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LEVEL OF SELECTED LIPID FRACTIONS IN EGG YOLK OF HENS FED WITH FODDERS SUPPLEMENTED WITH AMARANTH SEEDS

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This investigation was aimed at the assessment of the effect of amaranth addition to laying hens fodder on egg yolk lipid fractions. The examinations were conducted on 32-week-old laying hens of ISA SHAVER line, divided into 4 groups: control (I) – fed without supplementation and experimental (II, III and IV) fed with the addition of amaranth seeds (2%, 5% and 10%, respectively). Strict experiment lasted for 56 days. The following analyses were conducted: vitamin A, cholesterol and fatty acids content in egg yolk (after 4 weeks – series I and after 8 weeks – series II). Fatty acids content in amaranth seeds was analysed as well. There were not proved any statistically significant differences between particular groups. The highest vitamin A content in both series was assayed in the eggs of laying hens fed with the fodder containing 5% amaranth addition and ranged 1.96 μg and 4.37 μg/1 g yolk, respectively. Cholesterol level was the lowest in group III in both series (137 and 165 mg/1 g egg yolk, respectively).

Fatty acids content in egg yolk was similar in all groups, although in the case of polyunsaturated acids of n-6 group, especially linoleic acid, there was reported their slightly higher level in experimental groups, with a slight increase in n-6/n-3 acids ratio at the same time.

INTRODUCTION

One of the conditions of proecological animal rearing and breeding is their feeding with no antibiotics or chemitherapeutics applied. For many years investigations have been carried out into natural means addition to fodder, which can effect: animal state of health and productivity, nutritive value of animal-originating products, which in turn, enriches human diet in indispensable components, as well as providing environmental protection. Satisfactory results featured investigation on the use of alternative plants, such as *e.g.* herbs, *Scutellaria baicalensis* or *Echinacea angustifolia* in animal feeding [Bombik *et al*., 2002; Szymeczko *et al*., 2003; Króliczewska *et al*., 2004]. These plants possess prophylactic and therapeutic properties, as well as animal-originating products enriched in numerous valuable components. Health-supporting properties and the possibility of modifying animal products composition (including hens eggs) also characterise another plant coming from south America, namely amaranth (*Amaranthus sp*.). It features, among others, high content of protein of high biological value, rich composition of fatty acids, especially linoleic acids, vitamin E and tocotrienols, as well as considerable amount of dietary fibre [Bobel & Sokół, 2002]. There are many amaranth varieties. As it has been proved by appropriate examinations, these varieties are characterised by different contents of basic nutritive components, *i.e.* protein content can range from 11.8% to 19.3% [Bressani, 1988, 1994; Bressani & Garcia-Vela, 1990; Saunders & Becker, 1994], while crude fat content can amount from 0.8% to 13.0% [Teutonica & Knorr, 1985; Bressani, 1988; Becker, 1994].

In animal feeding amaranth is applied in different forms, *e.g.* fine-ground grain, or is subjected to the process of extrusion.

The aim of these investigations was to assess the effect of amaranth seeds addition to laying hens fodder on egg yolk lipid fractions.

MATERIALS AND METHODS

The investigations were conducted in vivarium conditions on 32-week-old laying hens of ISA SHAVER Line. The birds were randomly divided into 4 groups (20 birds in each): I – control group fed with fodder mixture DJ Type, II – experimental group fed with the same mixture supplemented with 2% of amaranth seeds, III – experimental group fed with DJ fodder mixture with 5% addition of amaranth seeds, and IV – experimental groups where hens were fed with DJ fodder supplemented with 10% of amaranth seeds. Strict experiment lasted for 8 weeks. The material for examination was collected twice, *i.e.* after 4 weeks (the first series) and after 8 weeks (the second series). The following assays were done regarding lipid fraction of egg yolk: vitamin A, cholesterol and fatty acids. Fatty acids content in amaranth seeds was also the subject to analysis.

Lipids were extracted from homogeneous yolk samples using a mixture of methylene chloride/methanol and further processed to esters by the methylation reaction [Folch *et al*., 1956]. Fatty acid profile analysis was performed by gas

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chromatography GC/MS. To conduct separation gas chromatograph coupled with mass spectroscopy by Agilent Technologies 6890N SC System; 5973 MS Detector was applied. The separation of fatty acids was carried out in a column DB-225 MS (60; 0.25, 0.25).

Analyses of cholesterol and vitamin A contents were carried out using an HPLC technique Agilent 1100 after methanol/ethanol extraction or ethanol/hexane/diether extraction, respectively. Cholesterol measurements was performed by an HPLC technique using XDB-C18 (4.6x250) column and detected at 210 nm. Vitamin A was detected spectrophotometrically after HPLC separation at 292 nm.

The results obtained were subjected to statistical analysis with the use of computer software Statistica ver. 7.0 to calculate significance of differences between the groups (p(0.05).

RESULTS

The results of investigation conducted with the use of amaranth added to laying hens fodder did not prove any statistically significant differences between the groups as for selected lipid fractions in egg yolk.

