Comparison of Bioactive Compound Content in Egg Yolk Oil Extracted from Eggs Obtained from Different Laying Hen Housing Systems

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***Abstract*—**Egg yolk oil is a natural source of bioactive compounds such as unsaturated fatty acids, oil soluble vitamins, pigments and others. Bioactive compound content in egg yolk oil depends from its content in eggs, from which oil was extracted. Many studies show that bioactive compound content in egg is correlated to the content of these compounds in hen feed, but there is also an opinion that hen housing systems also have influence on egg chemical content. The aim of this study was to determine which factor, laying hen housing system or hen diet, has a primary influence on bioactive compound content in egg yolk oil. The egg yolk oil was extracted from eggs obtained from 4 different hen housing systems: cage, barn and two groups of free range. All hens were fed with commercially produced compound feed except one group of free range hens which get free diet – pastured hens. Extracted egg yolk oils were analyzed for fatty acids, oil soluble vitamins and β-carotene content. α-tocopherol, ergocalcipherol and polyunsaturated fatty acid content in egg yolk oil was higher from eggs obtained from all housing systems where hens were fed with commercial compound feed. β-carotene and retinol content in egg yolk oils from free range free diet eggs was significantly ($p>0.05$) higher that from other eggs because hens have access to green forage. Hen physical activity in free range housing systems decreases content of some bioactive compound in egg yolk oil.

***Keywords*—**Egg yolk oil, vitamins, caged eggs, free range.

I. Introduction

HEN eggs are rich in bioactive compounds such as unsaturated fatty acids, oil soluble vitamins, pigments, sterols, phospholipids and others. Most of them are concentrated in yolk lipids. In previous studies lipid extraction from liquid egg yolk was studied to produce a highly valuable product which contains egg yolk bioactive compounds in the concentrated way. But bioactive compound content in eggs is different from different egg samples [1].

Many studies show that bioactive compound content in egg is correlated to the content of these compounds in hen feed. It is well known methods how to increase the nutritional and biological value of the eggs through the feed, so called “designer eggs”. But there is an opinion that animal housing system also has an influence on animal based food product chemical content, same increasing nutritional value and even affecting the taste of products [2]-[5].

In the last years a very big attention is pointed on animal welfare. But industrial production of animal based food products requires low cost production to satisfy consumer needs in cheap but qualitative food. To produce cheap means to use as less as possible resources for production of one product unit. One of the important production expenses is the size of the animal housing area. Usually, in industrial production, animals must live in limited space, without possibility to see daylight and breathe fresh air. Modern animal housing systems and EU legislation require providing animal with all necessary to satisfy its physiological needs. From the other side farmers themselves are interested in good animal housing practices to ensure better animal health for higher yield of products. Anyway, it is very important to keep a good balance between high production results and costs of production.

Nowadays there are 3 main bird housing systems for egg production: cages, barn and free range.

Cages, also called battery cages, mean that individual cages are connected together to columns and rows, building many levels allowing placing big amount of cages in small space. The hens are locked within the cage in small groups. Inside the cage all necessary equipment is provided: drinking system, feeding system, nest, roost, area for scratching and pecking. Each cage battery level also has egg collecting conveyor and manure conveyors. Henhouses are equipped with ventilation and lighting systems. Important environment parameters such as temperature and air quality are strictly controlled to ensure good animal welfare. Cage systems cause less problems occurred in hens [6] and is the most economical production method of eggs.

Barn housing system means that hens can move freely within a barn. The barn or the henhouse can be equipped with the same cage system mentioned before which contains drinking and feeding systems, nests, egg collecting belts, manure conveyor. Also ventilation and lighting are provided. But due to the higher mortality rate of laying hens, egg production in barn systems is lower than in cages [7].

Free range means that the birds are permitted outdoors daytime. Usually it is a field near the barn where hens can move freely in a quite big flock, breathe fresh air and get access to free feed: grass, insects, worms, soil. Free range housing method requires big areas and also egg collecting is not efficient, because, usually, egg collecting is not automated. Also keeping hens in big flocks cause health problems and increased mortality [8]. This non-efficient farming makes free range housing systems the most expensive production method

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of eggs. Price difference between free range eggs and caged eggs are more than double.

