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EFFECT OF SEABUCKTHORN BERRY FLOUR FROM HENS FOOD ON EGG YOLK CAROTENOIDIC PIGMENTS CONTENT

Delia DUMBRAVĂ[1], M. MATIUȚI[2], Mărioara DRUGĂ[1], Alfa-Xenia LUPEA,[3] I. IANCULOV[4], Camelia CLEP[1]

[1] USAMVB TIMIŞOARA, FACULTY OF AGROFOOD PRODUCTS;
[2]USAMVB TIMIŞOARA, FACULTY OF VETERINARY MEDICINE;
[3] "POLITEHNICA" UNIVERSITY TIMIŞOARA, FACULTY OF INDUSTRIAL CHEMISTRY AND ENVIRONMENT ENGINEERING;
[4] USAMVB TIMIŞOARA, FACULTY OF AGRICULTURE

ABSTRACT
This work presents the results of an experiment achieved on some hens lots, Italian breed, in the July-August 2004 period, when in their food was added seabuchthorn berry flour. They found the selective absorption, either the selective accumulation of carotenoids in the egg yolk, for the experiment conditions. The absorption was stronger in the case of dihydroxy xanthophylls (lutein, zeaxanthin) and weaker in the case of the carotenoidic hydrocarbons (α-carotene, β-carotene) and of the epoxycarotenoids (violaxanthin, loxanthin). For the same period was observed an increase of the egg production.

KEYWORDS
carotenoids, egg yolk, hens, food, seabuckthorn.

1. INTRODUCTION

Birds, like all the animals, haven't the capacity to synthesize the carotenoids "*de nuovo*". But they are capable to transform and to store the ingested carotenoids. This determine the colour of some tissues or organs in which these are stored, such as: the skin, body and the legs fat, the plumage, beak and the eggs yolk [1]. When the hens food is poor in carotenoids and when the absorption and the storage of the caroteoids are in deficit, we have to add some natural rich sources for carotenoids, for the achievement of good eggs productions with a higher biological value and an intense pigmentation of the egg yolk. The seabuckthorn (*Hyppophae rhamnoides*) berry flour is a rich source for carotenoids [2,3]. This study examined the degree of the carotenoids accumulation in the egg yolk and in the eggs production, following the hens feeding with or without supplement of seabuckthorn berry flour.

2. MATERIALS AND METHODS

Experimental lots and their feeding. Researches were achieved on three lots of 20 hens, Italian breed, of 27 weeks old, breeding in experimental conditions from one day chicken at an private farm from Dumbrăvița (Timiş). All hens lots has received the same base combined fodder. The Ist lot were the control, in the IInd and IIIrd lots were added seabuck-thorn berry flour in proportion of 2,5% respectively 4%. The experiment were preceded of an preexperi-mental period of two weeks. In table 1 are presented the hens food in the experiment period.

Table 1 – Composition of hens fooder in the experiment period (%)

Name of fooder product	Hens lot		
	I	II	III
Maize flour	69.00	66.50	65.00
Sun-flower groats	13.00	13.00	13.00
Vitaminic-mineral blend	11.70	11.70	11.70
Salt	0.30	0.30	0.30
Seabuckthorn berry flour	-	2.50	4.00

Hens lots were kept in closed paddocks in the best conditions of food, water, microclimate and hygiene.

The egg yolk carotenoidic pigments analysis were made from 5 in 5 days from the start of experiment, for 40 days of differentiate feeding and continued still 15 days after cessations of differentiate hens feeding, for establish the time in which the ingested carotenoidic pigments are eliminated from organism.

