

COMPOSITION OF SOME BOTANICAL MIXTURES AS POTENTIAL FEED ADDITIVES FOR LAYING HENS

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ABSTRACT: The aim of this study was to assess the nutritional quality of four botanical mixtures (AFC): AFC 1 (containing red corn, pumpkin pulp and marigold), AFC 2 (containing alfalfa meal, pumpkin pulp and marigold), AFC 3 (containing kale, alfalfa meal, marigold and spinach leaves), AFC 4 (containing buckthorn, red corn, pumpkin pulp and marigold), in terms of proximate analysis (crude protein, crude fat, crude fiber, ash), amino acid (AA) profile, vitamin E concentration and lutein and zeaxanthin content, in order to determine the potential of AFCs as feed additives in laying hens nutrition. The crude protein content for the analysed botanical mixtures ranged between 9.07-18.18% DM, and crude fiber between 10.41-30.83% DM. The amino acid profile of the mixture AFC 4 revealed a content of limiting essential amino acids required for laying hens: lysine 5.719% CP, methionine 1.058% CP and threonine 4.415% CP. The highest content of lutein and zeaxanthin was found in the mixture AFC 4 (66.659 mg/100 g), which also had the highest amount of vitamin E (640.93 mg/kg). With regard to safety of the botanical mixtures, lead and cadmium concentrations were determined. Concentration of lead ranged from 0.28-0.75 µg/g DM and 0.06-0.09 µg/g DM for concentration of cadmium, which was within the legislation of maximal limits of EU regulations. It can be concluded that the botanical mixture AFC 4 had the highest concentration of lutein, zeaxanthin and vitamin E, with an adequate content of essential amino acids. Furthermore, all four botanical mixtures had high amounts of xanthophylls and should be tested in laying hens trials in order to establish their effects on lutein and zeaxanthin concentration in egg yolk.

Keywords: *botanical mixtures, lutein, zeaxanthin, amino acids, vitamin E*

INTRODUCTION

Lutein and zeaxanthin are plant pigments, members of the xanthophylls family of carotenoids (Alves-Rodrigues and Shao, 2004). They are believed to be the only carotenoids vital to retinal function, being the only ones found in the macula of the retina where selectively accumulate. Within the eye, lutein has a protective role against age-related macular degeneration (AMD) which are the principal causes of

blindness (Abdel-Aal et al., 2013). The mechanisms by which lutein and zeaxanthin are thought to provide protection to the eye are through their roles as blue light filters and as antioxidants (Snodderly, 1995). Animals cannot synthesize lutein and zeaxanthin, though they have the ability to absorb carotenoids from their diet and deposit them into tissue. Xanthophylls biosynthesis only takes place in plants,

algae, bacteria and certain fungi (Kijlstra et al., 2012). Humans cannot synthesize both pigments but are able to deposit dietary pigments as absorbed or with slight modification of their structure (Larsen and Christensen, 2005). Therefore, their presence depends on entry into the organism through nutrition.

High concentrations of lutein and zeaxanthin occur in green leafy vegetables, such as spinach, kale, lettuce and broccoli (Perry et al., 2009). An important content of these xanthophylls was also reported in green bean, dill, parsley and endive (Maiani et al., 2009). Green leafy vegetables represent a rich source of lutein and zeaxanthin, but with a smaller availability when compared to eggs.

Eggs are a particularly useful source of xanthophylls pigments, as they naturally contain a relatively high proportion of lutein and zeaxanthin (Thurnham, 2007). A comparative study showed that serum lutein response following egg consumption was approximately two to three times higher as compared to lutein from spinach, because eggs provide a fat containing environment and relatively simple food matrix (Chung et al., 2004).

As with most fats and fat-soluble compounds, the composition of egg is responsive to manipulation of such nutrients in the layer diet (Leeson and Caston, 2004). Several authors reported that lutein supplementation in layer diet cause an increased lutein concentration in layers plasma (Wu et al., 2009) and in egg yolk (Leeson and Caston, 2004; Hammershøj et al., 2010; Kotrbáček et al., 2013; Englmaierová et al., 2013).