Hen's physical activity in free range and barn housing systems will require nutrients and bioactive compounds to fulfill its requirements for energy and physiological processes, so from this point of view, less nutrients and bioactive compounds will remain for eggs. But physiology of a bird, as of any other animal or human, is built the way that first of all embryo's (egg) nutritional needs are satisfied, so from that point egg chemical content must remain permanent at some minimum level.

The aim of this study was to determine which factor, laying hen housing system or hen diet, has a primary influence on bioactive compound content in egg yolk oil. The result of this study helps to understand which eggs (from which housing system) are most suitable as a raw material for egg yolk oil production in terms of bioactive compound content.

II. Materials and Methods

A. Materials

Eggs were collected from Lohmann Brown-Classic breed laying hens from three different laying hen housing systems: cages, barn and two groups of free range. The feed, commercially produced compound feed, was equal for all hens except second group of free range hens which had a free diet. The commercially produced compound feed contains 10400 IU kg^{-1} of vitamin A, 2600 IU kg^{-1} of vitamin D and 26 mg kg^{-1} of vitamin E. Eggs for experiment were collected in August 2014. The age of hens was from 50 to 70 weeks.

For the egg yolk oil extraction, thirty eggs from each group were cracked and yolks were separated from egg whites. Egg yolks in each group were pooled together and homogenized getting one pooled sample.

Extraction solvents (ethanol and hexane), used in egg yolk oil extraction, were analytical grade from Sigma Aldrich (Germany). Compressed nitrogen gas with purity 99.999 % HiQ Nitrogen 5.0 was from Linde AG (Germany).

B. Egg Yolk Oil Extraction

Lipid extraction with ethanol and hexane from liquid egg yolk was made by following steps. First, polar lipids were extracted with ethanol from liquid egg yolk and then neutral lipids were extracted from precipitate with hexane [9]. For polar lipid extraction, 200 g of homogenized liquid egg yolk was added to 400 ml of ethanol and stirred until egg yolk proteins denatured and completely dispersed. Extraction was done at 20°C for 30 minutes. Then, the mixture was filtered by vacuum filtration, and the supernatant was collected and transferred to a separatory funnel. The precipitate was extracted with 400 ml hexane vigorously mixing for 30 minutes at 20°C using a magnet stirrer. The extract was filtered by vacuum filtration and supernatant was collected and added to the same separatory funnel. Both ethanol and hexane extracts were thoroughly but gently, to avoid emulsion formation, mixed to extract polar lipids and impurities to a polar ethanol-water phase and neutral lipids to a non-polar hexane phase. Then the mixed extracts were left for 1 hour for phase separation. Bottom ethanol/water layer, containing polar lipids and water soluble compounds was drained from the separatory funnel through the open stopcock. Hexane extract was collected in clean container. Egg yolk oil was obtained from the hexane extract by evaporation of the hexane in the rotary evaporator IKA RV 10 Control V (IKA®-Werke GmbH and Co. KG) at the temperature of 70°C and 400 mbar pressure. After solvent evaporation in the rotary evaporator, as the last step of the solvent removal, the pure nitrogen gas was laid through the egg oil for 10 minutes in the same rotary evaporator with the same evaporation conditions by means of a plastic tube immersed in the oil "to be published" [10].

C. Analysis

Fatty acids were determined in accordance with the standard methods ISO 12966-2 and ISO 5508, GC-FID (gas chromatography with flame ionization detector) using Shimadzu GC 2010 Plus gas chromatograph with flame ionization detector (Shimadzu Corporation, Japan).

Oil soluble vitamins (α-tocopherol, ergocalcipherol and retinol) were determined by reverse phase high performance liquid chromatography (HPLC) with 100 % acetonitrile as a mobile phase. For HPLC analysis Shimadzu Nexera X2 liquid chromatograph with YMC Pack ODS-A column and diode array detector was used. The following wavelengths for vitamin detection were used: 325 nm for retinol, 250 nm for ergocalcipherol and 260 nm for α-tocopherol.

