Research on Chemical Composition and Nutritive Value of Green Fodder Used in Ecological Production of Eggs for Consumption

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The aim of this paper is to study the chemical composition of a green fodder used in laying hens feeding raised in ecological system; organic fodder being an important condition to obtain organic eggs for consumption. In order to evaluate the chemical composition variations of the green fodder throughout the year, there were taken plants samples in three different vegetation stages (May, September and October). To each period, were done 5 determinations for the following indicators: dry matter - DM (%), ash - Ash (%), crude protein - CP (%), ether extract - EE (%), crude fiber -CF(%), nitrogen free extract - (NFE%) calcium – Ca(%), phosphorus - P(%), magnesium - Mg (%), natrium -Na(%). And also, were analyzed the relevant indexes for the organic production: lead -Pb (mg/kg DM), cadmium - Cd (mg/kg DM), cooper -Cu (mg/kg DM), zinc - Zn (mg/kg DM) nitrites (ppm), nitrates (ppm), pesticides. After analysis, it was noticed that dry matter, minerals, organic matter and protein content varied very significantly between the first period and the third ($p \leq 0.001$), while the fat content, crude fiber and nitrogen free extract did not register significant differences ($p \geq 0.05$). Regarding the minerals elements analyzed, P and Mg differed significantly distinctly between the first and the last analyzed period. The content of green fodder in Pb, Cd, Cu and Zn was below the limits allowed by current legislation. The analyses for pesticides have highlighted the lack of them in the fodder. The quality parameters of the analyzed green fodder, expressed through levels of pollutants such as heavy metals, nitrites, nitrates, pesticides, allow us to notify that the green fodders achieve the conditions necessary for organic production.

Keywords: eggs, ecological system, heavy metals, pollutants, fodder

To obtain large agricultural and livestock production requires the use of chemicals in agriculture and also, the use of antibiotics, which may have a negative effect on the environment and consumer health. Thus, the food may contain foreign substances less compatible with metabolism (such as, pesticides, nitrites, nitrates, heavy metals) from soil, air, technological processes or sanitation (antibiotics). There were conducted various studies which show their impact on human health [1-5].

Pesticides may provoke risks for the environment, humans and animals because they have remanence in the organs of plants and animals [6-7]. They may inflict immediate reactions (skin and eye irritation, headaches, dizziness and nausea) or chronic illness (cancer, asthma, diabetes) [8-11], affecting the immune system [12].

The heavy metals (Pb, Cd, Cu, Zn etc.) which are accumulated in the body may cause chronic diseases [13, 14], neurodegenerative diseases [15, 16]; we have to keep in mind that children are the most vulnerable group [17-19].

Antibiotics used in plant and animal production are found out in the finished product and are ingested by humans which may cause antibiotic resistance [20-23].

Numerous studies show the existence of antibiotic resistance for *Staphylococcus aureus* [24-26], *Streptococcus pneumonia* [27], *Escherichia coli* [28] and other microorganisms in cows, poultry and pigs farms that use antibiotics in their diets.

Organic products may be an alternative in reducing antibiotic resistance [29-33]. The organic production system provides a healthier food compared to the conventional one, eliminating the risk of contamination with these pollutants, in full correlation with the conservation and development of the environment. The primary goal of organic farming is to obtain authentic food products through nature-friendly processes. This system prohibits the use of GMOs, pesticides, growth hormones, antibiotics [34].

EC Regulation 834/2007 [35] presents the organizational framework for obtaining and certifying organic products. Through the assigned measures, from the poultry feeds providing until eggs packing and storing, it has to be avoided the contamination of food sources with pesticides, antibiotics, heavy metals, nitrites, nitrates etc.

Worldwide and in our country, many vegetal and animal products are obtained in the ecological system; the production of the ecological egg being of great interest. The egg is an important source of protein, energy, minerals, vitamins [36-41] which are needed for a balanced diet. The food quality (including eggs) influences the consumer's health and through organic production system may be assured the conditions to obtain quality products [34, 42-44].

