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Nutritional characterization of some natural plants used in poultry nutrition

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ABSTRACT

Current nutritional strategies of livestock industry are focused on evaluating the effects of terrestrial sources rich in natural bioactive compounds that can be used in farm animal feed and the subsequent implications on the quality of resulting animal products. In this context, the present study aimed to characterize from a nutritional point of view some natural plants used as phyto-additives in poultry nutrition: oregano, mint, basil, sage, fenugreek, thyme, turmeric, cumin and rosemary. The results of this study on plants nutritional evaluation showed a varied proximate analysis. Of all the plants, cumin, fenugreek and basil were the richest source of crude protein. Thyme had a large ether extractives content, followed by rosemary, sage and cumin. The obtained results revealed that oregano has the strongest antioxidant capacity (849.77 mmols equiv. asc. acid; 863.57 mmols equiv. vit. E), the highest total polyphenols concentration (86.77 mg GAE/g) and lutein and zeaxanthin (304.23 μg/g) of the analysed plants. Nevertheless, all plants had high concentration of total polyphenols, except cumin, a large amount of xanthophylls and vitamin E. After oregano, sage and thyme have been noted for their antioxidant capacity and major antioxidant compounds. Basil and sage revealed the highest amount of essential trace elements.
Keywords: poultry, phyto-additives, polyphenols, vitamin E

INTRODUCTION

Nowadays, most modern diets are based on natural products and ingredients. Knowledge of a diverse, safe and quality diet must be constantly improved, given that the composition and consumption of food are important segments of food industry. At the same time, the food availability for humans, in competition with animal feed, is insufficient so that it is necessary to capitalize on the full potential of existing resources and identify other unknown and / or less known (FAO and WHO, 2020).

The current nutritional strategies of livestock industry are focused on evaluating the effects of terrestrial sources rich in natural bioactive compounds that can be used in farm animal feed and the subsequent implications on the quality of resulting animal products (Arroyo et al., 2019). Several herbs, some of them traditionally used since ancient times by humans, in the form of spices, herbs and their essential oils, can be used in animal nutrition. The efficient use of bioactive compounds from these natural plants, given their traditional use, can lead to new food applications development (Diniz do Nascimento et al., 2020). Among botanicals widely used in traditional medicine are: oregano, mint, basil, sage, fenugreek, thyme, turmeric, cumin and rosemary, plants studied for this paper.

Oregano *(Origanum vulgare* L.*)* is an aromatic perennial herb from Lamiaceae family, which has stood out over time due to its antioxidant and antimicrobial properties against pathogenic microorganisms such as Escherichia coli, Staphylococcus aureus or Salmonella typhimurium (Arcila-Lozano et al., 2004). Oregano and its oil are currently used to replace synthetic additives, making it a good alternative to antibiotics (Windisch et al., 2008). Park et al., (2015) highlighted the positive effects of oregano use in poultry nutrition on production parameters, immune system, gastrointestinal microflora modulation, or pathogens inhibition.

Mint (*Mentha spp*.) is a genus of about 25-30 species of plants in Lamiaceae family, also an aromatic and medicinal herb, spreading worldwide, with antioxidant, antimicrobial and antifungal properties (Singh and Pandey, 2018). The use of mint and its oil in poultry diets can improve the production parameters (Verma et al., 2020), meat sensory quality (flavour, tenderness and juiciness) (Quinche et al., 2019) and lipid oxidation of eggs (Aydın and Bölükbaşı, 2020).

Basil *(Ocimum basilicum* L.*)* is an herbaceous plant, member of Lamiaceae family, used for their antimicrobial, antioxidant, anti-inflammatory, antibacterial and antiviral properties. Basil and its oil have a good efficiency against intestinal parasites and bacterial infections (Alabi et al., 2018). Recent studies (Sheoran et al., 2017) highlighted the basil potential to increase the production performance and immune status of birds when is used in their feed.

