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ALGAL OIL AS SOURCE OF POLYUNSATURATED FATTY ACIDS IN LAYING HENS NUTRITION: EFFECT ON EGG PERFORMANCE, EGG QUALITY INDICES AND FATTY ACID COMPOSITION OF EGG YOLK LIPIDS*

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Abstract

The aim of this study was to evaluate the effect of algal oil with very high level of docosahexaenoic acid (DHA, $C_{22:6n-3}$) used as fat source in the diet for laying hens, on egg yolk lipids fatty acid composition, as well as egg production and egg quality indices, in comparison with other dietary fat sources. The experiment was carried out on 168 ISA Brown hens (25 to 60 wks of age), allocated to 7 groups of 12 replicates (cages), with two birds in each cage. The experimental diets were supplemented with 2% of different fat sources, i.e. soybean oil (SO), coconut oil (CO), rapeseed oil (RO), linseed oil (LO), camelina oil (CAO), fish oil (FO), or algal oil (AO). Laying performance indices, i.e. egg production, mean egg weight, feed intake, feed conversion ratio, or egg and eggshell quality parameters were not affected by used fat sources (P>0.05). Dietary fat sources significantly influenced egg yolk lipids fatty acids composition. Thus, AO addition caused some changes in the yolk lipid profile that were favourable from the dietary perspective, i.e., increased concentration of eicosapentaenoic acid (EPA) and DHA (P<0.05). However, boiled eggs from hens fed a diet with AO were characterized by an inferior flavour and taste to those from other groups. In conclusion, the results of this experiment have shown that the use of AO in the laying hens nutrition is an efficient way to increase the deposition of *n-3* long chain polyunsaturated fatty acids (*n-3* LCPUFAs) in eggs, without negative effect on egg performance, however further researches, aiming to establish optimal AO dietary level that does not adversely affect the organoleptic properties of eggs, are needed.

Key words: laying hens, algal oil, dietary fats, laying performance, egg quality, yolk fatty acids composition

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In the recent years the importance of improving the dietary properties of foods of animal origin (meat, milk, and eggs), mainly health-promoting modifications of their composition, has increased. The beneficial properties of dietary long-chain *n-3* polyunsaturated fatty acids (*n-3* LCPUFAs), namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), for human health are well-known, since they are essential for proper physiological functioning and exert many positive effects on the organism, being important in several metabolic processes, including the proper functioning of the nervous and immunological systems (Benatti et al., 2004; Calder, 2006), as well as having anti-hypertensive, anti-atherogenic, anti-carcinogenic, and anti-obesity properties (Simopoulos, 2004, 2008; Riediger et al., 2009; Yashodhara et al., 2009; Mori, 2014).

The most important source of *n-3* LCPUFAs for humans are fishes. However, in western countries, fish consumption is too low to meet the recommended levels of these acids, with a simultaneous imbalanced (too high) *n-6/n-3* PUFA ratio (Givens and Gibbs, 2008; Simopoulos, 2011). For this reason the health-promoting characteristics of animal-origin products can be enhanced by changing their proportion of fatty acids, i.e. their enrichment with *n-3* LCPUFAs (Fraeye et al., 2012; Barfourooshi et al., 2018; Liu and Kim, 2018; Stadnik et al., 2018; Franczyk-Żarów et al., 2019). As consumer acceptance of eggs enriched with *n-3* LCPUFAs is increasing worldwide, they can be good examples of functional food, i.e. food not only possessing traditionally understood nutritional value, but also food beneficially influencing the metabolic and health status of human, thus reducing the risk of chronic lifestyle diseases and having important roles during pregnancy and infant growth, especially brain and neural tissues development (Yashodhara et al., 2009; Fraeye et al., 2012; Zduńczyk and Jankowski, 2013). The most popular, nutritional method of enriching eggs with *n-3* LCPUFAs is to include in hen's diet such *n-3* PUFA sources as fish, flaxseed and rapeseed oils.

