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FEEDING VALUE OF LOCAL PHYTO-ADDITIVES, POTENTIAL INGREDIENTS IN POULTRY DIETS

Tatiana Dumitra PANAITE, Rodica Diana CRISTE, Margareta OLTEANU, Arabela Elena UNTEA, Mariana ROPOTA, Iulia VARZARU, Alexandra LUPU

National Research-Development Institute for Animal Biology and Nutrition (IBNA), 1 Calea Bucuresti, Balotesti, 077015, Ilfov, Romania

Corresponding author email: tatiana_panaite@yahoo.com

Abstract

Four plants have been characterized physico-chemical as vegetal phytoadditives (blueberry leaves, walnut leaves, marigold and buckthorn meal) in order to determine their nutritional value for inclusion in laying hens diets as possible alternatives to the use of antibiotics in poultry feed. Chemical determinations revealed a variable protein content ranging between 6.76% (blueberry leaves) and 14.14% (buckthorn meal). Marigolds had significantly higher iron content (1262.54 ppm) and blueberry leaves showed high concentrations of manganese (1410.10 ppm) and zinc (40.37 ppm). The walnut leaves were characterized by high concentrations of: calcium (2.01%), lutein + zeaxanthin (264.10 ppm), total polyphenols (53.94 mg EAG/g) and polyunsaturated fatty acids, especially α-linolenic acid (13.45 g/100 g total fatty acids). The studied phytoadditives will be evaluated in a digestibility trial for their nutritional assessment in laying hens diets.

Key words*: phytoadditives, plants, nutritional value, hens.*

INTRODUCTION

The ban on antibiotics as growth promoters in poultry diets made the nutritionists look for alternatives such as probiotics, organic acids, oligosaccharides, botanic materials or plant extracts, which will probably change the microbial profile of the gut content of hens reared under intensive conditions (Yegani and Korver, 2008). Phytoadditives are among the most recent alternatives to antibiotics used as growth promotersfor farm animals, having a beneficial effect on production (Windisch et al, 2008; Kroismayer et al., 2008) and animal health (Athanasiadou et al., 2007; Peric et al., 2010), improving the intestinalmicroflora (Mitsch et al., 2004), enhancing nutrient digestibilityand changing the morphology of the digestive secretion (Kreydiyyeh et al., 2003; Jamroz et al., 2003). There are many plants rich in important nutrients, which seem to have a highly positive effect on animal health and productivity, even on the human health. The walnut (*Juglans regia* L.) contains important amounts of phenol compounds (Labuckas et al., 2008). The highest the content of phenols, the strongest is the antioxidant activity. Their active principles support the bactericide, bacteriostatic, astringent, slightly hypotensive, hypoglicemiant, calming, cicatrizing, emollient, antitoxic, antimitotic, antiperspirant, antiexantematic and antirheumatic activities. The blueberry (leaves and fruits) contains a wide variety of antioxidant substances (Dulebohn et al., 2008; Castrejón et al., 2008; Piljac-Zˇegarac et al., 2009) which help preventing the cardiovascular disorders and protect against cancer (Smith et al., 2004; Seeram, 2008; Neto, 2007) and against cerebral vascular accidents (Wang et al., 2005), also preventing of urinary tract infections (Jepson and Craig, 2007). More recently, strong oxygen radical absorbance capacity (Ehlenfeldt and Prior, 2001), hypotensive effects (Sakaida et al., 2007), hypolipidemic effects (Nagao et al., 2008) and antileukemic activity (Skupień et al., 2006) of the leaves have been reported. However, the detail of the chemical constituents of the leaves has not yet been clarified. Many reports have suggested that blueberry leaves are rich in polyphenol compounds with high antioxidant activity (Wang et al., 2015; Feng et al., 2017). The polyphenols of blueberry leaves are mainly composed of proanthocyanidins, followed by caffeoylquinic acids and flavonolglycosides,

especially the oligomericproanthocyanidins, contribute to the biological activities of the blueberry leaves (Matsuo et al., 2010). According to the literature (Biswas et al., 2010; Kaushaland Sharma, 2011), the leaves, seeds and residues of buckthorn fruits are suitable as animal feeds because they are rich in nutrients (Panaite et al., 2016) and bioactive compounds (Lee et al., 2011) such asvitamins (Luhua et al, 2004; Ranjith et al, 2006), amino acids (Repyakh et al., 1990), lipids (Bekker and Giuschenkova, 1997), sugars (Yang, 2009) and flavonoids (Hakkinen et al., 1999). Some studies have also shown the presence of antioxidants (Püssa et al, 2007;Geetha et al., 2009). They are rich in carotenoids, xanthophyll, phenols andflavonoidsand a high content of essential oils (Yang et al., 2000; Singh et al., 2006). The feeding of buckthorn, in different forms (leaves, seeds or buckthorn fruits residues) produced a significant body weight increase of the animals (Hu and Guo, 2006; Biswas et al., 2010).