Currently there are organic farms which produce eggs according to this system [45]. In order to obtain organic eggs, it is necessary that the administered fodder (mixed feed, pasture) and the hens' maintenance to comply with the EC Regulation no. 834/2007 [35]. The alfalfa hay may also be used for the laying hens feeding [46] and also, the green fodder which is in the paddock may be taken into account as hen's feed to obtain the ecological eggs [47].

The nutritional value of green fodder varies according to the production system: ecological or conventional [48]. The chemical composition of organic green fodder varies depending on many factors, including species [49], soil, region, season [50] but also the plant stage of vegetation [51, 52]. The chemical composition of the plant determines the fodder nutritional value [53-56], and also, it influences

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the animal health and the species and categories productivity being necessary to know it.

The egg chemical compositions which are obtained from an ecological system depend on the chemical composition of the fodder (ecological mixed fodder, green fodder from the paddock and additional food in the paddock) [57, 58].

Heavy metals, pesticides, nitrites and nitrates are potentially relevant indicators for the ecological system and their level determination in fodder offers data regarding the pollution level.

There are numerous studies regarding the plants content in heavy metals [59-68]. The fodders contamination sources in heavy metals are various: mineral fertilizers, water irrigation, organic fertilizers, amendments, car aerosols, coal combustion, various industries [69-72].

In this context, the chemical composition analyses of the pasture from the paddock and also, the analyses of potential toxic elements level (heavy metals, nitrates, nitrites, pesticides) are determinant in knowing the quality of these feeding sources in order to produce organic eggs for consumption.

Experimental part

Material and method

For analysis, there were taken samples of green fodder from the hatched paddock with an area of 2 ha, which represents the ecological pasture of a farm with a stock of 2800 laying hens. The samplings were done throughout the year (period I, before flowering – in May; period II – in September; period III - in October) corresponding to different vegetation stages of the plant. The pasture floral composition was formed from: *Dactilis glomerata* 52.06%, *Medicago sativa* 20.46% and the remaining 27.48% was composed by *Achillea millefolium*, *Medicago falcata*, *Salvia nemorosa*, *Gallium verum*, *Plantago lanceolata*, *Trifolium repens*, *Lotus corniculatus*, *Centaurea orientalis*, *Glomerata campanula*, *Echium vulgare*, *Knautia arvensis*, *Capsella bursa pastoris* and *Taraxacum officinale*.

The chemical composition of ecological green fodder was analyzed on 15 samples (5 samples for each period).

The dry matter content (DM%) was determined by drying the fodder samples to 105°C for 6 h to the electric oven ESAC 50. The drying was repeated till constant mass, according to the standards SR ISO 6496:2001 [68] and SR ISO 712:2010 [74].

The ash (Ash%) was determined according to ISO 2171:2010 [75] and AOAC 1990 [76] by burning the samples at 550°C. There was used the calcination furnace Superterme STC 611.06.

Crude protein (CP%) was determined using the Kjeldahl method (mineralization, distillation, titration) as described in ISO 5983-1:2006 [77].

Determination of ether extract (EE%) was done using the Soxhlet method in accordance with ISO 6492:2001 [78]. There was used the Solvent Extractor SER 148-VELP.The crude fiber determination (CF%) was done according to SR EN ISO 68:2002. The semi-automatic method was used, using the VELP FIVE 6 fiber extraction system, and the reagents were: hydrochloric acid 0.5 mol/L, sulfuric acid 0.3 mol/L, acetone, n-octanol, antifoam agent.

The chemical parameters determination (DM, Ash., CF, CF, CP) were used to calculate the organic matter (OM%) and nitrogen free extract (NFE) with the following formula:

OM% = DM%-Ash%,

NFE% = OM% - (CP%+EE%+CF%).