Algal biomass is a very rich source of biologically active substances, among others long chain *n-3* LCPUFAs (Belay et al., 1996), whose positive health effects in the animal and human body are related mainly to reduction of the production of inflammatory eicosanoids, cytokines, and reactive oxygen species and the expression of adhesion molecules (James et al., 2000; Calder, 2006; Schmitz and Ecker, 2008; Lum et al., 2013; Świątkiewicz et al., 2015 a). The results of several studies indicated that supplementation of the diet for laying hens with algal biomass can be a very efficient way to manipulate composition of yolk lipids and increase of LCPUFAs *n-3*, mainly DHA concentration in eggs (Parpinello et al., 2006; Rizzi et al., 2009; Bruneel et al., 2013; Lemahieu et al., 2013, 2014, 2015; Park et al., 2015; Haberecht et al., 2018; Selim et al., 2018; Keegan et al., 2019; Moran et al., 2019). Moreover, algae can be an alternative for fish as a source of LCPUFAs *n-3*, which is important because of overfishing and depletion of feed stocks as well as increasing pollution of fish biomass with heavy metals (Świątkiewicz et al., 2015 b). However, in the previous studies usually algal biomasses with different content of crude fats were evaluated and to date there is a very limited amount of experimental data on the efficacy of algal oil in laying hens nutrition. Therefore the goal of our study was to evaluate the effect of dietary algal oil (AO) on fatty acids composition of egg yolk,

laying performance, and egg quality indices, in comparison with other dietary oils that can be used in laying hens nutrition (soybean oil (SO), coconut oil (CO), rapeseed oil (RO), linseed oil (LO), camelina oil (CAO), and fish oil (FO)).

Material and methods

Birds and experimental diets

The experiment was carried out with 168 17-week-old ISA Brown hens, obtained from a commercial source. Hens were placed in a poultry house in cages (two birds per each cage), on a wire-mesh floor under controlled climate conditions. The cage dimensions were 30 cm × 120 cm × 50 cm, equating to 3600 cm^2 of total floor space. During the pre-experimental period (17 to 24 weeks of age), a standard commercial laying hens' diet was offered *ad libitum*. All experimental procedures relating to the use of live animals were performed according to guidelines of The Local Cracow Ethics Committee for Experiments.

Table 1. Composition and nutrient content of experimental diet, g/kg air dry matter

Item	
Ingredient (g/kg):	
Corn	422.1
Wheat	210.0
Soybean meal	236.0
Experimental oil[3]	20.0
Limestone	90.0
Monocalcium phosphate	12.5
NaCl	3.0
DL-Methionine	1.4
Vitamin-mineral premix[1]	5.0
Nutrients composition:	
Metabolizable energy (MJ/kg[2])	11.60
Crude protein	170.0
Lys	8.35
Met	4.10
Ca	37.0
Total P	6.15
Available P	3.90

[1]The premix provided per 1 kg of diet: vitamin A – 10,000 IU; vitamin D_3 – 3,000 IU; vitamin E – 50 IU; vitamin K_3 – 2 mg; vitamin B_1 – 1 mg; vitamin B_2 – 4 mg; vitamin B_6 – 1.5 mg; vitamin B_{12} – 0.01 mg; Ca-pantothenate – 8 mg; niacine – 25 mg; folic acid – 0.5 mg; choline chloride – 250 mg; manganese – 100 mg; zinc – 50 mg; iron – 50 mg; copper – 8 mg; iodine – 0.8 mg; selenium – 0.2 mg, cobalt – 0.2 mg.

[2]Calculated according to European Table (Janssen, 1989) as a sum of the ME content of components.

[3]Following experimental oils were used: treatment 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

At week 25, the hens were randomly assigned to one of 7 treatments, each comprising 12 cages (replicates) with 2 hens in each cage. Hens were fed experimental diets until week 60. The composition of the experimental cereal-soybean diets is given in Table 1. During the experiment, the hens had free access to mash feed and water, and were exposed to a 14 L:10 D lighting schedule.