3. CAROTENOIDS DETERMINATION FROM FOODER AND EGGS

a. **Extraction.** For carotenoids extraction were used adequate methods, thus:

- extraction from fooder - after saponification with a KOH 8% solution,carotenoids were extracted with petroleum ether and then with a mixture of petroleum ether:benzene (5:1) [4, 5].
- extraction from seabuckthorn berry flour – were first extracted with a mixture of petroleum ether:ethanol 96% (8:1) to colourless and after saponification with KOH 8% in ethanol, with petroleum ether [2].
- extraction from eggs yolk - after treating with ace-tone, carotenoids were extracted with a mixture of petroleum ether:ethanol (17:1) and then extract was saponified [2].

b. **Separation.** The obtained extracts were pas-sed through a chromatographic column filled with an mixture of magnesium oxyde and fine sand (1:2) and was obtained three fractions, coreponding to the used eluents (petroleum ether, petroleum ether:diethyl ether = 1:1, diethyl ether). This fractions were rechro-matographiated on thin layer for major carotenoids separation [6].

c. **Identification** The isolated carotenoids were identified by their VIS spectra characteristic sand in case of 5,6- and 5,8-epoxicarotenoids was achieved and the colour reaction with HCl in diethyl ether (when this determine a blue colour).

d. **Dosing.** Carotenoids were measured by spec-trophotometric method, using an "SHIMADZU 1240" spectrophotometer [7].

4. RESULTS AND DEBATES

Carotenoidic pigments from base combined fooder and from seabuckthorn berry flour, determined at the start of experiment (mean values of three parallel determinations) are present in table 2.

In table 3 is presents the variation of egg yolk carotenoids content in the time of experiment. From table 3 data it comes out that hens absorb and store in yolks. in a greater or a smaller measure, almost all the carotenoids from fooder. Absorption and deposing of carotenoidic pigments take place selectively, depen-ding of their molecular structure. The easier are absor-bed and stored in eggs the dihydroxylic xanthophylls (zeaxanthin and lutein), then the monohydroxylic xanthophylls (α-Cripto-xantin, β-Criptoxanthin) and more difficult the carotenoidic hydrocarbons (α-Carotene, β-Carotene, □-Carotene, □-Carotene). In a small proportion are stored in eggs the epoxydic carotenoids (eloxanthin and violaxanthin). They were never found in the eggs yolk: the licopene (with acyclic structure) and the δ-Carotene (with pseudoiononic structure).

Table 2 – Carotenoids content from base combined fooder and from seabuckthorn (*Hyppophae rhamnoides*) berry flour

Carotenoid	λ_{max} (nm)	Solvent	$E_{1cm}^{1\%}$	Base combined fooder content (µg/g)	Seabuckthorn berry flour content (µg/g)	R_f*
α-Carotene	444	Petroleum ether	2800	0.98	1.82	0.65
β-Carotene	453	Petroleum ether	2592	3.02	49.01	0.50
ε-Carotene	440	Petroleum ether	3120	-	10.84	0.71
δ-Carotene	456	Petroleum ether	3290	-	7.11	0.22
γ-Carotene	462	Petroleum ether	3100	-	24.02	0.12
α-Cryptoxantin	446	Hexane	2636	0.86	-	0.75
β- Cryptoxantin	452	Petroleum ether	2386	1.75	89.17	0.69
Lutein	445	Ethanol	2550	5.33	19.03	0.36
Zeaxanthin	450	Ethanol	2540	4.12	34.17	0.25
Licopene	487	Benzene	3370	-	6.11	0.02
Flavoxanthin	432	Benzene	2550	-	5.32	0.22
Violaxanthin	454	Benzene	2240	0.61	9.05	0.18
Total carotenoids				16.61	255.65	

* – values were determined for α-Carotene, β-Carotene, ε-Carotene, δ-Carotene, γ-Carotene, licopene on plates of MgO actived (Merk) using a mixture of petroleum ether: benzene (50:50, v/v) and for cryptoxanthin, lutein, zeaxantine, flavoxanthin, violaxanthin on actived silica plates, using a mixture of methylene chloride:ethyl acetate (80:20, v/v) [6].