ß-carotene content was determined in accordance with the standard method ISO 17932:2011 using UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan).

The results were presented as the means and standard deviation for three replicates. Means were compared by t-test and analysis of variance (ANOVA). Significance was defined at $p<0.05$. Statistical analysis, tables and figures were carried out by Microsoft Excel 2010 version software.

III. Results and Discussions

A. Vitamins

Egg yolk oil is a source of oil soluble vitamins such as vitamin E, vitamin D and vitamin A.

Vitamin E is a very strong, lipid soluble antioxidant. Usually vitamin E content is expressed as a sum of tocopherols, but the results from previous studies show that vitamin E in egg yolk oil is presented as α-tocopherol and γ-tocopherol, besides γ-tocopherol content was very low [11]. α-tocopherol is the most bioactive form of vitamin E and therefore α-tocopherol was determined in this study as a vitamin E in all egg yolk oil samples.

α-tocopherol content in egg yolk can be affected through the hen feed and fluctuates from 10 till 967 mg kg^{-1} [2], [4], [12], [13]. α-tocopherol is a lipid soluble compound and it can be extracted from egg yolk in egg yolk oil.

Fig. 1 shows that α-tocopherol content in egg yolk oils was similar ($p<0.05$) for caged eggs and barn eggs, 205.56 ± 24.67 mg kg^{-1} and 225.54 ± 27.06 mg kg^{-1} respectively. α-tocopherol content in egg yolk oil extracted from free range eggs was 149.96 ± 17.99 mg kg^{-1}, that is significantly lower ($p>0.05$) than in oil from barn and caged eggs. But the lowest α-tocopherol content (101.32 ± 12.16 mg kg^{-1}) was determined in egg yolk oil extracted from eggs collected from free range free diet hens.

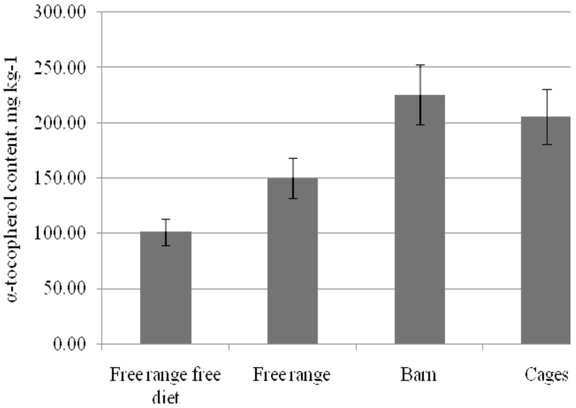

Fig. 1 α-tocopherol content in egg yolk oils from different hen housing systems

As in [14] vitamin E content in eggs from free range hens had twice higher amount of vitamin E than in caged eggs, but in their study free range hens were fed with mixed grass diet in comparison with caged hens which receive commercial compound feed. In our study we received the opposite result – vitamin E content was twice higher in egg yolk oil extracted from caged eggs that from free range free diet hen eggs.

Vitamin D is another representative of lipid soluble vitamins in egg yolk. There is a discussion among health care specialists which form of vitamin D is more important for health - cholecalcipherol (D_3) or ergocalcipherol (D_2). In our study it was important to determine difference in vitamin D content in different egg yolk oil samples, and ergocalcipherol was chosen as representative of vitamin D.

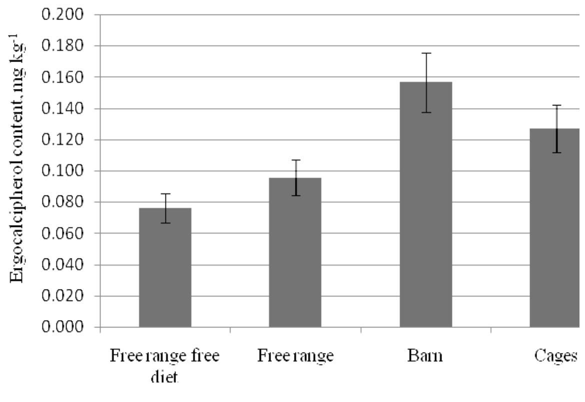

Fig. 2 Ergocalcipherol content in egg yolk oils from different hen housing systems

Fig. 2 clearly shows that ergocalcipherol content in egg yolk oil extracted from both free range eggs was significantly ($p<0.05$) lower than from barn and cage eggs.