Table 2. Fatty acids profile of oils used in the experiment, % of total fatty acids

Item	SO[1]	CO	RO	LO	CAO	FO	AO
$C_{8:0}$	0.0	7.46	0.0	0.0	0.0	0.0	0.0
$C_{10:0}$	0.0	5.91	0.0	0.0	0.0	0.0	0.0
$C_{12:0}$	0.0	49.9	0.0	0.0	0.0	0.05	0.11
$C_{14:0}$	0.0	19.7	0.0	0.08	0.08	3.55	3.22
$C_{16:0}$	8.62	8.61	4.75	9.50	6.22	11.2	37.8
$C_{16:1}$	0.10	0.02	0.20	0.10	0.09	2.95	0.23
$C_{18:0}$	4.29	1.92	1.44	5.99	2.17	2.42	1.11
$C_{18:1}$	35.7	5.10	59.5	25.0	14.1	39.6	1.66
$C_{18:2\ n-6}$	38.1	1.35	19.6	25.4	18.7	14.0	2.59
$C_{18:3\ n-3}$	12.1	0.01	13.3	33.3	53.2	8.14	0.25
$C_{20:4\ n-6}$	0.46	0.0	0.0	0.27	1.43	7.79	0.15
$C_{20:5}$ (EPA)	0.0	0.0	0.04	0.01	3.61	4.66	1.03
$C_{22:6}$ (DHA)	0.0	0.0	0.0	0.0	0.19	4.42	51.3
SFA[2]	13.8	93.5	6.95	16.0	8.64	17.6	42.2
UFA	86.2	6.49	93.0	84.0	91.4	62.4	57.6
MUFA	35.9	5.11	60.0	25.2	17.8	43.2	1.90
PUFA	50.3	1.37	33.1	58.9	73.5	39.2	55.7
PUFA *n-6*	38.1	1.36	19.7	25.5	20.1	21.9	2.93
PUFA *n-3*	12.1	0.01	13.4	33.3	53.4	17.2	52.7
PUFA *n-6*/PUFA *n-3* ratio	3.14	94.36	1.47	0.77	0.38	1.27	0.06

[1]SO – soybean oil, CO – coconut oil, RO – rapeseed oil, LO – linseed oil, CAO – camelina oil, FO – fish oil, AO – algae oil.

[2]SFA – saturated fatty acids, UFA – unsaturated fatty acids, MUFA – monosaturated fatty acids, PUFA – polyunsaturated fatty acids.

The experimental diets (Table 1) were supplemented with 2% of different fat sources, i.e. soybean oil (SO), coconut oil (CO), rapeseed oil (RO), linseed oil (LO), camelina oil (CAO), fish oil (FO), or algal oil (AO). The fatty acid profiles of used oils were shown in Table 2. The nutritional composition of the experimental diets was calculated based on the chemical composition of raw feedstuffs, the metabolisable energy value on the basis of equations from European Tables (Janssen, 1989). The chemical composition of the feed materials was analysed using conventional methods (AOAC, 2000). Amino acids were determined in acid hydrolysates, after the initial peroxidation of sulphur amino acids, in a colour reaction with a ninhydrin reagent using a Beckman System Gold 126AA automatic analyser. Calcium content

was determined using flame atomic absorption spectrophotometry and phosphorus content using the calorimetric method (AOAC, 2000).

Measurements

During the experiment, feed intake, number and weight of laid eggs were recorded and laying performance, daily egg mass, daily feed intake and feed conversion per 1 kg of eggs and per individual egg calculated. At weeks 36, 48 and 60, one egg from each hen was collected to determine eggshell quality, using the EQM system (Technical Services and Supplies, York, England) as described by Krawczyk et al. (2013). Another egg was collected for the measurement of shell breaking strength. The shell breaking strength was measured by using a texture analyser TA.XT Plus (Stable Micro Systems, Godalming, Surrey, UK) fitted with a 30 kgf load cell and 77-mm compression plate (P/75). The eggs were compressed at a constant test speed of 2 mm/min and the breaking strength was determined at the time of eggshell fracture.