Sage *(Salvia officinalis)* is a plant from Lamiaceae family with good antioxidant and antimicrobial properties. It has been shown that the inclusion of sage in broiler diets led to a significant reduction of plasma total cholesterol, triglycerides and low-density lipoprotein (Rasouli et al., 2019), and also reduced the total bacterial count and coliform bacteria in the intestines of the broiler (Majid et al., 2019).

Fenugreek *(Trigonella foenum-graecum* L.*)* is an annual plant herb from the Fabaceae family with antimicrobial, antifungal and antiviral properties. Recent research has shown that the use of fenugreek in laying hen diets improved their immune system, feed conversion ratio (Samani et at., 2020) and egg production adversely affecting their quality (Wahab et al., 2019).

Thyme *(Thymus vulgaris* L.*)* is a perennial aromatic plant, member of mint family, Lamiaceae, with strong antimicrobial and antiseptic properties. There is information in the literature (Yalçin et al., 2020) regarding the potential of antioxidant compounds from thyme that can prevent lipid peroxidation of egg yolk, reduce the cholesterol and total saturated fatty acids of yolk and improved omega-3 fatty acid content when it is included in hen diets.

Turmeric *(Curcuma longa* L.*)* is a perennial plant, member of ginger family Zingiberaceae with many positive effects on animal health and welfare. The bioactive compounds of turmeric, make it an excellent feed additive for animal nutrition due to its antioxidant, antibacterial, anti-inflammatory and antiviral, properties (Dono, 2013). Kosti et al., (2020) reported an increase in egg production and Lactobacillus spp. counts when they supplemented laying hen diets with turmeric powder.

Cumin *(Cuminum cyminum)* is a flowering plant from Apiaceae family with antioxidant, antimicrobial and anticholesterol properties (Hajati et al., 2014). It has been shown that the use of cumin in broiler diets improved body weight gain, feed conversion ratio, blood biochemistry (Rafiee et al., 2014) and has beneficial effects on chemical and physical attributes of meat (Majid et al., 2020).

Rosemary *(Salvia Rosmarinus)* is a woody, perennial herb from Lamiaceae family with strong antioxidant properties. Polat et al., (2011) reported a decrease in serum cholesterol level for broilers fed with rosemary, concluding that rosemary diet may positively influence the renal and hepatic function of birds. Similar results on serum cholesterol level were obtained in a study Alagawany et al., (2015) on laying hens, and in addition, the use of rosemary improved egg numbers, egg mass, yolk percent and yolk-to-albumen ratio, with a decrease in albumen percent.

The present study aimed to characterize from a nutritional point of view some natural plants used as phyto-additives in poultry nutrition.

MATERIALS AND METHODS

The plants characterized in this study were purchased in dried form from a local producer who grows these plants in the south (Măcrișului Valley Farm, Ialomița County) and southwest (Zătreni Valley Farm, Vâlcea County) of the Romania. The sampling and conditioning of plants were performed by producer.

In order to determine the total polyphenol content (TP) and total antioxidant capacity (TAC), it was necessary to obtain extracts from the studied plants. According to Untea et al., (2020), 1 g of dried plant powder was extracted in 10 ml of methanol and kept on a rotary shaker for 24 hours in the dark. The extract thus obtained was centrifuged for 10 minutes at 1500 rpm, and the resulting supernatant was used for analysis.

Proximate composition

Standardized methods according to Regulation (EC) no. 152/2009 and ISO standards were used in order to determine the main nutrients concentration from plants. Crude protein (CP) was determined by Kjeldahl method, according to SR EN ISO 5983-2: 2009, using a semiautomatic Kjeltec 2300 system–Tecator Instruments (Sweden). Ether extractives (EE) were determined by organic solvent extraction method according to SR ISO 6492: 2001, using a Soxtec 2055 Foss Tecator (Sweden). Crude fibre (CF) was determined by intermediary filtration method according to SR EN ISO 6865: 2002, using an automatic analyser Fibertec 2010, (Foss Tecator, Sweden). Crude ash (Ash) was determined by gravimetric method according to SR EN ISO 2171: 2010, using a Caloris CL 1206 oven (Romania).