There was no difference in ergocalcipherol content in barn egg yolk oil which contained 0.157 ± 0.019 mg kg^{-1} and cage egg yolk oil which contained 0.127 ± 0.015 mg kg^{-1} of ergocalcipherol. But egg yolk oil extracted from free range free diet hen eggs contained less vitamin D than yolk oil from free range compound feed hen eggs, 0.076 ± 0.009 mg kg^{-1} and 0.096 ± 0.011 mg kg^{-1}, respectively. As vitamin D content in eggs is correlated with hen feed supplemented with vitamin D [5], [15], we can conclude that there was deficiency of vitamin D in free range free diet hens feed and physical activity of hens in both free range systems consumes vitamin D for hen physiological needs for energy (Krebs cycle).

Animal form of vitamin A - retinol is presented in egg yolk in high concentrations. The higher content of retinol was determined in egg yolk oil from free range free diet hen eggs. Eggs for experiment were collected in summer (August) when free range free diet hens have access to fresh grass. Hens were pastured on living grass, and the fact can explain the high content (13.66 ± 1.64 mg kg^{-1}) of retinol in free range free diet hen eggs. We must admit that free range free diet hen egg yolk color was darker than yolks of other experimental eggs, what can be affected by usage of grass forage in hen diet [16]. It can be explained with high lutein and zeaxanthin content which was not determined in this study. The color of barn and caged egg yolks was lighter than free range free feed egg yolk and the retinol content in egg yolk oils was also lower, 10.07 ± 1.21 mg kg^{-1} for barn and 9.80 ± 1.18 mg kg^{-1} for caged eggs. Such difference in retinol content results shows that vitamin A content in egg yolk oil depends on hen feed (green forage).

As in [1] vitamin A content in eggs was not affected by the housing method. Vitamin A concentration in eggs can be significantly increased by addition of retinol in hen feed, but increased supplementation of vitamin A decreases α-tocopherol content in egg yolk [2], [4], [17]. Comparing our results for free range free diet group, we can confirm the relationship between α-tocopherol and retinol levels in egg yolk oil, where high concentration of retinol results to lower content of α-tocopherol.

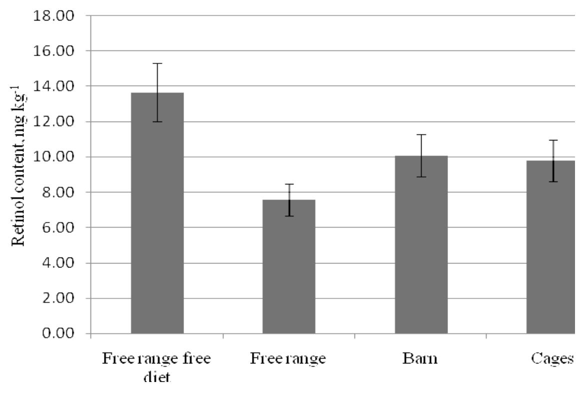

Fig. 3 Retinol content in egg yolk oils from different hen housing systems

The lowest content (7.56 ± 0.91 mg kg^{-1}) of retinol was in free range egg yolk oil. This hen flock was fed only with commercially produced compound feed without access to green forage that can explain such low concentration of retinol.

B. β-Carotene

β-carotene content in egg yolk oil plays an important role. It is not responsible only for very attractive orange color of egg yolk oil, but also, as a precursor of vitamin A, it gives additional nutritional value to egg yolk oil. Extraction process of egg yolk oil was based on different compound polarities, and hexane was used to extract non-polar compounds, such as β-carotene as much as possible.