At the end of the experiment (60 weeks of age), one egg from each cage (replication) was collected for determination of sensory parameters. After boiling for 10 min, the eggs were evaluated by a 6-person panel. The panelists ranked the flavour and taste of the eggs on a 4-point scale (from 2 to 5) for the degree of liking (2 – flavour and taste unacceptable, 3 – acceptable, 4 – good, 5 – very good).

Statistical analysis

The data were subjected to statistical analysis using a completely randomized design, in accordance with the GLM procedure of Statistica 5.0 (StatSoft, Inc., Tulsa, OK, USA). All data were analysed using one-way ANOVA. When significant differences in treatment means were detected by ANOVA (F-test), Duncan's multiple range test was applied to the individual means. Statistical significance was considered to be $P \leq 0.05$.

Results

Laying performance and egg quality

Mean egg production, averaged across all dietary treatments throughout the first phase of the laying cycle (26 to 43 wks of age) was 95.9%; daily egg mass, 56.8 g/hen; daily feed consumption, 113 g/hen; and feed conversion, 1.98 kg of feed/kg of eggs; throughout the second phase (44 to 60 wks): 93.5%, 58.1 g, 116 g/hens, and 2.00 kg/kg, respectively; and throughout the entire experimental period (26 to 60 wks): 94.6%, 57.5 g, 114 g and 1.99 kg/kg, respectively (Tables 3, 4 and 5). Dietary fats used in our experiment did not affect performance indices ($P>0.05$). Similarly, there were no significant differences in egg (albumen height, Haugh units, yolk colour) and eggshell (eggshell %, thickness, density, and breaking strength) quality parameters between the treatments at 36, 48 and 60 wks of hen's age (Tables 6, 7 and 8).

Table 3. Effects of dietary treatments on egg performance of hens during first phase of laying cycle, 26–43 weeks of age

	Dietary treatment*							P level	SEM
	1	2	3	4	5	6	7		
Number of eggs produced/100 hens per day	96.5	96.2	95.6	95.5	95.6	96.5	95.7	0.434	0.266
Daily mass of eggs (g per hen)	56.5	57.2	56.0	56.6	56.9	57.7	56.8	0.306	0.195
Egg weight (g)	58.5	59.5	58.5	59.3	59.5	59.8	59.3	0.335	0.178
Daily feed intake (g per hen)	113	113	112	113	113	113	112	0.438	0.253
Feed conversion (kg per kg of eggs)	2.00	1.97	2.01	1.99	1.99	1.95	1.98	0.378	0.007

*/ 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

Table 4. Effects of dietary treatments on egg performance of hens during second phase of laying cycle, 44–60 weeks of age

	Dietary treatment*							P level	SEM
	1	2	3	4	5	6	7		
Number of eggs produced/100 hens per day	93.3	93.7	93.6	93.5	93.7	93.5	93.5	0.865	0.302
Daily mass of eggs (g per hen)	58.0	59.1	57.5	58.4	57.9	59.0	56.9	0.345	0.279
Egg weight (g)	62.2	63.1	61.5	62.5	61.7	63.1	61.6	0.411	0.249
Daily feed intake (g per hen)	116	117	116	117	117	116	115	0.913	0.361
Feed conversion (kg per kg of eggs)	2.00	1.98	2.01	2.00	2.01	1.97	2.03	0.557	0.008

*/ 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

Table 5. Effects of dietary treatments on egg performance of hens during entire experimental period cycle, 26–60 weeks of age