Table 3 - Variation of egg yolk carotenoids content in the time of experiment

Days from beginning of expe-riment	0			5			10			15			20			25		
Lot	I	II	III	I	I	II	III	I	II	III	II	III	I	II	III	I	II	III
α-Carotene	0,21	0,18	0,23	0,19	0.20	0,27	0,17	0,24	0,28	0,18	0,28	0,32	0,20	0,24	0,31	0,17	0,29	0,33
β-Carotene	1,12	1,16	1,08	1,18	1.16	1,35	1,15	1,28	1,42	1,18	1,46	1,68	1,16	1,47	1,66	0,98	1,43	1,62
ε-Carotene	0,36	0,39	0,42	0,33	0.35	0,45	0,39	0,52	0,59	0,37	0,55	0,63	0,35	0,53	0,58	0,36	0,55	0,60
γ-Carotene	-	-	-	-	-	0,12	-	0,38	0,56	-	0,78	0,89	-	0,76	0,83	-	0,73	0,88
α-Criptoxanthin	0,33	0,30	0,36	0,31	0.32	0,48	0,36	0,46	0,57	0,38	0,48	0,59	0,32	0,45	0,56	0,35	0,48	0,53
β-Criptoxanthin	0,62	0,67	0,65	0,69	0.63	0,83	0,64	1,20	1,32	0,67	1,53	1,68	0,63	1,50	1,65	0,66	1,53	1,68
Lutein	7,12	7,22	7,18	7,20	6.86	7,46	7,08	7,96	8,23	7,13	8,86	9,05	6,86	8,81	9,12	6,91	8,92	8,96
Zeaxanthin	5,53	5,61	5,67	5,56	5.47	6,24	5,49	7,11	8,03	5,51	11,23	13,25	5,47	11,16	13,14	5,49	11,08	13,04
Eloxanthin	0,28	0,33	0,30	0,32	0.30	0,38	0,34	0,45	0,48	0,32	0,48	0,51	0,30	0,46	0,53	0,35	0,48	0,51
Violaxanthin	0,36	0,31	0,34	0,32	0.36	0,51	0,32	0,49	0,56	0,38	0,53	0,61	0,36	0,51	0,58	0,34	0,42	0,56
Total carotenoids	15,93	16,17	16,23	16,12	15.65	18,09	15,94	20,09	22,04	16,12	26,12	29,19	15,65	25,89	28,76	15,61	25,91	28,71
No of eggs/ day	10	10	11	10	10	17	11	19	20	12	20	20	10	20	20	10	20	20

The main carotenoids from the yolks in the I[st] lot (control): lutein is higher than zeaxanthin. After 15 days of differentiate feeding of lots is observed that, in case of egg yolks from the II[nd] and the III[rd] lot, zeaxanthin is accumulated in greater quantity than lutein.

The total carotenoidic pigments content from egg yolks of the II[nd] and the III[rd] lot is greater than that from the I[st] lot. This proved that the addition of Hyppophae rhamnoides berry flour in the hens food, in relatively small proportions, has favourable effects for the carotenoids accumulation in yolk and for his pigmentation. The highest efficiency in absorption and depositing of carotenoids in eggs was achieved in the case of the III[rd] lot. It was also observed an important increase of eggs production to the II[nd] and the III[rd] lots comparative with the I[st] lot.

Table 3 - Variation of egg yolk carotenoids content in the time of experiment (continued)

