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Efficacy of *Tragopogon graminifolius* medicinal powder as an inulin source for laying hens

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Abstract

In an experiment, the efficacy of *Tragopogon graminifolius (TG)* plant powder as a medicinal supplement for laying hens was investigated. A total of 192 Hy-line (W36) hens that had been laying for 52 to 63 weeks, were used. They were allocated to four replicates of four treatments (12 birds per in each replicate) in a completely randomized design. Diets were formulated to contain 0%, 2%, 4% and 6% *TG* powder. Dietary augmentation with *TG* powder affected the production and blood indexes of laying hens significantly. Egg weight, egg production percentage, egg mass and feed intake were greatest and feed conversion ratio, high yolk colour index, and Haugh unit were best when the hens were supplemented with 6% *TG* powder. Eggshell thickness decreased when this high level of *TG* powder was used. The lowest blood white cell count was observed with 2% TG in the diet. The *TG* levels did not change the blood indexes meaningfully. Based on these results, the provision of *TG* to laying hens at up to 6% of their diet improved the production traits.

Keywords: egg production, egg yolk, feed intake, haematology
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Introduction

Feeding organic products to animals is becoming more acceptable to consumers and the use of herbal supplements may make animal products more healthful (Torki *et al.,* 2021). Recently, the use of medicinal plants in poultry diets has increased (Darabighane *et al.,* 2017; Gadde *et al.,* 2017). they are added for a variety of reasons, including antimicrobial and antioxidant activities, growth promotion, reducing the use of pharmaceutical and chemical additives, improved bird health, increased beneficial properties of the poultry products, and appeal to customers (Oluwafemi *et al.,* 2020; Pliego *et al.,* 2020).

Tragopogon graminifolius is a valuable source of inulin (Falahi *et al.,* 2019), which acts as a prebiotic because of its beneficial nutritional effects and is widely used in the food industry worldwide (Birmani *et al.,* 2019; Stolzenbach *et al.,* 2020). Dietary supplementation with inulin improved egg production and eggshell thickness of laying hens (Shang *et al.,* 2020b; Bucław, 2017). Bell *et al.* (2017) recommended supplementation of the ration for laying hens with 1.0% of inulin powder as it increased egg production and shell thickness. Shang *et al. (*2020a) concluded that supplementation of laying hen with inulin also prolonged the shelf-life of eggs.

In layers and broilers, medicinal plants do not merely stimulate appetite and digestion, but can influence other physiological functions, help to sustain good health and welfare, and improve their performance (Torki *et al.,* 2021). Ghasemi Shansabadi *et al. (*2015) indicated that low-density thyme powder in the rations of Japanese quails increased their growth, prevented oxidation of fats, and was a highly effective antioxidant. The use of *Silybum marianum L*. powder at up to 2% in layer diets upgraded their performance, egg traits, blood biochemicals, and immunity significantly (Nobakht, 2015b). As an inulin source, the use of *Cichorium intybus* L powder as a medicinal additive for layers at rates up to 3% was

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shown to improve their performance, egg yolk colour index, Haugh unit, and blood cell immunity (Nobakht, 2015a).

The current experiment aimed to examine the effects of various levels of *Tragopogon graminifolius* medicinal plant powder as a source of inulin on egg traits, and blood indexes of laying hens.

Materials and Methods

The Animal Welfare Committee of Islamic Azad University (Maragheh Branch) approved the animal care protocol used in this experiment (Protocol no. 1396-IAU. 21.05.2017). A total of 192 laying hens between the ages of 52 and 63 weeks were allotted to four replications of four treatments (12 hens in each replicate) in a completely randomized design. The treatments consisted of 0% (control), 2%, 4% and 6% of *TG* powder provided as a supplement to the usual ration of the hens. Feed and water were provided ad libitum. UFFDA software (User-friendly Feed Formulation Done Again) (University of Georgia, USA) was used for diet formulation. Diets were formulated as recommended nutrient requirements of the Hy-Line (W36) strain (Table 1).