The use of 0.2% buckthorn flavonesin broiler diets improved the intestinal absorption of the proteins, lipids, Ca and P. Broiler performance was also improved, as shown by larger eviscerated carcasses, less abdominal fat (Michel et al., 2012).

The marigolds contain flavonoids, carotenoids, vitamin C, etheric oils, bitter substances, triterpene saponins, resins and mucilage.

They are a natural resource of de xanthophyll for broiler diets. In broilers, thezeaxanthin-influences the yellow value in all tissues, particularly in the abdominal fat, lutein and zeaxanthin being stored in the skin and adipose tissues in a proportion of 8-12% and 4-9%, respectively (Hamelin and Altemueller, 2012).

Furthermore, the carotenoids are essential to the immune system, have antioxidant effects and as cannot be synthesized by the birds, they have to be supplied in the diets (Breithaupt, 2007; Jung et al., 2012). The marigold extract (lutein) is a xanthophyll with strong antioxidant capacity, frequently used in layer diets (Koutsos et al., 2006).

Starting from the premises that the above-mentioned plants can be seen as Phytoadditives with positive effects on animal health and productivity, the purpose of the feeding trial was to evaluate the feeding properties of the plants in a digestibility trial for their nutritional assessment in laying hens diets.

MATERIALS AND METHODS

Our study characterised physically and chemically four plant phytoadditives (marigold flowers, blueberry leaves, walnut leaves and buckthorn meal) as possible alternatives to antibiotics in poultry feeding. The marigold flowers, blueberry leaves and walnut leaves were purchased from a company specialised in processing medicinal plants. The buckthorn meal was purchased from a company producing edible cold pressed oils. The samples were ground in a laboratory mill (Grindomix – GM 200) for 3 minutes, at 6500 rotations/min, homogenized and dried in a drying cabinet for 48 hours (T = 65^0C) and 24 hours (T = 103^0C). We used standardized analytical methods, according to Regulation (CE) no. 152/2009 (Sampling and analytical methods for the official inspection of feeds) and ISO standards.

Determination of the gross chemical composition of the plants

The dry matter (DM) was determined according to ISO standard 6496/2001 using the gravimetric method, by drying at 65-103^{0}C (Sartorius analytical scaleand BMT model ECOCELL Blueline Comfort); *crude protein* (CP) was determined according to ISO standard 5983-2/2009, using the Kjeldahl method (semiautomatic KJELTEC auto 1030 – Tecator); *ether extractives* (EE) was determined according to ISO standard 6492/2001 by extraction in organic solvents (SOXTEC-2055 FOSS – Tecator); *crude fibre* (ISO 6865/2002) was determined by intermediary filtration (FIBERTEC 2010–Tecator) and the *ash* (ISO 2171/2010 was determined using the gravimetric method (Caloris furnace CL 1206). By calculation, we determined the organic matter (formula 1) and the nitrogen-free extractives (formula 2).

$$OM\ (\%) = DMreal\ (\%) - Ash\ (\%) \quad \text{(formula 1)}$$

$$NFE\ (\%) = OM\ (\%) - (CP + EE + CF) \quad \text{(formula 2)}$$

Where: OM = organic matter; DMreal = real dry matter; Ash = ash; NFE = nitrogen-free extractives; CP= crude protein; EE = ether extractives; CF = crude fibre (formula 2)

Determination of the amino acids

Amino acids from samples were determined by high performance liquid chromatography (HPLC), using a method optimised and validated by Varzaru et al. (2013), and HPLC system Finnigan Surveyor Plus, HyperSil BDS C18 column, size 250 × 4.6 mm, 5µm (Thermo-Electron Corporation, Waltham, MA).