β-carotene distribution among the egg yolk oils from different hen housing methods was similar to retinol results. The highest content of β-carotene (136 ± 5 mg kg^{-1}) was determined in egg yolk oil obtained from free range free diet hen egg yolk. Barn and cage system eggs gave 48 ± 4 mg kg^{-1} and 54 ± 4 mg kg^{-1} respectively of β-carotene in egg yolk oil, but the lowest content of β-carotene (33 ± 3 mg kg^{-1}) was determined in free range egg yolk oil where hens were fed with commercial compound feed. Comparing results from both free range systems we can ascertain that β-carotene content in egg yolk oil is affected by the hen diet. The increase of β-carotene and retinol content in egg yolk by supplementing hen feed with β-carotene was also reported in other studies [2], [17]. As in [1] β-carotene content is higher in free range eggs than in eggs from caged hens, in case that hen diet contains higher amount of β-carotene.

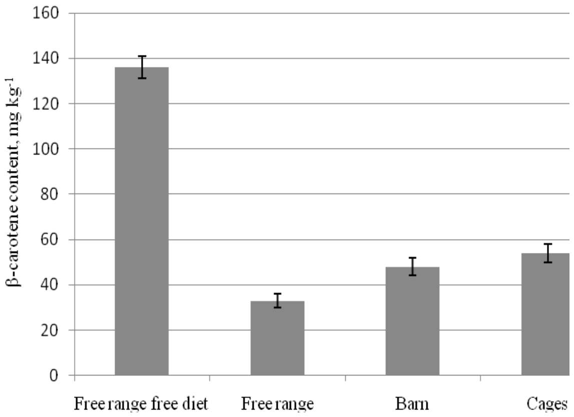

Fig. 4 β-carotene content in egg yolk oils from different hen housing systems

C. Fatty Acids

The total fatty acid profile of all egg yolk samples was similar that can be explained by the usage of hens with the same genetics. Fig. 5 shows total amount of saturated, monounsaturated and polyunsaturated fatty acids in egg yolk oils extracted from eggs collected from different hen housing systems. The lowest content of polyunsaturated fatty acids was determined in egg yolk oil from free range free diet eggs. Polyunsaturated fatty acid content in egg yolk oils from all other hen keeping systems was significantly ($p<0.05$) higher than from free range free diet group. The difference was in hen diets where all other hen groups except free range free feed group were fed with commercial compound feed.

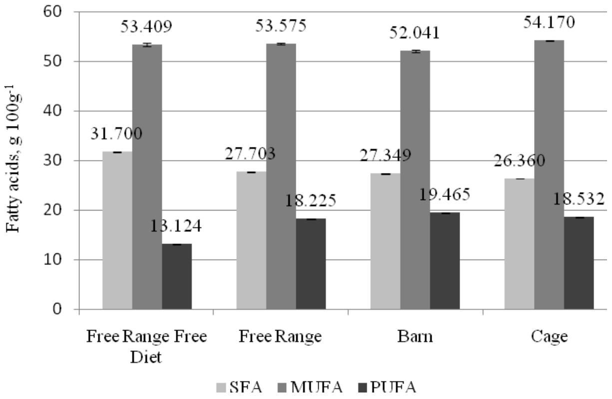

Fig. 5 Fatty acid profile of egg yolk oils from different hen housing systems SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

Fatty acid content in egg yolk oil samples presented in Table I.