The botanical mixtures were proposed for the study by a producer of natural supplements designed for human consumption, for the recovery of the by-products resulted from the production processes. Because these mixtures were studied with the purpose of using them as feed additives, several determinations were performed beside lutein and zeaxanthin, in order to assess their nutritional quality and safety. Thus, proximate and amino acid analysis was performed. Taking into account the EU regulations for feed additives

safety, the content of lead and cadmium was also determined. The vitamin E, which is an antioxidant found also in egg yolk, was determined in the botanical mixtures, as it can have the potential of adding nutritional value to the AFCs.

The overall aim of this study was to assess the nutritional quality of four botanical mixtures as potential feed additives in laying hens nutrition.

MATERIAL AND METHODS

The samples origin:

The botanical mixtures (AFC) were manufactured for this study by SC HOFIGAL Export-Import SA, Romania, and they were prepared from cultivated vegetable materials without using chemical stimulators (Table 1). It is intention that the AFCs with potential as feed additives, as established by this study, are manufactured by the producer in the appropriate amounts required to conduct an experimental study on laying hens.

Chemical analysis:

The sample preparation and determination of crude protein (CP) concentrations of four AFCs were performed according to SR EN ISO 5983-2 (2009), which involved a semiautomatic Kjeldahl method using a Kjeltec auto 2300 – Tecator (Foss, Sweden). The crude fat was extracted using an improved version of the method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal according to SR ISO 6492 (2001) method. The crude fibre was determined using a semiautomatic Fibertec-Tecator method (SR EN ISO 6865, 2002) and the ash by incineration at 550 °C according to SR EN ISO 2171 (2010) method.

The AFC samples were prepared and analysed for lead (Pb) and cadmium (Cd) concentrations applying flame atomic absorption spectrometry (FAAS) as described by Untea et al. (2012), after the microwave digestion. The used equipment was an atomic absorption spectrometer Thermo Electron – SOLAAR M6 Dual Zeeman Comfort (Cambridge, UK), with deuterium lamp for background correction and air-acetylene flame.

Table 1.
Botanical mixtures (AFC) composition*

AFC 1	AFC 2	AFC 3	AFC 4
50% red corn (<i>Zea mays</i>)	50% alfalfa meal (<i>Medicago sativa</i>)	50% alfalfa meal (<i>Medicago sativa</i>)	40% buckthorn leaves (<i>Hippophae rhamnoides</i> L.)
30% pumpkin pulp (<i>Cucurbita maxima</i>)	20% pumpkin pulp (<i>Cucurbita maxima</i>)	8% kale leaves (<i>Brassica oleracea</i>)	20% pumpkin pulp (<i>Cucurbita maxima</i>)
20% marigold flowers (<i>Tagetes erecta</i>)	30% marigold flowers (<i>Tagetes erecta</i>)	34% marigold flowers (<i>Tagetes erecta</i>)	20% marigold flowers (<i>Tagetes erecta</i>)
-	-	8% spinach leaves (<i>Spinacia oleracea</i>)	20% red corn (<i>Zea mays</i>)

*The data regarding the amount of the botanicals in mixtures were provided with the consent of the producer

In order to determine the amino acids profile of the AFC samples, an HPLC Surveyor Plus Thermo Electron (Massachusetts, United States), and Hyper-SilBDS C18 column (Thermo Electron, Massachusetts, United States), dimensions 250 mm × 4.6 mm × 5 µm were used. The samples were prepared as described by Varzaru et al. (2013).

Lutein and zeaxanthin were determined using an HPLC serie 200 (Perkin Elmer, USA) and a Nucleodur C18 ec column (Macherey-Nagel, Germany), dimensions 250mm × 4.6 mm × 5 µm, the HPLC techniques being the most efficient for carotenoids analysis in complex mixtures (Khachik, 2009). The mobile phase consisted of acetone and water as used by Cernelic et al. (2013). For the vitamin E determination the same equipment as in the case of the amino acid analysis was used. Before chromatographic analysis, the samples were prepared (saponificated and extracted with an organic solvent), according to the method described in the Commission regulation (EU) No. 152/2009 (2009).