Table 1 Feed ingredients and chemical composition of diets for laying hens that incorporated *Tragopogon graminifolius* medicinal powder

	Level of *Tragopogon graminifolius* medicinal plant, %			
Feed ingredients. %	0	2	4	6
Corn	50.	50.	50.	50.
Wheat	17.92	15.11	12.55	10.00
Soybean meal	19.74	19.78	19.83	19.85
Soybean oil	2.65	3.20	3.75	4.31
Tragopogon graminifolius	0	2	4	6
Oyster shell	7.37	7.39	7.41	7.44
Bone powder	1.75	1.69	1.63	1.56
Sodium chloride	0.31	0.31	0.30	0.30
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25
DL-Methionine	0.01	0.02	0.03	0.04
Calculated composition				
Metabolizable energy, kcal/kg	2900	2900	2900	2900
Crude protein, %	14.50	14.50	13.78	13.78
Calcium, %	3.40	3.40	3.40	3.40
Available phosphorus, %	0.33	0.33	0.33	0.33
Crude fibre, %	2.79	3.30	3.82	4.31
Sodium, %	0.16	0.16	0.16	0.16
Lysine, %	0.70	0.70	0.70	0.70
Methionine + cysteine, %	0.56	0.56	0.56	0.56
Tryptophan, %	0.18	0.18	0.18	0.18

[1] Vitamin A: 2.7 mg, vitamin D_3: 0.05 mg, vitamin E: 18 mg, vitamin K_3: 2 mg, thiamine: 1.8 mg, riboflavin: 6.6 mg, pantothenic acid: 10 mg, pyridoxine: 3 mg, cyanocobalamin: 0.015 mg, niacin: 30 mg, biotin: 0.1 mg, folic acid: 1 mg, choline chloride: 250 mg, antioxidant: 100 mg per kg of diet

[2] Ferrous sulfate: 50 mg, manganese sulfate: 100 mg; zinc oxide: 100 mg; copper sulfate: 10 mg, potassium iodide: 1 mg, sodium selenite: 0.2 mg per kg of diet

During the experiment the laying hens were kept on a regime of 16 hours of light and 8 hours of darkness. Temperature in the poultry house was set automatically between 18 ºC and 24 ºC. Eggs were collected daily, and data for feed intake, egg production percentage, egg mass, egg weight and feed conversion ratio were determined weekly. Mortality was recorded when it occurred, and hen days were determined at the end of each week based on the number of surviving hens. The eggs were classified as normal or damaged. Damaged eggs included fully cracked eggs, defined as an egg with a broken shell and destroyed membrane, eggs with a hairline crack, defined as an egg with broken shell, but intact membrane, and eggs without a shell, but with an intact membrane.

At the end of the experiment, three eggs were collected from each replicate and the traits of egg quality were determined. Specific gravity was determined by floating the egg in various concentrations of salt water (Farkhoy *et al.*, 1994). After the internal contents of the eggs had been emptied, eggshells were cleaned and kept at room temperature for 48 hours, then dried and weighed, and the average thickness was recorded. The colour of the yoke was determined with the Roche yolk colour fan (ORKA Food Technology, LLC., West Bountiful, Utah, USA), and the yolk index, egg albumin index, and Haugh units were determined, as described by Farkhoy *et al.* (1994). At the end of the experiment, two birds from each replicate were randomly chosen for blood collection. After the birds had been fasted for approximately six hours, 5 mL blood samples were collected from the brachial vein. One mL of the blood was transferred to tubes with EDTA for haematological analysis, including the numbers of red and white blood cells, haemoglobin concentration, packed cell volume, and the number of lymphocytes (Gross & Sigel, 1983). The remaining 4 mL of blood was centrifuged to obtain serum for biochemical analysis, including the concentrations of cholesterol, triglyceride, albumin, total protein, and uric acid. Kits for the Anision-300 auto-analyser system (Pars Azmoon Company, Tehran, Iran) were used in the analysis of blood biochemistry (Nazifi, 1997).

The results were calculated with the general linear model procedure of SAS (SAS Institute, Inc., Cary, North Carolina, USA). The linear model was:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where: Y_{ij} = a response variable for the ith treatment in the jth replicate, μ = overall mean, T_i = the effect of the level of *Tragopogon graminifolius* powder in the diet, and ε_{ij} = the residual deviation of the observation from the effect in the model.

Results and Discussion

The effects of *TG* on egg productivity of hens are shown in Table 2. Increasing levels of *TG* improved all of these measures of productivity (*P* <0.01). The highest values of egg weight, egg production, egg mass, and feed intake, and the best feed conversion ratio were observed when 6% *TG* was used in the diet.