The highest level of vitamin A was recorded for the group III (with 5% addition of amaranth), both in the first series (after 4-week examination) and in the second series (after 8-week examination) and it amounted 1.96 and 4.37 μg/1 g egg yolk, respectively (Table 1). In the other groups, in the first series the highest vitamin A level was determined in the group IV (10% amaranth addition) – 1.76 μg/1 g egg yolk and 4.19 μg/1 g egg yolk, whereas in the second series – in the group with 2% amaranth addition. After 8 weeks of investigation the lowest vitamin A content was assayed in the group with the highest contribution of amaranth addition.

Cholesterol content in egg yolk was relatively differentiated in all the groups (Table 2). Its lowest level was detected in group III, ranging from 137 mg/egg yolk after 4-week examination and slightly increasing up to 165 mg/egg yolk after 8 weeks. A similar increase occurred in the remaining experimental groups and appeared to be the highest in the group I (without amaranth addition, 189 mg/egg yolk), while in the second series it decreased to 168 mg/egg yolk.

Amaranth seeds are characterised by a rich composition of fatty acids (Table 3). It was possible to prove a high level of palmitic (20.89%), oleic (21.75%) and linoleic acids (43.13%).

After 4 weeks of the study (the first series), amaranth addition to laying hens fodder caused a decrease in saturated fatty acids content by 1.17% in group II, by 1.18% in group III and by 0.78% in group IV in comparison to the control group (Table 4), but after 8 weeks (second series) an increase in the saturated fatty acids content was observed in all experimental groups (about 1.66%). Linoleic acid contribution increased in groups with 5% and 10% amaranth addition by 0.86 and 1.1% respectively. In the experimental groups the sum of polyunsaturated fatty acids of n-3 group was slightly lower in comparison to the control group and amounted on average 0.63% in group II, 0.64% in group III and 0.65% in group IV, while in (control) group I – 0.67%. The average content of n-6 group acids in groups I and II was equal and reached 12.76%, whereas in group III it accounted for13.65% and in group IV for 13.99%. Due to a lower level of n-3 group acids and, at the same time, a higher level of n-6 group acids in experimental groups, n-6/n-3 acid group's ratio was higher as a result of previously mentioned data.

DISCUSSION

As it was proved in the investigation by different authors, amaranth seeds are of a high hypocholesterolemic value [Chatuverdi *et al*., 1993; Berger *et al*., 2003]. In our own investigation only the first series proved to feature a low cholesterol level in the group of laying hens fed with 5% amaranth addition to fodder (group III), while in the second series the level of cholesterol in group III showed a slight increase, although not statistically significant as compared with the first series.

Other authors [Berganza *et al*., 2003] carried out investigation of fat content, fatty acids and content in amaranth. They proved that amaranth oil contained 5.83–7.13% of fat,

TABLE 1. Vitamin A content in hen egg yolk (µg/1 g egg yolk).

Group	First series	Second series
I	1.59	4.01
II	1.70	4.19
III	1.96	4.37
IV	1.76	3.86

TABLE 2. Cholesterol content in hen egg yolk (mg/1 g egg yolk).

Group	First series		Second series	
	mg/1 g of yolk	mg/egg yolk	mg/1 g of yolk	mg/egg yolk
I	12.7	189	10.9	168
II	11.0	164	11.2	179
III	9.3	137	10.6	165
IV	9.4	144	11.3	183

TABLE 3. Fatty acids content in amaranth seeds (%).

Fatty acid	Chemical formula	Average content (%)
Myristic acid	$C_{14:0}$	0.127
Pentadecanoic acid	$C_{15:0}$	0.05
Palmitic acid	$C_{16:0}$	20.89
Palmitoleic acid	$C_{16:1}$	0.295
Heptadecanoic acid	$C_{17:0}$	0.082
Stearic acid	$C_{18:0}$	4.468
Oleic acid	$C_{18:1}$	21.741
Linoleic (LA) acid (n-6)	$C_{18:2}$	43.131
α-Linolenic (ALNA) acid (n-3)	$C_{18:3}$	0.761
Arachidic acid	$C_{20:0}$	0.766
Eicosenoic acid (n-6)	$C_{20:1}$	0.179
Lignoceric acid	$C_{24:0}$	0.266
Squalene	$C_{30:6}$	7.275

TABLE 4. Fatty acids composition in hen egg yolk (%).