Statistical analysis:

All determinations were performed in triplicate. Analysis of variance (ANOVA) and Fisher's least square difference (LSD) tests were applied to compare means at 5% significance level using the statistical data analysis software StatView for MS Windows (Statistical Analysis System, Version 6.0). Results were expressed as the mean of replications ± SD for all mea-

surements.

RESULTS AND DISCUSSION

The botanical mixtures examined in this study have not been investigated before, thus having an innovative character from the point of view of their composition. Several authors have studied different herbal formulas in order to observe their pigmentation ability on the egg yolk. Dried marigold petals and marigold concentrates were used as feed additives to improve the pigmentation of the poultry skin and the eggs of laying hens (Liu et al., 2011). Alfalfa concentrate, tomato powder and marigold extract, single or in mixtures, were investigated for their potential as feed additives in quail diet, increasing the yolk concentrations of lutein, zeaxanthin, lycopene and β-carotene in eggs produced by female quail fed diets supplemented with natural carotenoids (Karadas et al., 2006).

The crude protein content of the analysed botanical mixtures ranged between 9.70 - 18.18% DM, and crude fiber between 10.41 - 30.83% DM (Table 2). The highest concentration of crude protein was recorded in the mixture AFC 3 (18.18% DM), which also had the highest content of crude fiber (30.83% DM) and ash (8.29% DM), while the crude fat content for AFC 3 was the lowest one (0.81% DM) from the analyzed botanical mixtures.

The results for the proximate composition ranged between the values obtained by other authors for the ingredients of our botanical mixtures. Studing the effects of

diets including various proportions of marigold flower on the pigmentation of rainbow trout, Büyükçapar et al. (2007) reported that the proximate composition of the marigold flower was: 12.22% crude protein, 9.20% crude fat and 14.3% crude fiber. Depending on the plants stage of growth Tedeschi et al. (2001) recorded a crude protein content for alfalfa of 17.2 - 21.7%, and Homolka et al. (2008) a crude fibre of

25.4-40.1%. Spinach leaves were characterized by 19.82% crude protein, 3.32% crude fat, 4.92% crude fiber and 19.34% ash (Chaturvedi et al., 2013). The chemical composition of buckthorn leaves, determined by Kashif and Ullah (2013) revealed 11.06% crude protein, 5.81% crude fat, 17.31% crude fiber and 7.12% ash. In a study conducted by Pasha et al. (2013), the crude protein content of the

Table 2.
Proximate analysis of botanical mixtures (AFC)

AFC	dry matter	crude protein	crude fat	crude fiber	ash
	%	% DM	% DM	% DM	% DM
AFC 1	94.82 ± 2.8 ^a	9.70 ± 0.3 ^a	3.57 ± 0.1 ^a	10.41 ± 0.3 ^a	3.53 ± 0.1 ^a
AFC 2	96.05 ± 3.8 ^a	16.11 ± 0.6 ^b	1.67 ± 0.05 ^b	28.82 ± 1.2 ^b	6.78 ± 0.2 ^b
AFC 3	96.87 ± 3.3 ^a	18.18 ± 0.5 ^c	0.81 ± 0.02 ^c	30.83 ± 0.9 ^b	8.29 ± 0.2 ^c
AFC 4	94.76 ± 3.6 ^a	14.18 ± 0.5 ^d	4.01 ± 0.1 ^d	15.29 ± 0.4 ^c	5.40 ± 0.2 ^d

Results are expressed as a mean ± SD

Values with the different superscript in the same column are statistically different (P<0.05)

Table 3.
Lead and cadmium concentration in botanical mixtures (AFC)

AFC	Pb	Cd
	µg/g DM	µg/g DM
AFC 1	0.28 ± 0.01 ^a	0.08 ± 0.005 ^a
AFC 2	0.75 ± 0.04 ^b	0.08 ± 0.004 ^a
AFC 3	0.57 ± 0.03 ^c	0.09 ± 0.005 ^a
AFC 4	0.71 ± 0.04 ^b	0.06 ± 0.003 ^b

