *Escherichia coli* and *Campylobacter* Gram-negative bacteria.

Therefore, the results of previous studies on broccoli extract encouraged us to study the effect of adding leaves extract to drinking water on eggs production and intestinal microflora (Lactobacilli, *E. coli* and total bacteria count) of Japanese quail.

Material & Methods

This study was conducted for the period from 29 October 2019 to 12 December 2019 at Quail farm, College of Agriculture, University of Basrah. One hundred and thirty-two, 45 days-old of Japanese quail (males and females) were randomly distributed into four groups (33 birds for each). Each group has three replicates (11 birds for each). All birds reared in cages (replicates) with dimensions (100 × 51 × 49) cm. The birds were housed the same rearing methods. Room temperature was kept up at 25°C from the beginning of the study till its end. The groups were included: The first group was kept without any addition as the control. The extraction of broccoli leaves as 100, 200 and 300 mg.l⁻¹ were added to bird's drinking water of the second, third and fourth groups respectively. Chemical analysis of broccoli leaves powder (Table 1) was carried out according to AOAC (2016).

Table (1): Chemical analysis of broccoli leaves powder (% on a dry weight basis).

Component (%)	broccoli leaves powder%
Dry matter	98.6
Crude protein	12.24
Crude fat	4.3
Ash content	4.66
Crude fibre	23.2
Available carbohydrate	55.6
Organic matter	95.34

Table (2): Ingredients and nutrient composition of birds diet.

Ingredient %	%
Yellow corn	40.5
Wheat	24
Soybean meal (44%)	25
protein concentrates ¹ (44%)	4
Vegetable oil	0.5
Limestone	3
Dicalicum phosphate	2
*Premix	1
Total	100
Calculated composition	
Metabolizable energy (kcal.kg ⁻¹)	2900
Crude protein (%)	20.00
Crude fat (%)	3.93
Crude fibre (%)	3.49
Calorie: protein ratio	145
Calcium (%)	2.31
Phosphorus available (%)	0.46
Lysine (%)	0.38
Methionine (%)	1.06
Methionine + Cysteine (%)	0.83

¹Protein concentrate used from Al-Hayat Company, Jordanian Origin, to provide the following per kg of diet: 44% protein, 2800 kcal.kg⁻¹ME, 12% fat, 25% ash, 5% calcium, 2.9% phosphorus, 2.55% methionine + Cysteine, 2.8% lysine. *Premix contents: vitamins in amounts per kg diet: vit.A: 2500 IU, vit.D3: 5000IU, vit.E: 75mg, vit.K: 3mg, vit B1: 3 mg, vit B2: 8 mg, vit B6: 5 mg, vit B12: 0.016 mg, folic acid: 2mg, biotin: 0.20 mg, pantothenic acid: 13mg, Nicotinic acid :55 mg, Choline chloride 1600mg. Mineral composition (mg kg diet): Cooper :16 mg, Iodin:1.25mg, Iron:40mg, Manganese:120 mg, Selenium: 30mg, Zinc 100mg.

All birds were fed the same experimental diet. The experimental diet (Table 2) was containing an approximately 20% crude protein and 2900 kcal.kg⁻¹ metabolizable energy (NRC, 1994). The birds were given *ad libitum* access to food and water. The birds were housed in cages and the same rearing methods.

Alcoholic extract of broccoli leaves Preparation

The broccoli leaves were carefully washed and dried at a temperature of 45 °C. The dried leaves were ground to a thick powder shape. Fifty grams of the powder was blended into 250 ml of ethanol in a clean beaker. The beaker was covered in a water bath (37 °C). After 24 hours, the mixture was blended for one hour with a magnetic stirrer. Then, after drying, scrape it off and put it in the refrigerator for further use (Anessiny & Perez, 1993).

Eggs parameters

The eggs parameters including the accumulative number of eggs, an average of eggs weight, eggs mass and hen day production (HD%) were determined according to (Younis, 2014) after 30 days from study begins as follows:

$$HD\% = \frac{\text{No. of eggs for 30 days}}{\text{No. of hens} \times 30 \text{ days}} \times 100$$

$$\text{Accumulative number of eggs} = \frac{HD\%}{100} \times \text{No. of days}$$

$$\text{Eggs mass} = \text{accumulative number of eggs} \times \text{eggs weight}$$

Feed intake and feed conversion ratio

Feed intake was recorded daily. Feed conversion ratio was calculated by dividing the feed intake by eggs mass. it were calculated according to Zduńczyk *et al.* (2013).

Microbiological analysis

After 45 days from study begins, fresh caecal and duodenum samples from euthanized (three females of treatment) were diluted 10-fold by weight in buffered peptone water. Samples were mechanically homogenized at room temperature and used to enumerate lactobacilli, *E. coli* and total bacteria. All microbiological analyses were performed in three duplicates and the average values were used for statistical analysis. Lactobacilli were enumerated using MRS agar and incubated anaerobically at 37 C° for 48 h. MacConkey's agar was used to quantify *E. coli* after 24 hours of culture at 37 °C. Total bacteria were identified using a nutrient agar and incubated at 37 °C for 24 h. After the incubation periods, colonies of the respective bacteria were counted according to Harrigan & McCance (1978).