Content of total polyphenols (TP)

The total polyphenol content of sample extracts was performed spectrophotometrically using an improved version of Folin–Ciocalteu method, as described by Untea et al., (2020). The results regarding the total polyphenol content were expressed as mg gallic acid equivalents per gram of dried sample (mg GAE/g).

Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the extracts was determined using the phosphomolybdenum method as described by Untea et al., (2020). The absorbance was recorded at 695 nm and the results were expressed as ascorbic acid equivalents for hydrophilic compounds (mmols equiv. asc acid) and vitamin E equivalents for lipophilic compounds (mmols equiv. vit E) per gram of dried sample. For turmeric, cumin and rosemary was used the DPPH radical-scavenging activity method as described by Varzaru et al., (2020). The absorbance of the solution was recorded at 517 nm using a spectrophotometer (Jasco V-530, Japan Servo Co. Ltd., Japan). Trolox was used as standard. The results were expressed as mM Trolox equivalents (mmols equiv. Trolox).

Lutein and zeaxanthin determination

The content of lutein and zeaxanthin was determined using a high-performance liquid chromatograph (Perkin Elmer 200 series, Shelton, CT, USA) equipped with UV detector (445 nm). Chromatographic determination was performed under isocratic conditions according to the method described by Varzaru et al., (2020). The results were expressed as as μg/g of dried sample.

Vitamin E determination

The vitamin E concentration was determined using a high-performance liquid chromatograph (HPLC Finningan Surveyor Plus, Thermo-Electron Corporation, Waltham, MA) equipped with PDA-UV detector (292 nm). Chromatographic determination was performed under isocratic conditions according to the method described in Regulation (EC) no. 152/2009 and by Varzaru et al., (2020). The results were expressed as μg/g of dried sample.

Trace elements determination

The calcium (Ca) was determined by the titrimetric method, according to SR ISO 6490-1:2006 and the phosphorus (P) was determined spectrophotometrically, according to Regulation (EC) no. 152/2009, using a molecular absorption spectrophotometer Jasco V-530. Copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) were determined according to the method described by Untea et al. (2012) using atomic absorption spectrometry method. The results were expressed as μg/g of dried sample.

Statistical analysis

The obtained results were calculated using descriptive data analysis from Microsoft Excel for Windows.

RESULTS AND DISCUSSION

Proximate analysis of plants

The results of proximate analysis of plants are given in Table 1. The highest concentration of crude protein was recorded in cumin, which also had the highest content of crude fibre from the analysed plants. High concentrations of crude protein were also found in fenugreek and basil, the latter having at the same time the highest ash content. Thyme was noted with a large ether extractives content, compared to the other plants analysed, followed by rosemary, sage and cumin.

The literature includes various results on proximate analysis of these plants studied in the present paper. This is due to the variety of plants, geographical origins, environmental and climatic conditions, soil type, harvesting time, temperature and period of drying (Carvalho-Filho et al., 2006). El-Ghorab et al., (2010) reported in a comparative study on chemical composition and antioxidant activity of some plants including cumin, a content of 15.70%±0.32 crude protein; 11.50%±0.38 ether extractives;

37.20%±0.37 crude fibre and 9.30%±0.24 crude ash. Previous studies (Seidavi et al., 2020) on black cumin seeds using in broiler diets have shown their positive effects on production performance at a 3-5% level of inclusion in the diet. The inclusion of black cumin oil (1-2%) in laying hen diets reduced the number of intestinal *E. coli.* and also improved the production performance of birds, namely egg mass and egg quality overall.