TABLE I
FATTY ACID CONTENT IN EGG YOLK OILS FROM DIFFERENT HEN HOUSING SYSTEMS

Fatty acid	Fatty acid content, g $100g^{-1}$			
	Free range free diet	Free range	Barn	Cages
Palmitic acid	24.079 ± 0.036	20.854 ± 0.071	20.009 ± 0.058	19.354 ± 0.042
Stearic acid	6.528 ± 0.080	5.778 ± 0.022	6.233 ± 0.030	5.861 ± 0.017
Tricosylic acid	1.093 ± 0.002	1.071 ± 0.002	1.107 ± 0.002	1.145 ± 0.001
Palmitoleic acid	5.359 ± 0.055	3.275 ± 0.033	3.404 ± 0.037	3.318 ± 0.024
Oleic acid	48.050 ± 0.398	50.300 ± 0.174	48.637 ± 0.221	50.852 ± 0.099
Linoleic acid	11.117 ± 0.077	15.442 ± 0.053	16.453 ± 0.062	15.618 ± 0.042
α-linolenic acid	1.251 ± 0.001	1.815 ± 0.002	2.045 ± 0.002	1.784 ± 0.002
Docosahexaenoic acid	0.756 ± 0.001	0.998 ± 0.002	0.967 ± 0.001	1.130 ± 0.001
Omega-3	2.007 ± 0.001	2.813 ± 0.002	3.012 ± 0.002	2.914 ± 0.002
Omega-6	11.117 ± 0.077	15.442 ± 0.053	16.453 ± 0.062	15.618 ± 0.042
Omega-6/Omega-3	5.5/1	5.5/1	5.5/1	5.4/1

One of the important nutritional values of egg yolk oil is a content of omega-3 and omega-6 polyunsaturated fatty acids [18]. In egg yolk oil omega-3 fatty acids are presented as α-linolenic acid and docosahexaenoic acid. The highest omega-3 fatty acids content was determined in egg yolk oil extracted from barn eggs (3.012 ± 0.002 g $100g^{-1}$) and the lowest in oil extracted from free range free diet hen eggs (2.007 ± 0.001 g $100g^{-1}$). Egg enrichment with omega-3 fatty acids using a source of polyunsaturated fatty acids in hen diet was reported by other researchers [3]. Pastured hens do not have access to high fat content feed that can explain the low content of omega-3 fatty acids in free range free diet eggs. As in [1] omega-3 fatty acid content in free range eggs was lower than in caged eggs. Our results confirm that caged eggs and also barn eggs have more omega-3 fatty acids in comparison to free range free feed eggs. Barn and cage eggs results were very close to each other and eggs contain 3.012 ± 0.002 g $100g^{-1}$ and 2.914 ± 0.002 g $100g^{-1}$ of omega-3 fatty acids, respectively.

Omega-6 fatty acid in egg yolk is presented by linoleic acid whose content in egg yolk oil fluctuates from 11.117 ± 0.077 g $100g^{-1}$ till 16.453 ± 0.062 g $100g^{-1}$.

The ratio between omega-6 and omega-3 fatty acid is also very important for human health [18]. The best ratio of omega-6/omega-3 reported by health specialists is 4:1 or even 2:1. The good balance of omega-6/omega-3 (5.5/1) in egg yolk oil increases its nutritional value.

IV. Conclusion

1. Higher bioactive compound concentration in egg yolk oil depends on hen feed content, but not on the hen housing system. Vitamin E, vitamin D and polyunsaturated fatty acid content in egg yolk oil was higher from eggs obtained from all housing systems where hens were fed with commercial compound feed. β-carotene and retinol content in egg yolk oils from free range free diet eggs was significantly ($p>0.05$) higher that from other eggs because hens have access to green forage.
2. Hen physical activity decreases bioactive compound content in egg yolk oil. From all hen groups which were fed with the same commercially produced compound feed, the lowest ergocalcipherol, α-tocopherol, retinol and β-carotene content was determined in egg yolk oil from free range eggs.
3. Eggs obtained from battery cage laying hen housing method is most acceptable for production of egg yolk oil in case of bioactive compound content and price.

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References

[1] K. E. Anderson, "Comparison of fatty acid, cholesterol, and vitamin A and E composition in eggs from hens housed in conventional cage and range production facilities," *Poult. Sci.*, vol 90, no 7, pp. 1600–1608, 2011.

[2] Y. H. Jiang, R. B. McGeachin, C. A. Bailey, "α-tocopherol, b-carotene, and retinol enrichment of chicken eggs," *Poult. Sci.*, vol. 73, no. 7, pp. 1137–1143, 1994.

[3] N. M. Lewis, S. Seburg, N. L. Flanagan, "Enriched eggs as a source of N-3 polyunsaturated fatty acids for humans," *Poultry Sci.*, vol. 79, no. 7, pp. 971–974, 2000.