Phosphorus content was determined spectrophotometric using the vanadium-molybdenum reagent by measuring the absorbance's at a wavelength of 430 nm on a Shidmadzu Uvmini 1240 spectrophotometer. The calcium, magnesium and sodium content was determined using the atomic absorption spectrometry method according to SR EN ISO 6869:2002 [79]. There was used the atomic absorption spectrometer with AA-6300 SHIMATZU flame.

The content of heavy metals (lead, cadmium, cooper and zinc) was determined according to SR EN 14082:2003 [80], AOAC, 1990 [76] using atomic absorption spectrometry (AAS) on a GBC-AVANTA type spectrometer.

Calibration curves for lead were made in 5 points (0.5; 1; 2.5; 5 and 7.5 ppm) for cadmium in 5 points (0.2; 0.5; 1; 1.5 and 2 ppm) for copper at 3 points (1, 2 and 4 ppm) and for zinc in 4 points (1, 2, 3 and 4 ppm). The wavelengths to which the metal concentration was determined were Pb: λ = 217 nm; Cd: λ = 228.8 nm; Cu: λ= 324.7 nm; Zn: λ = 213.9 nm. The results were obtained using the formula:

$E\ mg \cdot kg^{-1} = (CV \cdot 1000)/(M \cdot 1000^{x})$,

where:

E - the content of the analyzed element; ±

C - the quantity taken from the standard curve, $\mu g \cdot mL^{-1}$;

V - total volume of the sample solution (50 mL);

M - the quantity of sample taken at work, (g); 1000 - content reporting factor per 1000g; 1000^{x} - the conversion factor of ìg in mg.

The determination of organochlorine and organophosphorus pesticide residues was performed by the gas chromatography method according to SR EN ISO 14181:2001 [81], SR EN ISO 14182:2001 [82] for nutrients. The following steps were taken: fat extraction and purification, pesticide residues extraction, purification and concentration of the fat from the sample, and then reading the gas chromatograph. A mixture of 80 organochlorine and organophosphorus pesticides was used as the standard solution.

Nitrates and nitrites were determined according to SR 13175:1993 [83]. The method consisted in nitrites extraction from the analyzed samples through extract deproteinising and highlighting the red nitrification reaction of nitrites with sulphanilamide chloride and naphthyl-1-ethylene diamide dichlorohydrate. Photocolorimetry was done at a wavelength of 538 nm. The nitrates determination was done by its reducing to nitrites in the presence of cadmium followed by the colouration and colorimetry reaction. For this determination was used the UVmini1240 SHIMADZU spectrophotometer.

The calculation of the energy value (kcal/kg DM) of the fodder samples was done using the NRC system formula, 1994: EM = 35.3 x CP%+79.5 x EE%+40.6 x FE%+199 [84].

The data obtained from chemical analyses were statistically processed and interpreted. There have been calculated the position and variance estimators such as, arithmetic mean, variance, standard deviation, standard deviation of arithmetic mean, and the variance coefficient. Establishing the significance of the differences for the values obtained from the fodder samples from the three periods has been made using the statistical program IBM SPSS 21.0 and also, the Tukey test with two variables and T-Test (2-tailed).

Results and discussions

The dry matter content of the analyzed samples oscillated between 21.98 and 25.15%. These values are close to those mentioned by Singh (24.12-25.9%) [85].

At the beginning of the plant growing, the water content of the plants is higher and decreases towards the final stages of vegetation. Thus, there are significant differences between the periods I (May) and II (September) ($p \leq 0.05$) and distinctly significant between the periods I and III (October) ($p \leq 0.01$) in terms of the dry matter content (table 1).

The ash content of pasture varied between 9.02% (October) and 11.85% (May), with very significant differences between those periods ($p \leq 0.001$). The ash content of the plants sampled in May differed very significantly, compared to the ones obtained in September (10.09%).