	Dietary treatment*							P level	SEM
	1	2	3	4	5	6	7		
Number of eggs produced/100 hens per day	94.9	95.0	94.6	94.5	94.7	95.0	93.8	0.746	0.196
Daily mass of eggs (g per hen)	57.2	58.1	56.8	57.5	57.4	58.4	56.7	0.201	0.205
Egg weight (g)	60.3	61.2	60.0	60.9	60.6	61.4	60.5	0.326	0.181
Daily feed intake (g per hen)	115	115	114	115	115	114	113	0.729	0.229
Feed conversion (kg per kg of eggs)	2.00	1.98	2.01	2.00	2.00	1.96	2.00	0.136	0.007

*/ 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

Table 6. Effect of dietary treatments on egg quality indices at 36 weeks of age

	Dietary treatment*							P level	SEM
	1	2	3	4	5	6	7		
Albumen height (mm)	11.0	10.8	11.3	11.7	11.4	11.9	11.7	0.324	0.134
Haugh units	103	102	104	106	104	106	105	0.351	0.553
Yolk colour (points on DSM scale)	3.50	3.42	3.66	3.42	3.58	3.66	3.36	0.323	0.057
Eggshell (%)	11.1	11.1	11.2	11.6	11.3	11.5	11.6	0.272	0.074
Eggshell thickness (μm)	0.390	0.373	0.376	0.376	0.383	0.376	0.376	0.660	0.003
Eggshell density (mg/cm^2)	89.6	87.4	87.0	89.0	89.3	92.7	91.9	0.442	0.808
Eggshell breaking strength (N)	57.2	59.1	55.4	56.6	53.5	56.9	59.4	0.347	0.755

*/ 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

Table 7. Effect of dietary treatments on egg quality indices at 48 weeks of age

	Dietary treatment*							P level	SEM
	1	2	3	4	5	6	7		
Albumen height (mm)	9.03	9.07	8.98	8.83	8.85	9.12	8.98	0.895	0.125
Haugh units	93.4	94.5	93.2	92.4	90.4	94.1	94.0	0.667	0.674
Yolk colour (points on DSM scale)	2.33	2.58	2.50	2.42	2.58	2.33	2.50	0.556	0.054
Eggshell (%)	10.8	11.5	11.2	11.4	11.3	11.2	11.4	0.083	0.068
Eggshell thickness (μm)	0.369	0.374	0.371	0.372	0.381	0.379	0.376	0.770	0.003
Eggshell density (mg/cm^2)	89.3	86.3	89.6	89.2	91.5	87.5	91.7	0.326	0.686
Eggshell breaking strength (N)	53.4	56.7	56.8	57.5	57.4	56.7	56.4	0.920	0.909

*/ 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

Table 8. Effect of dietary treatments on egg quality indices at 60 weeks of age

	Dietary treatment*							P level	SEM
	1	2	3	4	5	6	7		
1	2	3	4	5	6	7	8	9	10
Albumen height (mm)	9.03	9.23	8.97	8.95	8.85	9.12	8.98	0.887	0.116
Haugh units	93.7	95.1	93.4	93.0	91.3	94.1	94.5	0.520	0.623
Yolk colour (points in DSM scale)	2.33	2.58	2.50	2.41	2.58	2.33	2.25	0.556	0.054

Table 8 – contd.

1	2	3	4	5	6	7	8	9	10
Eggshell (%)	10.8	11.5	11.2	11.4	11.3	11.2	11.4	0.083	0.068
Eggshell thickness (μm)	0.369	0.374	0.371	0.372	0.381	0.379	0.376	0.877	0.003
Eggshell density (mg/cm^2)	86.3	89.6	89.2	91.6	89.4	88.6	91.7	0.326	0.686
Eggshell breaking strength (N)	53.4	56.7	56.8	57.5	57.4	56.7	56.4	0.920	0.909