Determination of the minerals

Plant samples of 0.4 g each were processed as described previously (Untea et al., 2012) and analyzed for Ca, Mg, Cu, Fe, Mn, Zn concentrations applying flame atomic absorption spectrometry (atomic absorption spectrometer Solaar M6 Dual Zeeman Comfort (Thermo Electron Ltd., Cambridge, UK) after the microwave digestion (Speedwave MWS-2 Comfort, Berghof, Eningen, Germany). The phosphorus content was determined by UV-Vis spectropho-tometry (UV-Vis spectrophotometer Jasco V530 Tokio, Japan).

Determination of the fatty acids in the plants

The fatty acids were determined by gas chromatography, as shown by Panaite et al., (2016), by transformation in methyl esters of the fatty acids from the sample, followed by the separation of the components in the chromatographic column, identification by comparison with standard chromatograms and quantitative determination of the fatty acids according to SR CEN ISO/TS 17764 -2: 2008.

Determination of the polyphenol concentration

The total phenol content of plants extracts was measured spectrophotometrically according to the Folin-Ciocalteu's method, as described by (Conrad et al., 2001) with slight modifications. The absorbance was measured at 732 nm and the results were reported as mg gallic acid equivalents per 100 mL of sample (mg GAE/ mL).

Determination of the total Antioxidant Capacity (TAC) by phosphomolybdenum method

The total antioxidant capacity of the plant extracts was evaluated by the method of Prieto et al. (2010). The antioxidant activity was expressed for the samples as ascorbic acid equivalents.

RESULTS AND DISCUSSIONS

Table 1 shows the basic chemical composition of the phytoadditives. The crude protein ranged between 6.76% CP (blueberry leaves) and 14.14% CP (buckthorn meal). Also the blueberry leaves had the highest concentration of fibre (33.66% CF), whole the buckthorn meal had a high content of fat (15.38% EE) and the lowest proportion of calcium (0.06%), manganese (19.60%) and zinc (27.76 %).

Table 1 data shows that the buckthorn meal is rich in protein and fat. The buckthorn meal protein content was lower than the values reported by (Kaushal and Sharma, 2011) who reported values of 27.7% to33.2%for crude protein, of 15.0% to 21.9% for crude fibre, and of2.7% to 3.6% for the ash. Sharma (2010) reported 90.06%dry matter (DM); 26.00% crude protein (CP); 4.50% ether extractives (EE); 14.00% crude fibre (CF); 2.50% ash; 53.0% NDF; 0.75% calcium (Ca); 1.25% phosphorus (P) and 2906 kcal/kg metabolisable energy (ME) in the buckthorn meal. Similar values for the buckthorn meal chemical composition were also reported by (Fanatico et al.,2005; Fanatico et al., 2006).

Compared to the buckthorn meal (0.06%), the marigold flowers (0.49%) and blueberry leaves (0.50), the concentration of calcium in the walnut leaves (2.01%) was much higher, similar to the findings of other studies (Ercisli et al., 2005). The marigold flowers had the highest concentration of copper (12.16%) and iron (1262.54 mg), while the blueberry leaves had the highest concentration of manganese (1410.10 mg) and zinc (40.37 mg). The iron concentration in the blueberry leaves (62.86 mg) was lowest of all studied plants. Nevertheless, this value is in agreement with the data reported by Criste et. al., (2013) in an inter-laboratory study in Romania, in which participated seven laboratories, the results ranging between 61.43 to 100.86 mg/kg. It is well known that the iron efficiency uptake in wild plants depends on the Fe source, which is different from most greenhouse experiments (Criste et al., 2013).The concentration of lutein-zeaxanthinin the analysed plant material was highest in the walnut leaves and buckthorn meal, followed by the blueberry leaves. Forty-one different carotenoids have been reported in

various cultivars of sea buckthorn berries, the major types being zeaxanthin, cryptoxanthin, and carotene (Pintea et al., 2005). On the other hand, Andersson et al. (2009) showed that the concentration of xanthophyll in the white buckthorn fruits varies with the geographical area. The lowest concentration of lutein and zeaxanthin was determined in the marigold flowers (11.221ppm). As it is known, lutein has a yellow-orange color, and it has been used for many years in poultry diets as a mean to pigment egg yolks. The content of polyphenol (Table 1), both in the walnut leaves(53.94 mg EAG/g) and in the blueberry leaves(52.82 mg EAG/g) shows that both of them are rich in polyphenol.