Organic matter content was lower in the first period of analysis (88.15%) and maximum in the third period (90.98%), values between which were very significant differences ($p \leq 0.001$). Distinctly significant differences also existed between the values obtained in the other periods. The organic matter is formed from proteins, fat, cellulose and nitrogen free extract.

In the poultry nutrition, the protein level of the diet, assured by the protein content of the components, is very important. The crude protein content of the analyzed samples was between 28.08% during first period and 15.12% during the third period ($p \leq 0.001$). The plants sampled in spring had almost double the content in CP when compared with those sampled in autumn. Almeida [86] and Horsted [87] mentioned values of 15.9% and 16.7% respectively, and Singh values of 18.21-19.37% [85].

In terms of ether extract content, the average values established for the analyzed fodder were between 2.01% and 2.26% and were within the limits mentioned by the specific literature, 2.2% [87] and 3.42% [88]. Differences between fat content were insignificant for all analyzed periods ($p \geq 0.05$).

The average crude fiber values for the analyzed samples in all studied periods were between 21.9% and 30.09%, which are similar to those presented in the specific literature (26.34-29.8%) [85, 86]. The differences were insignificant for the all studied periods.

Regarding the nitrogen free extract content, it was noticed that the average values were between 35.91% and 43.76%, and the statistical differences between all three periods were insignificant ($p \geq 0.05$).

Mineral elements such as, Ca, P, Mg and Na contribute to the egg formation and it is necessary to know the content of the fodder in those minerals.

Table 1

CHEMICAL COMPOSITION OF ORGANIC GREEN FODDER SAMPLES (% DM) (N = 5)

Specifications	No of period / harvest month						Interpretation of T-test diferences (2-tailed)	
	I/May		II/September		III/October			
	$\overline{X} \pm S_{\overline{x}}$	V%	$\overline{X} \pm S_{\overline{x}}$	V%	$\overline{X} \pm S_{\overline{x}}$	V%		
DM%	21.98±0.2	2.26	23.06±0.04	0.4	25.15±0.03	0.31	I-II	t=3.33; p=0.044*
							I-III	t=11.01; p=0.002**
							II-III	t=-53.9; p= 1.47^{ns}
Ash	11.85±0.13	2.48	10.09±0.05	1.15	9.02±0.01	0.36	I-II	t=-9.05; p=0.003**
							I-III	t=-16.52; p=0.0005***
							II-III	t=20.25; p=0.0003***
OM	88.15±0.13	0.34	89.91±0.03	0.09	90.98±0.05	0.12	I-II	t=8.52; p=0.003**
							I-III	t=15.24; p=0.001***
							II-III	t=-10.74; p=0.002**
CP	28.08±0.07	0.58	16.02±0.05	0.7	15.12±0.02	0.29	I-II	t=-14.67; p=0.001***
							I-III	t=11.39; p=0.001***
							II-III	t=- 114.55; p=1.47 ^{ns}
EE	2.26±0.08	8.01	2.12±0.06	6.46	2.01±0.04	4.38	I-II	t=-0.99; p=0.39^{ns}
							I-III	t=-2.66; p=0.079^{ns}
							II-III	t=1.12; p=0.34^{ns}
CF	21.9±0.07	0.71	28.16±0.04	0.28	30.09±0.03	0.22	I-II	t=63.24; p=8.71^{ns}
							I-III	t=83.3; p=3.81^{ns}
							II-III	t=- 128.76; p=1.03 ^{ns}
NFE	35.91±0.08	0.49	43.61±0.04	0.23	43.76±0.07	0.35	I-II	t=83.22; p=3.82^{ns}
							I-III	t=45.6; p=2.32^{ns}
							II-III	t=1.86; p=0.16^{ns}
Ca	1.3±0.003	0.49	1.28±0.02	2.97	1.26±0.01	2.15	I-II	t=-1.3; p=0.29^{ns}
							I-III	t=-2.54; p=0.08^{ns}
							II-III	t=0.41; p=0.71^{ns}
P	0.56±0.02	3.69	0.57±0.01	8.81	0.49±0.01	5.04	I-II	t=-0.28; p=0.79^{ns}
							I-III	t=-4.94; p=0.008**
							II-III	t=3.12; p=0.04*
Mg	0.155±0.04	5.86	0.142±0.01	10.2	0.139±0.03	5.57	I-II	t=-1.21; p=0.31^{ns}
							I-III	t=-9.04; p=0.003**
							II-III	t=0.32; p=0.77^{ns}
Na	0.219±0.01	8.74	0.198±0.004	4.23	0.204±0.003	2.75	I-II	t=-1.42; p=0.25^{ns}
							I-III	t=-1.13; p=0.34^{ns}
							II-III	t=-0.98; p=0.4^{ns}