*/ 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

Fatty acids profile of egg yolk lipids and sensory properties of boiled eggs

There were significant differences in fatty acids profile of egg yolk lipids between dietary treatments (Tables 9 and 10). Dietary inclusion of AO positively influenced dietetic properties of eggs, i.e. increased level of such LCPUFA as EPA and DHA, as well decreased *n-6*/*n-3* ratio ($P<0.05$) without any effect on cholesterol concentration. Beneficial changes in egg yolk fatty acid profile were also noted in hens fed the diet containing LO, CAO and FO (as compared to SO, CO, and RO diets), however this effect was to a significantly lesser extent than in AO treatment. As compared to other experimental treatments, the use of LO and AO negatively affected the sensory properties, i.e. flavour and taste, of boiled eggs (Table 11).

Table 9. Effect of dietary treatment on concentrations of selected fatty acids and cholesterol in egg yolk lipids (%)

	Dietary treatment*							P level	SEM
	1	2	3	4	5	6	7		
$C_{16:0}$	27.3 c	28.2 d	26.3 b	25.2 a	26.2 b	26.6 b	28.4 d	0.0001	0.193
$C_{18:0}$	8.41	7.69	7.90	8.26	7.89	7.92	7.84	0.064	0.070
$C_{18:1}$	41.5 a	43.6 b	46.7 c	42.7 ab	43.2 b	45.9 c	43.3 b	0.0001	0.317
$C_{18:2\ n-6}$	19.9 b	10.3 a	11.2 a	11.1 a	11.6 a	10.8 a	10.0 a	0.021	0.869
$C_{18:3\ n-3}$	1.055 c	0.510 a	1.092 c	5.360 e	3.588 d	1.095 c	0.763 b	0.0001	0.265
$C_{20:4\ n-6}$	1.780 f	1.546 d	1.662 e	1.007 b	1.183 c	1.177 c	0.737 a	0.0001	0.055
$C_{20:5}$ (EPA)	0.0167 a	0.0117 a	0.0233 a	0.1217 c	0.0950 b	0.0983 b	0.1333 c	0.0001	0.008
$C_{22:6}$ (DHA)	1.120 b	0.863 a	1.343 b	2.207 c	2.073 c	2.513 d	4.860 e	0.0001	0.197
Cholesterol (mg/g of yolk)	10.45	10.08	10.53	10.75	10.95	10.58	10.75	0.196	0.090

*/ 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

Table 10. Effect of dietary treatment on concentrations of main groups of fatty acids in egg yolk lipids (%)

	Dietary treatment*							P level	SEM
	1	2	3	4	5	6	7		
SFA	36.2 c	38.3 d	34.7 ab	33.9 a	34.6 ab	34.9 b	36.7 c	0.0001	0.247
UFA	63.8 b	61.6 a	65.3 cd	66.1 d	65.4 cd	65.0 c	63.3 b	0.0001	0.247
MUFA	44.8 a	48.3 c	49.9 d	46.2 b	46.8 b	48.8 cd	46.8 b	0.0001	0.295
PUFA	18.9 de	13.4 a	15.4 b	19.9 e	18.6 d	15.7 bc	16.6 c	0.0001	0.361
PUFA n-6	16.8 c	12.0 b	12.9 b	12.2 b	12.8 b	12.0 b	10.8 a	0.0001	0.295
PUFA n-3	2.19 b	1.39 a	2.48 b	7.69 e	5.76 d	3.71 c	5.76 d	0.0001	0.337
n-6/n-3	7.66 f	8.61 g	5.25 e	1.58 a	2.23 c	3.24 d	1.88 b	0.0001	0.415

*/ 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

Table 11. Effect of dietary treatment on sensory properties of boiled eggs (points)

	Dietary treatment*							P level	SEM
	1	2	3	4	5	6	7		
Flavour	4.81 a	4.65 a	4.79 a	4.42 b	4.70 a	4.63 a	3.85 c	0.0001	0.053
Taste	4.75 a	4.60 a	4.68 a	4.14 b	4.61 a	4.62 a	3.41 c	0.0001	0.074

*/ 1 – soybean oil, 2 – coconut oil, 3 – rapeseed oil, 4 – linseed oil, 5 – camelina oil, 6 – fish oil, 7 – algae oil.