Regarding the fenugreek, available data (Naidu et al., 2011) highlights a content of 27.57 g ±0.09 crude protein; 6.71 g ±0.01 ether extractives and 3.90 g/100 g ±0.14 ash. It has been shown that the use of fenugreek seeds in broiler diets, especially at an inclusion rate of 2.0%, significantly increased productive performance. At the same time, abdominal fat was reduced and drumstick meat weight and dressed breast, thigh, carcass was improved. Considering the economic efficiency of new feeds, the inclusion of fenugreek seeds in broiler diets led to a reduction in their costs and improved production (Toaha et al., 2016).

Table 1. Proximate analysis of plants

Sample	Crude protein	Ether extractives	Crude fibre	Crude ash
		%		
Oregano	11.95	1.88	9.28	8.95
Mint	17.09	1.65	12.30	13.81
Basil	18.06	2.56	10.88	17.34
Sage	14.19	3.40	20.24	10.23
Fenugreek	18.71	1.24	22.94	11.39
Thyme	14.67	7.32	24.63	9.69
Turmeric	10.91	2.86	5.35	9.29
Cumin	21.58	3.06	30.09	5.82
Rosemary	5.35	3.62	22.25	6.61

Antioxidant capacity and major antioxidant compounds of the plants extracts

As can be seen in Table 2, the plant with the strongest antioxidant capacity and major antioxidant compounds overall was oregano, followed by sage and thyme. Jałoszyński et al., (2008) evaluated the antioxidant activity of dehydrated oregano by three methods: freeze drying, convective drying and vacuum-microwave drying. The obtained results ranged between 63.96 mg - 394.06 mg GAE/100 g, the conclusion of the study being that the method used for plant drying greatly influences the total polyphenols concentration. Spiridon et al., (2012) reported for oregano a polyphenol content of 67.8±3.41 mg GAE/g; flavonoids 31.6±4.25 mg R/g and an antioxidant activity of 114±4.18 mg AA/g.

Available literature data (Ri et al., 2017) showed that oregano powder supplementation in broiler nutrition improved average daily food intake and thus the average daily gain. A decrease in the content of malondialdehyde and higher total antioxidant activity (T-AOC) in the serum of broilers were also observed. In terms of meat quality, oregano can slow down the lipid peroxidation processes at an inclusion rate of 2.5 - 5%, although it also influences the meat color parameters, but without affecting their sensory quality and the level of acceptability by consumers (Velasco et al., 2017). Beneficial effects of oregano using (powder and oil) in broiler diets have also been reported by Vlaicu et al., (2020). The inclusion in diets of 0.01% oregano oil level and 0.005% oregano oil together with 1% oregano powder significantly ($p<0.05$) improved the overall production performance. The number of *Enterobacteriaceae* and *Escherichia coli* count was significantly lower in the intestine of the experimental groups, while the number of *Lactobacillus spp.* was significant higher compared to the control group. Similar results of dietary supplementation of laying hen diets with oregano essential oil have been reported by Denli et al., (2019). The use of oregano essential oil improved the egg weight and decreased the egg shell bacterial contamination through the reduction of *Escherichia coli* and Coliforms. It is also known that oregano improves the intestinal microflora of broilers under heat stress (Turcu et al., 2018).

Table 2. Antioxidant capacity and major antioxidant compounds of the plants extracts

Sample	Total polyphenols (mg GAE/g)	TAC (mmols equiv. asc. acid)	TAC (mmols equiv. vit. E)	Lutein and zeaxanthin (μg/g)	Vitamin E (μg/g)
Oregano	86.77	849.77	863.57	304,23	134,12
Mint	25.19	301.42	264.43	196,35	77,85
Basil	11.37	313.61	275.50	230,31	113,33
Sage	50.20	575.30	562.67	257,66	160,76
Fenugreek	3.74	175.86	128.80	165,79	88,13
Thyme	34.13	442.48	416.72	236,81	188,93
Turmeric	21.36	-	-	3,37	12,02
Cumin	6.73	-	-	6,24	23,76
Rosemary	53.42	-	-	70,63	156,12