Specification	No. of period	Harvest month	$\overline{X} \pm S_{\bar{x}}$	V%	Interpretation of T-test diferences (2-tailed)	
ME	I	May	2836±9.23	0.73	I-II	t=-9.36 p=0.003**
	II	September	2708±4.89	0.40	I-III	t=-13.25 p=0.001***
	III	October	2673±159	0.13	II-III	t=4.11 p=0.03*

Table 2
AVERAGE ENERGY VALUE OF ORGANIC GREEN FODDER SAMPLES (KCAL/ kg DM) (N = 5)

Table 3
AVERAGE CONTENT IN HEAVY METAL OF ORGANIC GREEN FODDER SAMPLES (MG/kg DM) (N = 5)

Specification	No period / harvest month						Interpretation of T-test diferences (2-tailed)	
	I/May		II/September		III/Octomber			
	$\overline{X} \pm S_{\bar{x}}$	V%	$\overline{X} \pm S_{\bar{x}}$	V%	$\overline{X} \pm S_{\bar{x}}$	V%		
Pb	0.44±0.02	9.87	0.32±0.01	9.91	0.29±6.38	6.39	I-II	t=-4.004 p=0.03*
							I-III	t=-4.54 p=0.02*
							II-III	t=1.37 p=0.26ns
Cd	0.028±0.001	5.04	0.026±0.001	8.10	0.021±0.001	10.04	I-II	t=-1.94 p=0.15 ns
							I-III	t=-3.48 p=0.04*
							II-III	t=3.6 p=0.04*
Cu	2.53±0.04	3.58	2.61±2.17	2.17	2.64±0.03	2.91	I-II	t=1.07 p=0.36 ns
							I-III	t=1.15 p=0.33 ns
							II-III	t=-0.79 p=0.49 ns
Zn	2.828±0.04	3.43	2.941±0.07	4.99	2.961±0.04	3.38	I-II	t=1.02 p=0.35 ns
							I-III	t=1.16 p=0.33 ns
							II-III	t=-0.2 p=0.85 ns

The calcium content of organic fodder varied between 1.26% (period III) and 1.3% (period I), with no significant differences between periods.

The average values of phosphorus content (0.49% - period III, 0.57% - period II) were higher than those presented in the specific literature: 0.2% for sun-dried alfalfa and 0.35% for dehydrated pasture [88], respectively 0.34-0.38% [85]. There were significant differences between the second and third period ($p \leq 0.05$) and distinctly significant between the first and third period ($p \leq 0.01$).

Magnesium content in the fodder sampled in period I (0.155%) was significantly higher compared to that presents in third period (0.139%), but lower than the values presented by Blair (0.29%) [88] or Mielmann (0.32%) [89].

The organic green fodder contained sodium in proportions ranging from 0.198% to 0.219%, with no significant differences between the analyzed periods.

The energy value of the green fodder varied depending on the vegetation stage, having the maximum metabolizable energy value (2836 kcal ME/kg DM) to the beginning of flowering, after which the value decreased due to the continuous increase in the cellulose content (2673 kcal ME/kg DM) during the vegetation period (third stage) (table 2).