Table 2 Effects of dietary augmentation with *Tragopogon graminifolius* plant powder on the performance of laying hens

Dietary level, %	Egg weight, g	Productivity, %	Daily production, g	Feed intake, g	Feed conversion ratio
0	60.71^{d}	56.51^{c}	34.30^{d}	110.11^{b}	3.21^{a}
2	60.93^{c}	57.99^{c}	35.34^{c}	110.43^{ab}	3.12^{b}
4	61.39^{b}	60.61^{b}	37.21^{b}	110.71^{a}	2.98^{c}
6	61.66^{a}	62.49^{a}	38.53^{a}	110.72^{a}	2.87^{d}
SE	0.04	0.47	0.28	0.10	0.02
P-value	0.0001	0.0001	0.0001	0.0088	0.0001

^{a,b,c,d} Within a column, means followed by a common superscript were not different with probability *P* =0.05

Table 3 shows the results of the use of *TG* powder on egg quality. Supplementation with up to 6% *TG* powder improved the yolk colour and Haugh unit significantly and reduced shell thickness (*P* <0.01), whereas *TG* powder had no significant effect on shell percentage, egg white percentage, and egg yolk percentage (*P* >0.05).

Table 3 Effects of dietary augmentation with *Tragopogon graminifolius* plant powder on the quality of eggs produced by laying hens

Medicinal plant	Yolk colour	Shell weight, %	White weight, %	Yolk weight, %	Haugh unit	Shell thickness, mm
0	2.00[c]	10.62	63.19	26.20	73.20[c]	0.34[a]
2	3.11[b]	10.34	63.13	26.51	73.92[b]	0.33[b]
4	4.66[a]	10.80	62.37	26.84	74.55[a]	0.33[b]
6	4.67[a]	11.46	60.85	27.71	74.77[a]	0.33[b]
SE	0.02	0.36	0.84	0.74	0.12	0.01
P-value	0.0001	0.2415	0.2457	0.5395	0.0001	0.0086

[a,b,c] Within a column, means followed by a common superscript were not different with probability *P* =0.05

Tables 4 and 5 present the effects of augmenting the diets of laying hens with *TG* powder on their blood biochemical profile. Among these variables, only the use of *TG* had a significant effect on the number of white blood cells (*P* <0.05). The highest number of white blood cells characterized those hens that received 4% of the powder, whereas those that received 2% had the lowest number of white blood cells.

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Table 4 Effects of using *Tragopogon graminifolius* plant powder on the blood lipid profile, total protein and uric acid concentrations of laying hens

Dietary level, %	Triglyceride, mg/dL	Cholesterol,mg/dL	Album, g/dL	Total protein, g/dL	Uric acid, g/dL	High density lipoprotein, mmol/L
0	1939.21	145.25	2.84	5.31	3.28	1.61
2	1524.92	124.24	3.05	4.85	2.09	6.08
4	1780.60	142.72	2.85	5.19	2.25	2.67
6	1292.40	78.63	3.18	4.90	2.25	2.72
SE	285.27	19.19	0.10	0.30	0.43	1.18
P-value	0.4425	0.1435	0.0931	0.6664	0.2574	0.1158

Table 5 Effect of using *Tragopogon graminifolius* plant powder on blood cell numbers

Dietary level, %	Haematocrit, %	Haemoglobin, %	Red blood cells, $x10^6/mm^3$	White blood cells, $x10^6/mm^3$	Heterophil (H), %	Lymphocyte (L), %	H/L
0	33.00	11.50	2.80	25167[ab]	6.67	92.67	0.07
2	32.67	10.64	2.73	22666[b]	9.00	90.00	0.10
4	30.67	12.40	3.04	27000[a]	14.00	86.00	0.17
6	33.00	12.10	2.75	25000[ab]	10.67	89.00	0.12
SE	0.90	0.62	0.10	799	2.29	2.24	0.03
P-value	0.2723	0.2680	0.1753	0.0315	0.2214	0.2849	0.2491

[a,b] Within a column, means followed by a common superscript were not different with probability *P* =0.05

The use of *TG* powder increased feed intake, so the hens received more nutrients and had improved laying performance, probably because of the general positive effects of medicinal plants, such as promoting antimicrobial properties, stimulating the secretion of digestive enzymes, increasing dietary fibre, and enhancing the speed of gastrointestinal passage (Shirani *et al*., 2019; Jin *et al.,* 2020; Youssef *et al.,* 2021). *Tragopogon graminifolius* makes feed more appetizing and this might increase feed intake (Leila *et al.,* 2011). However, in a study with mice, a methanolic extract of *TG* reduced feed intake (Zeeni *et al.,* 2014).