Fatty acids	Chemical formula	Group I		Group II		Group III		Group IV	
		Series		Series		Series		Series	
		First	Second	First	Second	First	Second	First	Second
Myristic acid	$C_{14:0}$	0.311	0.267	0.316	0.271	0.317	0.278	0.304	0.288
Pentadecanoic acid	$C_{15:0}$	0.044	0.043	0.048	0.046	0.045	0.045	0.036	0.047
Palmitic acid	$C_{16:0}$	27.836	26.052	27.322	26.226	26.978	26.500	27.701	27.326
Palmitoleic acid	$C_{16:1}$	3.477	2.942	3.315	3.111	3.222	3.194	3.685	3.155
Heptadecanoic acid	$C_{17:0}$	0.155	0.163	0.154	0.152	0.155	0.158	0.142	0.149
Stearic acid	$C_{18:0}$	8.292	8.521	8.369	8.765	8.175	8.708	8.168	7.929
Oleic acid	$C_{18:1}$	46.438	47.174	46.112	46.543	46.079	45.680	45.079	44.868
Linoleic (LA) acid (n-6)	$C_{18:2}$	10.909	11.652	10.818	11.740	12.028	12.249	11.826	12.922
α-Linolenic (ALNA) acid (n-3)	$C_{18:3}$	0.217	0.250	0.242	0.210	0.239	0.199	0.215	0.246
Eicosenoic acid (n-6)	$C_{20:1}$	0.157	0.230	0.168	0.195	0.147	0.191	0.201	0.176
Eicosatrienoic acid (n-6)	$C_{20:3}$	-	0.073	0.081	-	-	0.070	-	0.085
Arachidonic acid (AA) (n-6)	$C_{20:4}$	1.501	1.379	1.367	1.508	1.439	1.522	1.516	1.635
Docohexaenoic (DHA) acid (n-3)	$C_{22:6}$	0.390	0.477	0.429	0.412	0.389	0.459	0.383	0.457
n6/ n3	-	20	18	18	21	21	21	22	21

17.06–21.35% of palmitic acid, 3.05–3.80% of stearic acid, 20.26–32.01% of oleic acid, 33.52–43.88% of linoleic acid and squalene values ranged 2.26–5.94%, while our own investigation determined higher content of stearic acid (4.47%) and squalene (7.28%) in amaranth seeds. Punita & Chatuverdi [2000] applied palmitic acid and amaranth seeds in laying hens feeding. Those authors proved, among others, cholesterol reduction by about 14% and increased content of linoleic acid by about 100%. Reklewska *et al*. [1995], using amaranth in laying hens, demonstrated level of triacylglycerides and saturated fatty acids content in hen's egg yolk, which was similar to our results, as well as, a lower number of eggs obtained from laying hens fed with amaranth in their fodder. Tillman & Waldroup [1987], applying amaranth in laying hens feeding in the amount of 0, 10, 20 and 30%, stated considerably higher number of eggs, although of lower egg weight and, at the same time, decreased egg shell resistance and daily fodder intake by hens.

The highest content of vitamin A (2.0 μg/g of yolk) was determined in eggs laid down by hens fed with 5% of amaranths whilst the lowest level of (retinol) vitamin A, *i.e.* 1.6 μg/g, was found in eggs collected from layers fed with standard diet. Acker & Ternes [1994], in their studies on the influence of addition of vitamin A to broiler fodder also demonstrated that vitamin A accumulated in liver and hen's egg yolk. The results showed that vitamin A concentration in eggs was 1.9-5.5 μg/g of yolk. The concentration of vitamin A in the yolk of the hens' eggs was markedly increased by its dietary supplementation. However, the concentrations of both vitamin E and carotenoids in the yolks were significantly reduced by high dietary contents of vitamin A [Surai *et al*., 1998].

CONCLUSIONS

On the basis of the analysed content of selected lipid fractions in the egg yolk it is possible to state that the most advantageous proved to be 5% addition of amaranth seeds to laying hens fodder. In this group there was recorded an increased content of vitamin A and a satisfactory level of cholesterol, as well as fatty acids percentage. In spite of no statistically significant differences between the groups, it is advisable to use amaranth seeds in hens fodder as a natural addition of high nutritive value.

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POZIOM WYBRANYCH FRAKCJI LIPIDOWYCH W ŻÓŁTKU JAJA KUR ŻYWIONYCH PASZĄ Z DODATKIEM NASION AMARANTUSA

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Celem badań była ocena wpływu dodatku amarantusa do paszy dla niosek na frakcje lipi-dowe żółtka jaja kur. Badania przeprowadzono na 32-tygodniowych kurach nioskach linii ISA SHAVER, podzielonych na 4 grupy: kontrolną (I) – żywienie bez dodatku oraz doświadczalne (II, III i IV) – żywione z dodatkiem nasion z amarantusa (odpowiednio 2, 5 i 10%). Doświadczenie ścisłe trwało 56 dni. Dwukrotnie (po 4 – I seria i 8 tygodniach – II seria) przeprowadzono analizy zawartości witaminy A, cholesterolu i kwasów tłuszczowych w żółtku jaja kur oraz kwasów tłusz-czowych w nasionach amarantusa. Nie wykazano statystycznych różnic pomiędzy grupami. Najwyższą zawartość witaminy A w obu seriach oznaczono w jajach niosek karmionych paszą z 5% dodatkiem amarantusa i wynosiła odpowiednio 1,96 μg oraz 4,37 μg/1 g żółtka (tab. 1). Również poziom cholesterolu najniższy był w grupie III w obu seriach (odpowiednio 137 i 165 mg/żółtka jaja), (tab. 2). Zawartość kwasów tłuszczowych w żółtku jaj była podobna we wszystkich grupach, choć w przypadku kwasów wielonienasyconych z rodziny n-6, w szczególności kwasu linolowego, wykazano nieco wyższy ich poziom w grupach doświadczalnych, przy równoczesnym niewielkim wzroście stosunku kwasów n-6/ n-3 (tab. 4).