Days from beginning of expe-riment	30			35			40			45			50			55		
Lot	I	II	III	I	I	II	III	I	II	III	II	III	I	II	III	I	II	III
α-Carotene	0,19	0,25	0,28	0,16	0,21	0,32	0,21	0,23	0,30	0,15	0,20	0,24	0,18	0,21	0,30	0,20	0,18	0,24
β-Carotene	0,89	1,40	1,58	1,12	1,43	1,61	1,16	1,39	1,64	1,13	1,18	1,29	0,99	1,16	1,23	1,14	1,12	1,20
ε-Carotene	0,33	0,51	0,58	0,37	0,54	0,51	0,33	0,48	0,56	0,35	0,43	0,53	0,38	0,41	0,45	0,36	0,39	0,43
γ-Carotene	-	0,70	0,86	-	0,68	0,81	-	0,65	0,86	-	0,52	0,73	-	0,33	0,41	-	0,12	0,18
α-Criptoxanthin	0,32	0,42	0,49	030	0,45	0,51	0,36	0,48	0,52	0,32	0,39	0,46	0,30	0,34	0,38	0,34	0,32	0,33
β-Criptoxanthin	0,64	1,48	1,69	0,60	1,52	1,70	0,69	1,55	1,62	0,63	1,42	1,51	0,60	0,83	0,96	0,67	0,71	0,77
Lutein	6,85	8,84	9,01	6,96	8,93	8,98	6,89	8,78	8,93	7,16	8,23	8,41	6,96	8,13	8,26	6,90	7,18	7,31
Zeaxantin	5,52	10,92	12,84	5,48	10,83	12,98	5,50	10,81	13,08	5,55	10,31	12,68	5,46	10,04	11,92	5,43	7,12	7,23
Eloxantin	0,28	0,43	0,46	0,31	0,46	0,49	0,32	0,35	0,52	0,30	0,44	0,46	0,30	0,36	0,41	0,26	0,28	0,33
Violaxantin	0,32	0,48	0,52	0,34	0,45	0,52	0,31	0,48	0,53	0,35	0,40	0,45	0,34	0,32	0,43	0,31	0,36	0,40
Total carotenoids	15,34	25,43	28,31	15,64	25,50	28,43	15,77	25,20	28,56	15,94	22,35	26,76	15,51	24,13	24,75	15,61	17,78	18,42
No of eggs/ day	10	20	20	11	20	20	11	20	20	12	20	20	10	18	19	10	16	18

In the first 5 days of the differentiate feeding of the hens we didn't find differences significant for the carotenoids content in the egg yolk of the three lots. The maximum carotenoids quantity accumulates in yolk after almost 15 days from the start of the experiment. After that, the carotenoidic pigments quantity oscillates in all the three lots.

The remanence period of the carotenoids in the egg yolk is about 5-10 days for the hens in the IInd and the IIIrd lots.

The yolk colours are determined by the content ratio of the orange pigments (β–Carotene, γ-Carotene, β–Cryptoxanthin, Zeaxanthin) and the yellow pigments (α-Carotene, α-Cryptoxanthin, ε-Carotene, Violaxanthin, Eloxanthin, Lutein) (see table 4).

Table 4 – Ratio between orange pigments (P_o) and yellow pigments (P_y) content and egg yolks colour in the time of experiment

Days from beginning of experiment	Lot	P_o/P_y	Yolk colour	Days from beginning of experiment	Lot	P_o/P_y	Yolk colour
0	I	0.86	Light yellow	30	I	0.85	Light yellow
	II	0.85	Light yellow		II	1.33	Intense orange
	III	0.85	Light yellow		III	1.49	Dark orange
5	I	0.86	Light yellow	35	I	0.85	Light yellow
	II	0.86	Light yellow		II	1.31	Intense orange
	III	0.89	Intense yellow		III	1.51	Dark orange
10	I	0.84	Light yellow	40	I	0.87	Light yellow
	II	0.98	Yellow orange		II	1.33	Intense orange
	III	1.06	Light orange		III	1.51	Dark orange
15	I	0.84	Light yellow	45	I	0.85	Light yellow
	II	1.34	Light orange		II	1.33	Intense orange
	III	1.49	Dark orange		III	1.54	Intense orange
20	I	0.86	Light yellow	50	I	0.83	Light yellow
	II	1.35	Intense orange		II	1.27	Light orange
	III	1.50	Dark orange		III	1.42	Intense orange
25	I	0.84	Light yellow	55	I	0.86	Light yellow
	II	1.33	Intense orange		II	1.04	Light orange
	III	1.49	Dark orange		III	1.03	Light orange

5. CONCLUSIONS

- Seabuckthorn berry flour represents a rich source of carotenoids for the hens food.
- Seabuckthorn berry flour carotenoids contribute to increase the eggs yolk biological value, to their adequate pigmentation, to increase the eggs production, even for small proportions in hens food. The most accumulated in yolks are: zeaxanthin and lutein, pigments with a very high biological value [8, 9, 10].
- The best results were obtained with an addition of 4% seabuckthorn berry flour in the hens food.

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