Results are expressed as a mean ± SD

Values with the different superscript in the same column are statistically different (P<0.05)

Table 4.
Amino acid profile of botanical mixtures

Amino acid	AFC 1	AFC 2	AFC 3	AFC 4
	g/100 g CP			
Aspartic acid	9.670 ± 0.3 ^a	10.770 ± 0.3 ^b	13.713 ± 0.4 ^c	12.059 ± 0.4 ^d
Glutamic acid	25.515 ± 0.8 ^a	16.089 ± 0.5 ^b	16.216 ± 0.5 ^b	17.997 ± 0.5 ^c
Serine	8.474 ± 0.3 ^a	8.672 ± 0.3 ^a	9.807 ± 0.4 ^b	9.598 ± 0.4 ^b
Glycine	5.206 ± 0.2 ^a	6.511 ± 0.2 ^b	7.321 ± 0.3 ^c	6.685 ± 0.2 ^b
Threonine	3.742 ± 0.1 ^a	5.140 ± 0.2 ^b	4.824 ± 0.1 ^c	4.415 ± 0.1 ^d
Arginine	6.423 ± 0.3 ^a	5.307 ± 0.2 ^b	6.023 ± 0.3 ^{a,d}	5.656 ± 0.2 ^{b,d}
Alanine	4.485 ± 0.2 ^a	3.873 ± 0.1 ^b	4.587 ± 0.2 ^a	4.316 ± 0.2 ^a
Tyrozine	1.670 ± 0.08 ^a	1.533 ± 0.07 ^a	1.738 ± 0.08 ^a	2.116 ± 0.1 ^b
Valine	6.052 ± 0.3 ^a	6.406 ± 0.3 ^{a,b}	6.991 ± 0.4 ^b	6.396 ± 0.3 ^{a,b}
Phenylalanine	4.938 ± 0.2 ^a	4.761 ± 0.2 ^a	5.539 ± 0.3 ^{b,c}	5.226 ± 0.3 ^{a,b}
Isoleucine	2.979 ± 0.1 ^a	3.191 ± 0.2 ^{a,b}	3.520 ± 0.2 ^b	3.293 ± 0.2 ^b
Leucine	5.351 ± 0.3 ^a	4.531 ± 0.2 ^b	5.297 ± 0.3 ^a	5.007 ± 0.3 ^{a,b}
Lysine	4.072 ± 0.2 ^a	5.078 ± 0.2 ^b	6.007 ± 0.4 ^c	5.719 ± 0.3 ^c
Cystine	1.402 ± 0.09 ^a	1.124 ± 0.07 ^b	0.946 ± 0.05 ^b	1.058 ± 0.07 ^b
Methionine	1.825 ± 0.1 ^a	1.080 ± 0.1 ^b	1.106 ± 0.1 ^b	1.051 ± 0.9 ^b

Results are expressed as a mean ± SD

Values with the different superscript in the same row are statistically different (P<0.05)

pumpkin pulp was 9.68%, similar with the value registered for the mixture AFC 1, but with the crude fiber (0.84%) and crude fat (0.83%) lower than the ones obtained for AFC 1.

The contaminants analysis of the four mixtures (Table 3) showed that the concentrations of lead and cadmium were below the maximal limits (5 ppm for lead and 1 ppm for cadmium) allowed by the legislation in force (Regulation no. 358/15.04.2003, harmonized with EU regulations).

The method used for amino acid analysis involved determination of 15 amino acids. The AA profile contains data regarding the limiting amino acids in animal nutrition (Table 4). The amino acid determination from the four mixtures revealed that the

mixture AFC 1 had the highest concentrations of sulphur essential amino acids important in human nutrition: methionine (1.825% CP) and cystine (1.402% CP). The highest content of lysine was found in the mixture AFC 3 (6.007% CP), which also had the highest content of CP. The amino acid analysis of the alfalfa meal (Tkáčová et al., 2011) revealed a lysine content of 4.23% CP, which is closer to the value obtained for AFC 1, and a methionine content of 1.46% CP, higher than the values obtained for mixtures AFC 2, AFC 3 and AFC 4.