Statistical analysis

A one-way variance analysis (ANOVA) was applied to analyse data. L.S.D was used to compare among means at the level of 0.05 (SPSS, 2016). The experiment carried out with triplicates.

Results & Discussion

The results presented in table (3) showed a significant improvement (P<0.05) in eggs production (HD%), the accumulative number of eggs per 30 days and eggs mass in the third and fourth groups compared to the first and second groups. While, the results showed no significant differences in the feed consumption, feed conversion ratio and eggs weight among groups. Medicinal plants or their extracts are used to stimulate growth and improve the physiological and reproductive parameters of birds (Al-Salhi & Al-Waeli, 2019 ; Sultan *et*

al., 2019; 2020; Al-Salhie & Makki, 2020). These findings may be due to the increased activity of broccoli compounds and phytochemicals that promote general health (Vallejo *et al.*, 2004). Rana (2008)

indicated that broccoli is rich in important vitamins (A and C) and minerals as calcium, iron, phosphorous, potassium and sodium as well as, it contain proteins, carbohydrates, that promote general health.

Table (3): Effect of adding broccoli leaves extract to drinking water on some productive parameters of Japanese quail birds (Mean± SE).

Groups Parameters	G1	G2	G3	G4
Accumulative number of eggs. 30 days ⁻¹	25.53 ^b ±0.18	25.42 ^b ±0.08	27.42 ^a ±0.08	27.77 ^a ±0.21
Eggs weight (g)	11.09 ^a ±0.78	11.08 ^a ±0.12	11.34 ^a ±0.07	11.54 ^a ±0.21
Hen day production(HD)%	85.11 ^b ±0.60	84.76 ^b ±0.27	91.42 ^a ±0.27	92.57 ^a ±0.72
Eggs mass (g)	283.12 ^b ±1.35	281.88 ^b ±3.84	311.20 ^a ±2.89	320.48 ^a ±3.11
Feed consumption(g)	271.28 ^a ±7.45	271.53 ^a ±4.14	276.98 ^a ±12.05	277.86 ^a ±4.91
Feed Conversion ratio (g.g ⁻¹)	0.97 ^a ±0.02	0.97 ^a ±0.03	0.88 ^a ±0.04	0.86 ^a ±0.01

^{a, b}: Means in the same row with different letters show significant differences (P<0.05).

Broccoli contains vitamin C as it protects cells against oxidative stress caused by free radicals (Gliszczynska-Swiglo *et al.*, 2006; Munyaka *et al.*, 2010). The exact mechanism underlying the effects of broccoli leaves extract on improving eggs production is still uncertain and may be due to its amino acid contents in the broccoli leaves (Campas-Baypoli *et al.*, 2009). Broccoli leaves contained high levels of carotenoids and antioxidants (Wu *et al.* 1992).

Nimalaratne *et al.* (2012) indicated that broccoli leaves contain carotenoids, as they can be used in poultry diets to obtain a dark-coloured eggs yolk. Such findings were compatible with Mustafa & Baurhoo (2018) who clarified that the feeding of dried broccoli floret to layers up to 120 g^{kg} of diet had no negative effects on feed intake, feed conversion ratio and eggs production. Hu *et al.* (2011) indicated that when adding dried broccoli

leaves powder at different levels 0, 30, 60 and 90 g.kg⁻¹ feed of laying hens diets, this did not significantly affect productive performance. In previous study on broiler chicken, no significant differences were observed when adding broccoli leaves and stems at levels that reached 12% of the diet. The addition had no significant effect on performance and growth, while the quality of breast meat improved significantly (Hu *et al.*, 2012).

Table (4) revealed the data of caecal and duodenum microflora. Statistical analysis showed a significant decrease (P<0.05) in

caecal and duodenum *E. coli* in third and fourth groups compared with the other studied groups. However, the highest significant increase (P<0.05) of duodenum lactobacilli was recorded in the fourth group. The total duodenum bacteria showed non-significant differences among groups. While There was a significant decrease in total caecal bacteria in the third group compared to other groups. It may be due to the role of active substances in broccoli leaf extract in preventing intestinal abrasion and preventing the survival of harmful microorganisms in the intestines of the digestive system (Allahghadri *et al.*, 2010).

Table (4): Effect of broccoli leaves extract to drinking water on caecal and duodenum microflora of Japanese quail (Mean± SE).