In contrast to the total polyphenol content obtained in this study for fenugreek, Naidu et al., (2011) reported a concentration of 85.88±0.01 mg GAE/g. Results of other research highlighted a total polyphenols content of sage that varies depending on harvesting time between 85.33 ±3.08 mg GAE and 110.52±4.64 mg GAE/g; for rosemary 94.29±0.59 mg GAE and 104.44±2.55 mg GAE/g (Baydar et al., 2009). Regarding mint, Kalemba-Drożdż et al., (2020) reported 8.90-10.68 mg/100 g polyphenols concentration, respectively 0.03-0.18 μg/100 g carotenoids. Similar results to those obtained in this study for basil were reported by Nguyen et al., (2010) who tested three cultivars of basil (*Ocimum basilicum* L.) leaves: 8.37±1.76 mg GAE/g for Sweet Thai; 11.58±3.34 mg GAE/g for Dark Opal, and 12.54±1.83 mg GAE/g for Genovese.

Majid et al., (2019) included different levels of fenugreek and sage in broiler diets, obtaining an improvement in production performance and broiler health, highlighted by the numbers of total bacteria and colon bacteria decreasing. The results of another study (Abbas, 2010) showed that broilers fed with basil (3 g/kg diet) had significantly higher body weight compared to those who received fenugreek (3 g/kg diet). In fact, both diets significantly improved serum cholesterol levels compared to control diet. It would suggest that the supplementation of broiler diets with basil improved productive performance. It has been shown that supplementing broiler diets with black cumin seed, fenugreek and turmeric powder at (1 and 2 g/kg diet) improved breast meat yield. The inclusion of fenugreek powder had a significant effect on gizzard and giblet ratio and reduced the abdominal fat ratio, compared to the control group and turmeric diet. However, turmeric can be used for its beneficial results on carcass yield and lean breast meat (Yesuf et al., 2017). In another study (Giannenas et al., 2016) sage (0.5%) and oregano (5%) essential oil were used as natural antioxidants in broiler diets. The obtained results indicated a slowdown in the lipid peroxidation processes for breast and thigh meat, corroborated with the inclusion rate in diet. Kishawy et al., (2016) evaluated the effects of sage oil and peel extract (PPE) on reducing the damaging effect of rancid soybean oil in broiler diets. The use of sage oil improved weight gain and feed conversion ratio, decreased the serum cholesterol, triacylglycerols and abdominal fat, and improved the immunity parameters of broilers.

DPPH radical-scavenging activity of turmeric, cumin and rosemary can be seen in Figure 1. Of all the three plants analysed, turmeric stood out with the highest total antioxidant capacity, followed by cumin. Research (Durrani et al, 2006) regarding the inclusion of turmeric in broiler diets reported improved production performance (chick weight gain, feed conversion) and better economic efficiency. Also, the inclusion of different turmeric levels in broiler diets (0.25%; 0.50%; 1%) revealed that 0.50% turmeric improved body weight, feed conversion ratio and feed consumption. Significant differences were recorded for dressing percentage in all treatments, compared to the control (Abd Al-Jaleel, 2012). Similar results were reported by Al-Kassie et al., (2011) who evaluated the effect of the mixture of cumin and turmeric in broiler diets at different levels of inclusion (0.25%; 0.50%; 0.75% and 1%). The inclusion of cumin and turmeric mixture at levels of 0.75% and 1% in diets improved body weight gain, feed intake and feed conversion ratio. At the same time, this mixture reduced the cholesterol level, Hb, RBC, WBC, and H/L ratio concentration. In a comparative study (Ashayeri Zadeh et al., 2009) the effect of garlic powder, black cumin seeds powder and wild mint powder were tested. The broilers fed with black cumin seeds powder had the highest body weight gain and feed conversion ratio compared with the other diets. Also, this diet led to the lowest abdominal fat percentage, the highest carcass and breast percent, compared to the control and wild mint diets. Gurbuz and Ismael (2016) evaluated the effect of peppermint and basil (0.5%; 1%; 1.5%) as a feed additive in broiler diets. The obtained results showed significant higher production performances and liver weight in 1.5% peppermint diet. The authors suggested that using peppermint and basil can improve the growth performance and feed conversion ratio of broilers.