Walnut leaves constitute a good source of phenolic compounds, suggesting that it could be useful in the prevention of diseases in which free radicals are implicated. The walnut leaves also are a rich source of polyphenol, where flavonols are major compounds, varying between 54.8% and 62.9% of total phenolics (Pereira, et al., 2007). Bilberries are rich sources of various phenolic compounds and carotenoids (Zotatti et al; 2016).

In the other study, Hokkanen et al. (2009) detected several bioactive compounds in bilberry leaves, such as flavan-3-ols, isomers of cinchonain, proanthocyanidins and coumaroyliridoids. On the other hand, bilberry leaf aqueous extracts are useful as antibacterials and against inflammation, especially inflammation of the oral cavity (Wang et al., 2000).

Table 2 shows that the amino acids (essential – lysine, valine and isoleucine, and nonessential – glutamic acid, arginine and tyrosine) content of the buckthorn meal was higher than the concentration of amino acids determined in the walnut leaves, marigold flowers and blueberry leaves. Close values for lysine (0.780%) in the buckthorn meal, were also determined in the walnut leaves (0.688%), which also had the highest concentration of methionine (0.433%) and cystine (0.133%).

The poultry cannot synthesize essential amino acids, which is why these amino acids have to be included in poultry diets for body proteins synthesis, supporting thus the growth and development of the body mass (Mehri et al., 2012; Kheiri and Alibeyghi, 2017).

Table 1. Chemical composition of the plant materials

Item	Marigold leaves	Blueberry leaves	Walnut leavses	Buckthorn meal
Basic chemical composition, (%)				
SU	89.81	88.37	88.99	90.20
SO	79.02	86.93	79.27	88.48
PB	13.78	6.76	12.83	14.14
EE	5.55	1.38	2.21	15.38
CF	15.09	33. 66	17.41	21.19
NFE	44.6	45.13	46.82	37.77
Ash	10.79	1.44	9.72	1.72
Minerals, (% or mg)				
Macrominerals, (%)				
Ca	0.49	0.50	2.01	0.06
P	0.28	0.19	0.30	0.30
Trace minerals, (mg)				
Cu	12.16	6.95	7.11	9.02
Fe	1262.54	62.87	366.54	405.35
Mn	35.24	1410.10	159.31	19.60
Zn	29.56	40.37	30.17	27.76
Xanthophyll, (ppm)				
Lutein+zeaxanthin	11.221	70.591	264.096	168.757
Total polyphenols, (mgEAG/g)				
Polyphenols	13.55	52.82	53.94	31.9

Where: DM - dry matter; OM–organic matter; CP– crude protein; EE – ether extractives; CF – crude fibre; NFE – nitrogen-free extractives;; Ca - calcium; P – phosphorus; Cu – copper; Fe – iron; Mn – manganese; Zn – zinc; *Analyses performed by the Laboratory of Chemistry and Nutrition Physiology – IBNA Balotești.

In terms of arginine content, an α-aminoacid used for protein synthesis, the buckthorn meal had the highest concentration, 1.526 % arginine, from the total amount of protein. In poultry nutrition, arginine:lysine ratio is very important,influencing meat quality and the appearance or severity of muscle myopathy in broiler breast (Zampiga et al., 2018).

Table 2. Amino acid profile in the plant materials

Item	Marigold leaves	Blueberry leaves	Walnut leavses	Buckthorn meal
Essential amino acids, (%)				
Threonine	0.523	0.498	0.713	0.557
Valine	0.811	0.385	0.724	0.760
Phenylalanine	0.631	0.420	0.968	0.855
Isoleucine	0.523	0.322	0.692	0.737
Leucine	0.797	0.685	1.411	1.304
Lysine	0.397	0.451	0.688	0.780
Methionine	0.372	0.261	0.433	0.410
Nonessential amino acids, (%)				
Ac. aspartic	2.461	0.970	1.643	1.732
Ac. glutamic	2.397	1.148	2.156	2.918
Serine	0.706	0.511	0.920	0.889
Glycine	0.572	0.601	0.883	0.584
Arginine	0.555	0.359	0.831	1.526
Alaina	0.624	0.447	0.933	0.775
Tyrosine	0.163	0.156	0.430	0.465
Cystine	0.112	0.082	0.133	0.122
Total	11.643	7.296	13.559	14.415

* Analyses performed by the Laboratory of Chemistry and Nutrition Physiology – IBNA Balotești.