Discussion

Laying performance and egg quality

The results of our experiment indicate that supplementation of the hen's diet with all of the used experimental oils, also with rich sources of LCPUFA *n-3* as FO or AO, have no negative influence on laying performance, feed intake, feed conversion ratio, and egg quality indices. Similarly, Cheng et al. (2004) found no effect of algal oil (0.5 or 2.0% in the diet) on egg production. Our findings confirm the results of several earlier studies on the use of algae biomass in laying hens nutrition, as the source of LCPUFA *n-3*, showing no effects of algae on egg performance (Bruneel et al., 2013; Lemahieu et al., 2013) or egg quality indices (Lemahieu et al., 2013). Recently, Josling et al. (2019) found no effect of fatty acid composition and saturation degree of diet on eggshell quality, however femur bones characteristics were positively affected in layers fed the diet supplemented with fish oil, i.e. diet with high content of *n-3* PUFA. Park et al. (2015) even observed a positive effect of microalgae (*Schizochytrium*) biomass at 1% dietary level on egg production and eggshell thickness (without effect on eggshell breaking strength). More recently positive influence of *Spirulina platensis* biomass (0.1–0.3% in the hens' diet) on performance indices and eggshell thickness was reported (Selim et al., 2018). However, in the earlier study by Herber and Van Elswyk (1996), layers fed the diet containing 4.8% marine algae mass had decreased egg production as compared to control group. Several authors indicated that inclusion of rich LCPUFA sources can impair egg weight, prob-

ably because of the fact that decrease in circulating triglycerides in blood due to high PUFA *n-3* consumption may limit the availability of lipids for yolk formation (Van Elswyk, 1997; Gonzalez-Esquerra and Leeson, 2000), but in our experiment such adverse effect of dietary oils rich in these acids was not observed.

Yolk fatty acids composition and sensory properties of boiled eggs

Algal oil is unique and different from other marine fats because of very high concentration of docosahexaenoic acid (DHA, $C_{22:6n-3}$) and, simultaneously, relatively low level of other *n-3* fatty acids. The results of the analysis of egg yolks from hens fed with different oil sources in our study confirmed the high efficacy of algal oil as a source of LCPUFA *n-3*, especially DHA, in laying hens nutrition. Thus, the concentration of DHA in eggs from hens fed with AO was much higher than for the other experimental oils. To date there is limited amount of experimental data on the efficacy of AO, used as dietary supplement for layers, in enriching of yolk lipids in LCPUFA *n-3*. Cheng et al. (2004) used 0.0, 0.5 or 2.0% of algal oil in the diet of layers, and, similarly to our results, found that DHA concentration in egg yolks increased significantly with the dietary supplementation of DHA. Similar dietary levels of AO (0.77 or 1.77%) were used by Cachaldora et al. (2005) who also reported high efficacy of LCPUFA *n-3* retention in egg yolks in hens fed the diet supplemented with this oil.