Parameters	Groups	G1	G2	G3	G4
	Intestinal parts				
Total bacteria log CFU ^g	duodenum	5.88 ^a ±0.15	6.19 ^a ±0.12	6.05 ^a ±0.06	6.22 ^a ±0.11
	caecum	6.54 ^{ab} ±0.17	6.80 ^a ±0.14	6.21 ^b ±0.14	6.83 ^a ±0.11
E-Coli bacteria log CFU ^g	duodenum	2.44 ^a ±0.05	2.27 ^a ±0.08	1.45 ^b ±0.04	1.32 ^b ±0.05
	caecum	2.66 ^a ±0.06	2.31 ^a ±0.13	1.74 ^b ±0.13	1.64 ^b ±0.09
Lactobacilli bacteria log CFU ^g	duodenum	4.54 ^d ±0.03	5.03 ^c ±0.04	5.22 ^b ±0.05	5.83 ^a ±0.07
	caecum	5.15 ^b ±0.08	5.26 ^b ±0.04	5.10 ^b ±0.03	5.65 ^a ±0.04

a, b, c, d Means in the same row with different letters show significant differences (P<0.05).

On the other hand, these findings may be due to herbs additives working to promote the growth of beneficial bacteria in the intestine, such as *Lactobacillus* bacteria, and to reduce harmful bacteria. Also, herbs can improve the stimulation of immune body system as they enhancing the nutrient absorption rate by improving villi growth and improving the activity of the digestive enzymes (Rahimi *et al.*, 2011). Such findings were compatible with Liu *et al.* (2018b) who indicated that adding fermented broccoli to the diets by 5% and 10% reduced the account of *E. coli* bacteria, Gram-negative bacteria, *Salmonella* and *C. perfringens* in caecal broiler.

Conclusions

It is concluded that alcoholic extract of broccoli leaves at levels 200 and 300 mg.¹ have the potential to enhance productive characteristics (eggs production HD %, accumulative eggs per 30 days and eggs mass). On the other hand, it increased duodenum lactobacilli and decreased caecal and duodenal *E. coli* population of breeder Japanese quails.

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Conflict to interest

There is no conflict of interest.

Ethical approval

All applicable institutional, national and international guidelines for the care and use of animals were followed.

ORCID: **Khalid C.K. Al-Salhie**
<https://orcid.org/0000-0003-1121-7056>

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تأثير اضافة مستخلص اوراق البروكلي *Brassica oleracea L. var. italica* لمياه الشرب في انتاجية

البيض والفلورا المعوية لطيور السمان الياباني *Coturnix japonica*

ضحى صالح عبار العاشور و خالد جلاب كريدي الصالحي

قسم الانتاج الحيواني، كلية الزراعة، جامعة البصرة، العراق

المستخلص: أجريت الدراسة الحالية لمعرفة تأثير إضافة مستخلص أوراق البروكلي لمياه الشرب في إنتاجية البيض والفلورا المعوية (البكتريا الكلية ، بكتريا العصيات اللبنية وبكتريا القولون *E. coli*) لطيور السمان الياباني. استخدم مائة واثنان وثلاثون طيراً بعمر 45 يوماً من السمان الياباني (ذكور واناث)، ووزعت عشوائياً على أربع مجاميع (33 طائراً لكل منها)، بواقع ثلاث مكررات لكل مجموعة (11 طائر لكل منها). وكانت المجاميع كالاتي: المجموعة الأولى دون أي إضافة وعُدت كمجموعة سيطرة ، واضيف مستخلص أوراق البروكلي بتركيز 100 و 200 و 300 ملغم لكل لتر من مياه الشرب لطيور المجاميع الثانية والثالثة والرابعة على التوالي. أظهرت النتائج تحسناً معنوياً ($P < 0.05$) في نسبة إنتاج البيض (HD%)، وعدد البيض التراكمي وكتلة البيض بعد 30 يوماً من المعاملة في المجموعتين الثالثة والرابعة مقارنة بالمجاميع الأخرى. بينما لم تظهر فروق معنوية ($P < 0.05$) في كمية العلف المستهلك وكفاءة التحويل الغذائي ووزن البيض بين مجاميع الدراسة المختلفة. أظهرت النتائج انخفاض معنوي ($P < 0.05$) في بكتريا القولون في كل من الاثني عشر والاعورين في المجموعتين الثالثة والرابعة مقارنة بالمجاميع الأخرى، بينما ارتفعت بكتريا العصيات اللبنية في الاثني عشر معنوياً ($P < 0.05$) في المجموعة الرابعة مقارنة بالمجاميع الأخرى. لم تظهر فروق معنوية في البكتريا الكلية في الاثني عشر بين مجاميع الدراسة المختلفة، بينما انخفضت البكتريا الكلية في الاعورين معنوياً ($P < 0.05$) في المجموعة الثالثة مقارنة بالمجاميع الأخرى. استناداً إلى النتائج ، يمكن الاستنتاج بأن المستخلص الكحولي لأوراق البروكلي بمستويات 200 و 300 ملغم لكل لتر يمكن أن يرفع من الصفات الإنتاجية (إنتاج البيض HD%)، وعدد البيض التراكمي وكتلة البيض). من ناحية أخرى، فقد ادت الاضافة الى زيادة بكتريا العصيات اللبنية في الاثني عشر فضلاً عن خفض بكتريا القولون (*E. coli*) في الاعورين والاثني عشر لطيور السمان الياباني.

الكلمات المفتاحية: اوراق البروكلي، انتاج البيض، الفلورا المعوية، السمان.