The buckthorn leaves, which represent 40% of the mixture AFC 4, were analyzed by Christaki (2012) who found a lysine content of 3.52% CP, and methionine and cystine concentration of 0.62% CP.

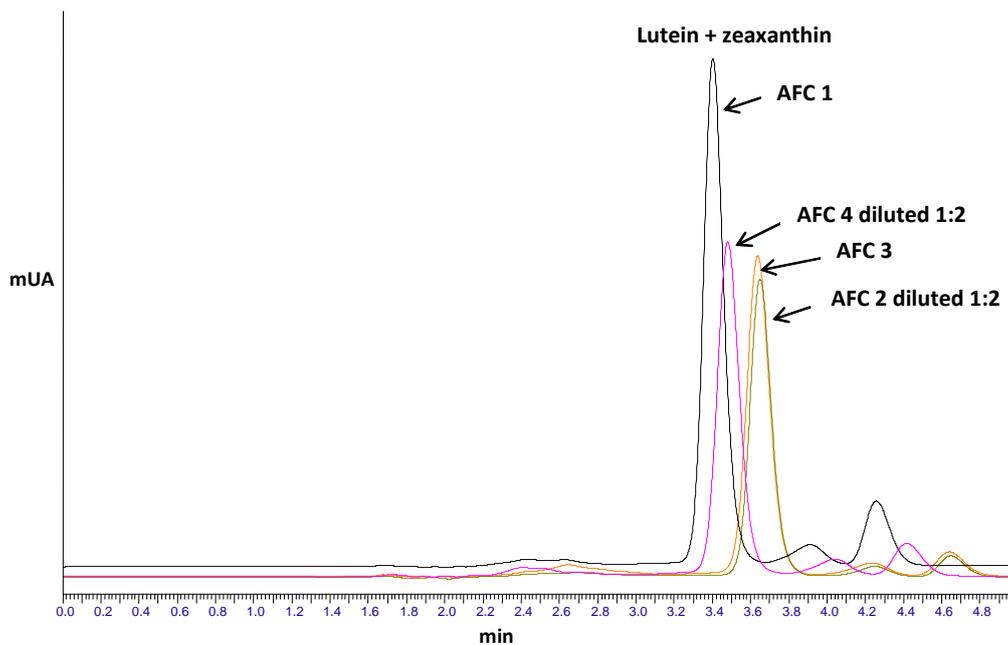


Figure 1. Overlaid chromatograms from the lutein and zeaxanthin analysis of four botanical mixtures

Table 5.

Lutein, zeaxanthin and vitamin E concentration in botanical mixtures

Sample	Lutein + zeaxanthin mg/100 g DM	Vitamin E mg/100 g DM
AFC 1	47.175 ± 1.11 ^a	17.129 ± 0.5 ^a
AFC 2	57.503 ± 0.67 ^b	13.500 ± 0.4 ^b
AFC 3	34.530 ± 0.66 ^c	6.671 ± 0.2 ^c
AFC 4	66.659 ± 1.83 ^d	64.093 ± 2.2 ^d

Results are expressed as a mean ± SD

Values with the different superscript in the same column are statistically different (P<0.05)

These results are below the values determined for lysine, methione and cystine in the mixture AFC 4.

Lutein and zeaxanthin chromatographic analysis in four botanical mixtures is presented in Figure 1.

The concentrations of lutein and zeaxanthin ranged between 34.530 – 66.659 mg/100 g DM. (Table 5). The highest content of these xantophylls was found in AFC 4 (66.659 mg/100 g DM, which also had the highest amount of vitamin E (640.93 mg/kg DM) (Table 5).

Lutein is present in significant amounts in maize, alfalfa and petals of marigold, from which it is extracted for commercial use (Lavecchia and Zuurro, 2008). Analyzing different cultivars of marigold, Li et al. (2007) observed considerable variations in their lutein ester contents, ranging from 161.0 to 611.0 mg/100 g of flower (on DM basis). The lutein and zeaxanthin values obtained for four botanical mixtures were lower than those reported by Li et al. (2007) for marigold, but an increased content can be observed when comparing the data with ones recorded by Elgersma et al. (2012) when analyzing alfalfa (12.9 mg/100 g).