The most important polyunsaturated fatty acid, theα-linolenic acid, was determined in the

highest amount in the walnut leaves (13.45%), followed by the marigold flowers (5.54%) and blueberry leaves (5.47%). However, the blueberry leaves had the best omega6/omega3 ratio, of 1.22, followed by the walnut leaves, with 0.75, and marigold flowers, with 1.6. Walnuts contain about 10% linolenic acid which has been associated with reduced risk in several prospective studies possibly due to antithrombotic and antiarrhymic effects of thelinolenic acid (Dolecek, 1992; Ascherto et al., 1996).

The fatty acids concentration of the plant materials (Table 3), shows that the highest concentration of caproic acid (C6:0) was in the blueberry leaves (3.10%) and in the walnut leaves (3.94%), being absent in the buckthorn meal. Some studies show that the supplements ofcaproic acid (3g/kg feed) for broilers, decreased significantly the number of colony-forming unis in the caecum of broilers, 3 days after the birds were challenged with *Salmonella Enteritidis* (Van Immerseel et al., 2004)

Table 3. Fatty acids profile of the plant materials

Item		Marigold leaves	Blueberry leaves	Walnut leavses	Buckthorn meal
Butyric	C 4:0	0.00	0.58	0.72	-
Caproic	C 6:0	1.76	3.10	3.94	-
Caprilic	C 8:0	8.36	0.71	0.05	-
Nonanoic	C 9:0	2.94	-	-	-
Capric	C 10:0	4.13	3.96	0.08	0.04
Undecanoic	C 11:0	0.48	0.00	0.08	-
Lauric	C 12:0	3.56	0.31	0.00	-
Tridecanoic	C 13:0	0.16	-	-	-
Miristic	C 14:0	19.14	8.48	0.73	0.41
Miristoleic	C 14:1	0.63	1.07	0.00	
Pentadecanoic	C 15:0	0.41	1.10	0.10	0.00
Pentadecenoic	C 15:1	0.40	1.17	0.07	22.01
Palmitic	C 16:0	25.38	46.51	29.84	-
Palmitoleic	C 16:1	0.91	0.43	1.73	13.46
Heptadecanoic	C 17:0	0.29	0.24	0.84	0.00
Heptadecenoic	C 17:1	0.42	0.00	0.25	0.00
Stearic	C 18:0	3.95	3.84	11.29	1.91
Oleic cis	C 18:1	6.02	9.02	22.09	36.85
Linoleic cis	C 18:2n6	7.22	4.53	10.02	21.14
Arachic	C 20:0	0.08	0.00	0.10	-
Eicosenoic	C20 (1n9)	0.08	0.00	0.08	-
Linolenic α	C 18:3n3	5.54	5.47	13.45	2.34
Heneicosanoic	C 21:0	-	0.14	0.00	-
Octadecatetraenoi	C18:4n3	0.55	1.22	0.85	1.36
Eicosadienoic	C20(2n6)	0.00	0.00	0.15	0.50
Behenic	C 22:0	2.06	0.83	0.00	-
Eicosatrienoic	C20(3n6)	0.00	-	-	-
Erucic	C22 (1n9)	0.00	-	-	-
Eicosatrienoic	C20(3n3)	0.00	-	-	-
Arachidonic	C20(4n6)	0.14	0.18	0.00	-
Docosadienoic	C22(2n6)	0.45	1.62	0.75	-
Tricosanoic	C 23:0	0.00	1.04	0.00	-
Eicosapentaenoic	C20(5n3)	0.37	0.00	0.17	-
Lignoceric	C24:0	0.61	0.00	0.26	-
Nervonic	C24 (1n9)	0.00	1.79	0.97	-
Docosatetraenoic	C22(4n6)	2.54	1.81	0.00	-
Altiacizigrasi		1.44	0.85	1.40	-
Total acizigrasi		100	100	100	100
Clasele de acizigrasi din grasime					
SFA		73.31	70.84	48.01	24.37
MUFA		8.45	13.48	25.20	50.30
PUFA, din care:		16.80	14.83	25.39	25.33
Ω3		6.46	6.69	14.47	3.70
Ω6		10.34	8.14	10.92	21.63
Ω6/Ω3		1.60	1.22	0.75	5.85

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CONCLUSIONS

The data reported in this paper support the fact that the analysed plants can be seen as phytoadditives with positive effects on animal health and productivity, even on human health. Furthermore, it results that they are rich sources of nutrients and that they meet the nutritional requirements for use as ingredients in layer diets.

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