Inulin is an important active ingredient of *TG* merit and has prebiotic properties that can improve performance through functionalities. These include reducing the harmful microbial population of the

gastrointestinal tract (Liu *et al.,* 2018; Xia *et al.,* 2019), increasing the height of intestinal villi (*Li et al*., 2018; Ding *et al.,* 2021), and increasing immunity (Song *et al.,* 2020; Gong *et al.,* 2020). Thus, it improves the condition of the gastrointestinal tract and increases digestion, absorption, and utilization of dietary nutrients, with a downstream effect of improving performance. The positive results of augmentation with *TG* in the present study were consistent with other studies that used inulin in poultry diets (Elrayeh & Yildiz, 2012; Park & Park, 2012; Abdelqader *et al.,* 2013).

The positive effects of *TG* on the laying capacity of hens may arise from its antioxidant properties (Sasmakov *et al.,* 2012). By protecting nutrients from oxidation, antioxidants facilitate their greater absorption from the gastrointestinal tract. As a result, fewer nutrients are excreted and the conditions for their utilization for improving egg production are provided.

Improved intestinal health might be another reason for the observed improvement of performance by the laying hens in this study. Farzaei *et al.* (2014) used *TG* as an effective treatment for intestinal inflammation in Wistar mice. Reducing intestinal inflammation could provide for greater nutrient absorption.

Medicinal plants such as *TG* are also rich in vitamins A and C and minerals such as iron, potassium, zinc, and copper, which can improve performance (Milani *et al.,* 2011). Green plants such as *TG* are rich in pigments such as carotenoids (Marounek & Pebriansyah, 2018; Upadhyay, 2018). The use of green plants in the diet of laying hens could result in the transfer of their pigments to the egg yolk, making it more colourful (Nobakht, 2015a; Nobakht, 2015b).

Haugh units are an important indicator of quality of egg whites (Kraus *et al.,* 2021; Malfatti *et al.,* 2021). Egg white is rich in high-quality protein. Because an increase in the level of *TG* improved performance, the amount of absorbed protein possibly also increased, resulting in improved condition of egg whites and an increase in the Haugh unit.

The use of reduced shell thickness *TG* in comparison with the control, because of the increase in egg size. However, the weight of the eggshell did not increase as a percentage of egg weight, indicating that there was no disturbance in calcium uptake by chickens. The use of *TG* had no significant effects on blood biochemical parameters. The ratio of lymphocytes to heterophils remained low with the increasing levels of supplemental *TG,* indicating that this dietary augmentation did not materially increase the level of stress on the birds (Gross & Siegel, 1983). Another report indicated the use of inulin
reduced blood cholesterol and triglyceride levels (Elrayeh & Yildiz, 2012).

The use of an aqueous extract of the aerial parts of the *TG* over four weeks improved the lipid profile significantly (triglyceride, total cholesterol, low density lipoprotein, and high density lipoprotein) of mice (Zeeni *et al.,* 2014) unlike the results in the present study. However, numerous factors might have contributed to these differences, including the type of animal, test conditions, and production status. In the present study *TG* powder increased the white blood cell count. However, the differences were numerically small, and possibly the body was prepared to deal with harmful external factors (Signore *et al.,* 2018; Manav *et al.,* 2019). Flavonoids, and vitamins A and C are among the active ingredients in *TG* that can effectively increase immune indicators such as the number of white blood cells.

Conclusion

The use of *TG* powder at up to 6% of the diets of laying hens improved performance and egg quality without meaningful adverse effects on haematology or serum lipids. Therefore, *TG* powder can be recommended as a feed additive for laying hens.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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Authors' Contributions

AN supervised the experiment, VP and TA advised the project. AN conducted the laboratory analyses. TA and VP supervised the experiment and finalized the manuscript. İC edited the article.

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