Guan et al. (2005) found that buckthorn fresh leaves are rich in total carotenoids (26.3 mg/100g), but the registered content was lower than lutein and zeaxanthin content found in the mixture AFC 4, which had 40% of buckthorn. A study conducted by Kopsell et al. (2009) revealed that lutein content in kale can vary from 4.8-13.4 mg/100 g, and in spinach between 6.6 – 13 mg/100 g. Dias et al. (2009) reported a lutein concentration of 0.52- 6.4 mg/100 g in kale and 3.6 – 5.6 mg/100 g in turnip leaves.

Interspecific interaction can affect leaf/stem ratio and the vitamin composition of plant organs. Leaf proportion is the largest factor affecting the α -tocopherol content in forages (Ballet et al., 2000). The buckthorn is known for the high vitamin E concentrations in seed oil (207 mg/100 g) and pulp oil (171 mg/100 g) (Zeb, 2004).

CONCLUSIONS

The nutritional quality assessment of the four proposed botanical mixtures, showed that the mixture AFC 4 (containing buckthorn, red corn, pumpkin pulp and marigold) had the highest concentration of vitamin E, lutein and zeaxanthin, with an adequate content of essential amino acids.

All the analyzed botanical mixtures had a higher content of xantophylls compared to spinach, kale and buckthorn leaves, which are considered to be rich sources of lutein and zeaxanthin. The botanical mixtures should therefore be tested in laying hens trials in order to establish the effects on lutein and zeaxanthin concentration in egg yolk.

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САСТАВ НЕКИХ БИЉНИХ СМЕСА КАО ПОТЕНЦИЈАЛНИХ АДТИВА У ХРАНИ ЗА КОКЕ НОСИЉЕ

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Сажетак: Циљ овог истраживања био је да се утврди квалитет четири биљне смесе (АФЦ): АФЦ 1 (која је садржавала црвени кукуруз, пулпу бундеве и невен), АФЦ 2 (брашно луцерке, пулпа бундеве и невен), АФЦ 3 (кељ, брашно луцерке, невен и лишће спанаћа) и АФЦ 4 (пасјаковина, црвени кукуруз, пулпа бундеве и невен), као потенцијалних адитива у исхрани кока носилја. Одређен је основни хемијски састав смеса (силови протеини, сирови маст, сирови влакна и пепео), њихов аминокиселински састав, садржај витамина Е, као и садржај лутеина и зеаксантина. Садржај сирових протеина у биљним смешама био је у опсегу 9,07-18,18% у сувој материји (СМ), док је садржај сирових влакна износио 10,41-30,83% у СМ. Садржај лимитирајућих есенцијалних киселина у смеси АФЦ 4 је одговарао потребама кока носилја и садржавао је 5,719% лизина у сировим протеинима (СП), 1,058% метионина у СП и 4,415% треонина у СП. Највећи садржај лутеина и зеаксантина је утврђен у смеси АФЦ 4 (66,659 mg/100 g), која је такође имала и највећи садржај витамина Е (640,93 mg/kg). У циљу утврђивања здравствене безбедности испитиваних смеса, одређене су концентрације олова и кадмијума. Концентрација олова је била у опсегу 0,28-0,75 µg/g у СМ, а кадмијума 0,06-0,09 µg/g у СМ, односно биле су у границама дозвољеним прописима ЕЗ.

Може се закључити да је биљна смеша АФЦ 4 имала највишу концентрацију лутеина, зеаксантина и витамина Е, са адекватним садржајем есенцијалних аминокиселина. Све четири смесе су имале висок садржај ксантофила, па би их требало испитати у огледима на кокама носилјама, како би се утврдио њихов утицај на концентрацију лутеина и зеаксантина у жуманцу јајета.

Кључне речи: биљне смесе, лутеин, зеаксантин, аминокиселине, витамин Е

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