[4] A. V. Mori, C. X. Mendonca Jr., C. R. M. Almeida, M. C. G. Pit, "Supplementing hen diets with vitamins A and E affects egg yolk retinol and α-tocopherol levels," *J. Appl. Poult. Res.*, vol. 12, no. 2, pp. 106–114, 2003.

[5] L. Yao, T. Wang, M. Persia, R. L. Horst, M. Higgins, "Effects of vitamin D_3-enriched diet on egg yolk vitamin D_3 content and yolk quality," *J. Food Sci.*, vol. 78, no. 2, pp. C178–183, 2013.

[6] C. M. Sherwin, G. J. Richards, C. J. Nicol, "Comparison of the welfare of layer hens in 4 housing systems in the UK," *Br. Poult. Sci.*, vol. 51, no. 4, pp. 488–499, 2010.

[7] D. V. Thomas, V. Ravindran, "Comparison of layer performance in cage and barn systems," *Journal of Animal and Veterinary Advances*, vol. 4, no. 5, pp. 554–556, 2005.

[8] D. C. Lay Jr., R. M. Fulton, P. Y. Hester, D. M. Karcher, J. B. Kjaer, J. A. Mench, B. A. Mullens, R. C. Newberry, C. J. Nicol, N. P. O'Sullivan, R. E. Porter, "Hen welfare in different housing systems," *Poult. Sci.*, vol. 90, no. 1, pp. 278–294, 2011.

[9] D. U. Ahn, S. H. Lee, H. Singam, E. J. Lee, J. C. Kim, "Sequential separation of main components from chicken egg yolk," *Food Sci. Biotechnol.,* vol. 15, no. 2, pp. 189–195, 2006.

[10] A. Kovalcuks, "Purification of egg yolk oil obtained by solvent extraction from liquid egg yolk," in *Annual 20th International Scientific Conference Research for Rural Development*, submitted for publication.

[11] A. Kovalcuks, M. Duma, "Interaction of selenium and vitamin E in eggs and egg yolk oil," in *Annual 19th International Scientific Conference Research for Rural Development*, Jelgava, Latvia, 15–17 May 2013, pp. 136–140.

[12] S. Grobas, J. Mendez, C. Lopez Bote, C. De Blas, G. G. Mateos, "Effect of vitamin E and A supplementation on egg yolk α-tocopherol concentration," *Poult. Sci.*, vol. 81, no. 3, pp. 376–381, 2002.

[13] K. R. Kang, G. Cherian, J. S. Sim, "Tocopherols, retinol and carotenes in chicken egg and tissues as influenced by dietary palm oil," *J. Food Sci.*, vol. 63, no. 4, pp. 592–596, 1998.

[14] H. D. Karsten, P. H. Patterson, R. Stout, G. Crews, "Vitamins A, E and fatty acid composition of the eggs of caged hens and pastured hens," *Renew. Agric. Food Syst.*, vol. 25, no. 1, pp. 45–54, 2010.

[15] P. Mattila, J. Valaja, L. Rossow, E. Venäläinen, T. Tupasela, "Effect of vitamin D2- and D3-enriched diets on egg vitamin D content, production, and bird condition during an entire production period," *Poult. Sci.*, vol. 83, no. 3, pp. 433–440, 2004.

[16] K. Horsted, M. Hammershøj, B. H. Allesen-Holn, "Effect of grass–clover forage and whole wheat feeding on the sensory quality of eggs," *J. Sci. Food Agric.*, vol. 90, no. 2, pp. 343–348, 2010.

[17] C. X. Mendonca Jr., C. R. M. Almeida, A. V. Mori, C. Watanabe, "Effect of dietary vitamin A on egg yolk retinol and tocopherol levels," *J. Appl. Poult. Res.*, vol. 11, no. 4, pp. 373–378, 2002.

[18] P. F. Surai, T. T. Papazyan, N. H. C. Sparks, B. K. Speake, "Simultaneous enrichment of eggs with PUFAs and antioxidants: prospects and limitations," in *Wild-type food in health promotion and disease prevention: the Columbus Concept*, F. de Meester, R. R. Watson Ed. Totowa, NJ: Humana Press Inc., 2008, pp. 139–153.