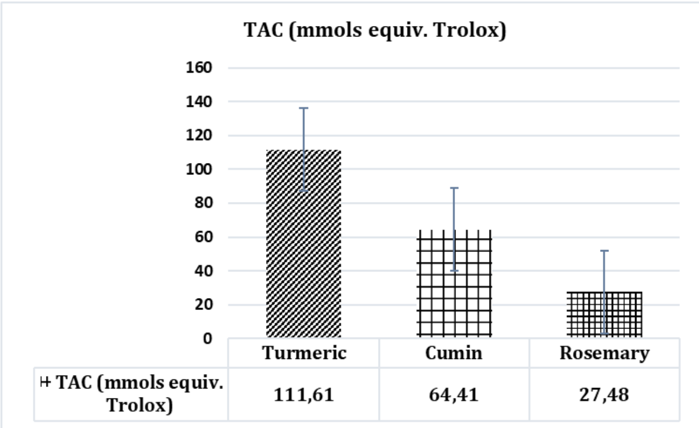

Figure 1. Total antioxidant capacity of the plants extracts (mm equiv. Trolox)

Essential trace elements composition of plants

From the recorded results (Table 3), it was observed that basil is a plant rich in essential trace elements, obtaining in this study the highest level of calcium, manganese, zinc and copper. The highest content of basil minerals is directly related with the highest crude ash concentrations from the studied plants. High levels of trace elements were also determined for sage which had the large amount of iron and remarkable levels of zinc and copper from the analysed plants. Results of previous studies showed for basil a content of 2.16-3.09% Ca; 0.40-64% P (Nurzyńska-Wierdak et al., 2011); 205.30 mg/kg Fe; 22.20 mg/kg Mn and 272.80 mg/kg Zn (Leal et al., 2008). Kalemba-Drożdż et al., 2020 reported a content of 0.18-9.70 mg Ca; 0.04-0.21 mg Fe and 0.01-0.04 mg Zn /100 ml sample of mint (fermented, macerate, tincture, decoction).

Farm activities are related with environmental pollution the with nitrogen (N), phosphorus (P) and traces element that animals excrete. One of the measures to prevent pollution is to reduce the elements excretion by improving their bioavailability and the inclusion of phyto-additives in farm animal diets (Lu et al., 2017).

Table 3. Essential trace elements composition of plants

Sample	Ca	P	Cu	Fe	Mn	Zn
		%			µg/g	
Oregano	1.54	0.37	7.86	317.03	60.79	24.98
Mint	2.22	0.36	9.62	337.54	101.94	28.22
Basil	3.29	0.45	11.49	326.77	139.98	39.32
Sage	1.34	0.44	12.05	903.38	45.20	38.41
Fenugreek	1.09	0.55	7.11	322.63	29.34	31.07
Thyme	0.76	0.43	7.16	818.00	39.65	33.06
Turmeric	0.32	0.43	6.23	618.72	84.18	27.53
Cumin	0.76	0.56	9.14	85.53	29.28	36.29
Rosemary	1.41	0.08	3.85	371.38	20.20	31.22

Note: Ca = calcium, P = phosphorus; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc.

Some of the studied plants can be considered good sources of trace minerals (ex. sage and thyme for iron or mint and basil for manganese) even if the bioavailability of minerals from vegetal sources is considered to be very low (Lopez et al., 2002) due to their rich phytate content. Other plants can stimulate the absorption of mineral from animals' diet by other pathways, including components with antioxidant potential (ex. oregano).

CONCLUSION

The results of this study showed that cumin, fenugreek and basil were the richest source of crude protein, oregano, sage and thyme have the strongest antioxidant potential and basil and sage revealed the highest amount of essential trace elements.

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