Statistical differences were noticed between all periods: very significant between the first and the third period, distinctly significant between I and II and significant between II and III.

All values obtained for the concentration of toxic heavy metals (Pb and Cd) were below the maximum permitted by the present legislation [90] (30 mg Pb/kg DM and 1 mg Cd/kg DM) (table 3).

The average concentration in Pb was 0.29 mg Pb/kg DM in samples from the last vegetation period and significantly higher in the second (0.32 mg Pb/kg DM) and first period (0.44 mg Pb/kg DM) of vegetation ($p \leq 0.05$).

The cadmium level in the analyzed samples was 0.021 mg Cd/kg DM during the third growing season and significantly higher in periods I (0.028 mg Cd/kg DM) and II (0.026 mg Cd/kg DM), but below the maximum level admitted by legislation ($p \leq 0.05$).

Concentration in Cu was between 2.53 mg Cu/kg DM for the samples taken in the first period and 2.64 mg Cu/kg DM for those taken during the third period without significant differences ($p \geq 0.05$). Those levels were lower than those presented by Blair [88] for green fodder for organic poultry (6.7 mg Cu/kg DM) and then those recommended by the Lohman Brown 2011 free-range technology guidebook (5 mg Cu/kg DM) [91].

The ecological green fodder content in zinc varied between 2.828 mg Zn/kg DM for the samples taken during the first growing season and 2.961 mg Zn/kg DM for those sampled in the third period, with no statistical differences between the values. These values were lower than those mentioned in the specific literature (19 mg Zn/kg DM) [88] or those recommended by the Lohman Brown 2011 free-range technology guidebook (60 mg Zn/kg DM) [91].

Table 4

NITRATE AND NITRITE CONTENT OF ORGANIC GREEN FODDERSAMPLES (PPM) (N=5)

Specification	No period / harvest month						Interpretation of T-test diferences (2-tailed)	
	I/May		II/September		III/October			
	$\overline{X} \pm S_{\overline{x}}$	V%	$\overline{X} \pm S_{\overline{x}}$	V%	$\overline{X} \pm S_{\overline{x}}$	V%		
NO_3^-	377.64±0.06	0.03	365.25±2.93	1.79	363.12±1.11	0.68	I-II	t=-3.23;p=0.05*
							I-III	t=-9.96; p=0.002**
							II-III	t=0.67; p=0.55ns
NO_2^-	0.83±0,01	2.87	0.69±0.03	9.58	0.61±0.01	5.09	I-II	t=-2.92; p=0.06ns
							I-III	t=-7.97; p=0.004**
							II-III	t=2.4; p=0.1ns

All the analyzed samples had a nitrite content (table 4) lower than the maximum permitted limit present in EU Regulation No 574/2011 (15 ppm) [90], but there were still distinctly significant differences between fodder obtained in May and the ones in October. Regarding the nitrate content, the values were about 11 times lower than the one considered safe for consumption (max 4400 ppm), to all analyzed periods.

The organochlorine and organophosphorus pesticide residue levels were below the maximum admissible limit (0.05 mg/kg and 0.001 mg/kg respectively - EU Regulation No 574/2011) [90] for all analyzed periods. These data show that the quality parameters of green fodder, expressed through the levels of pollutants with importance for the organic system, were maintained throughout the analyzed period.

Conclusions

The chemical composition of the green fodder administered to chickens reared in organic system varied between analyzed periods, depending on the stages of vegetation. With the plants maturation, the content in protein, ash, fat and minerals decreased and while the content in dry mater, organic matter, cellulose and nitrogen free extract increased. Knowing these seasonal fluctuations in fodder chemical composition is useful for establishing daily intake and estimating the egg production.

The contents of heavy metals, pesticides, nitrite and nitrate of pasture were below the permissible levels, which provide certainty in the ecological feeding of hens which produce eggs for consumption. These determinations may represent key points when controlling the feeding conditions achievement in an ecological system.

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