Many more previous studies were aimed to evaluate the algal biomass, as a rich source of LCPUFAs *n-3*, in laying hens nutrition. Generally, results of these experiments are in agreement with the findings of our study and indicated that algal biomass is very efficient as feed additive increasing LCPUFAs *n-3* concentration in egg yolks. For example, Bruneel et al. (2013) reported a significantly increased concentration of DHA in egg yolks of layers fed a diet containing *Nannochloropsis gaditana* biomass and indicated that these algae can be used for the production of designer eggs enriched with DHA as an alternative to current sources of LCPUFA *n-3*. Lemahieu et al. (2013) indicated that the high enrichment of egg yolks with PUFA *n-3*, simultaneously with increased yolk colour, can be achieved by the dietary supplementation of *Phaeodactylum* or *Isochrysis* algal biomass. Their subsequent experiments proved the suitability of *Isochrysis* biomass as an LCPUFA *n-3* source in layer's diet and showed that a supplementation of 2.4% *Isochrysis* leads to the highest enrichment of yolk with LCPUFA *n-3* (Lemahieu et al., 2014, 2015). In a much earlier experiment by Herber and van Elswyk (1996) a golden marine algae biomass incorporated in the hen's diet promoted very efficient deposition of DHA in the yolks. A similar effect on increased DHA yolk content was obtained by diet supplementation with marine microalgae *Schizochytrium limacinum* biomass (Rizzi et al., 2009). More recently Keegan et al. (2019) increased the egg DHA concentration and decreased the *n-6*/*n-3* ratio, improving the nutritional value of the eggs, by the addition of 0.5, 1.0 or 1.5% *Aurantiochytrium limacinum* biomass to the diet for laying hens.

In our study, the sensory properties of boiled eggs were negatively affected by LO and AO, but, surprisingly, not by FO. Generally, this observation is in agreement with several earlier experiments, where it was reported that excess of LCPUFA *n-3*

in the diet for laying hens can impair the sensory quality of LCPUFA enriched eggs by producing the off-flavour often described as "fishy" by the panelists. For instance, Lawlor et al. (2010) showed that high dietary level of fish oil (6%) is an effective way for increasing LCPUFA *n-3* content of yolk lipids, but it leads to some adverse sensory attributes of hard boiled eggs. On the other hand, Parpinello et al. (2006) did not find any negative effect of the use of algal biomass, as a source of LCPUFA *n-3* in the diet of laying hens, on sensory indices of eggs.

In conclusion, the results of this study indicated that the use of AO in the laying hens nutrition is an efficient way to increase the deposition of LCPUFA *n-3* in eggs, without any negative effect on egg performance indices. However, further researches, aiming to establish optimal AO dietary level that does not adversely affect the sensory attributes of eggs, are needed.

References

AOAC (2000). Official methods of analysis. 17th ed. Association of Official Analytical Chemists. Gaithersburg, USA.

Barfourooshi H.J., Towhidi A., Sadeghipanah H., Zhandi M., Zeinoaldini S., Dirandeh E., Akers R.M. (2018). Effect of dietary fish oil on mammary gland development and milk production of Holstein cow. Ann. Anim. Sci., 18: 973–990.

Belay A., Kati T., Ohta Y. (1996). Spirulina (*Arthrospira*): potential application as an animal feed supplement. J. Appl. Phycol., 8: 303–311.

Benatti P., Peluso G., Nicolai R., Calvani M. (2004). Polyunsaturated fatty acids: biochemical, nutritional and epigenetic properties. J. Amer. Coll. Nutr., 23: 281–302.

Bruneel C., Lemahieu C., Fraeye I., Ryckebosch E., Muylaert K., Buyse J., Foubert I. (2013). Impact of microalgal feed supplementation on omega-3 fatty acid enrichment of hen eggs. J. Funct. Foods, 5: 897–904.

Cachaldora P., De Blas J.C., de Blas J.C., García-Rebollar P., Álvarez C., Méndez J. (2005). Effects of type and level of supplementation with dietary *n-3* fatty acids on yolk fat composition and *n-3* fatty acid retention in hen eggs. Spanish J. Agricult. Res., 3: 209–212.

Calder P.C. (2006). *n-3* polyunsaturated fatty acids, inflammation, and inflammatory diseases. Amer. J. Clin. Nutr., 83: 1505S–1519S.

Cheng C.H., Shen T.F., Chen W.L., Ding S.T. (2004). Effects of dietary algal docosahexaenoic acid oil supplementation on fatty acid deposition and gene expression in laying Leghorn hens. J. Agricult. Sci., 142: 683–690.

Fraeye I., Bruneel C., Lemahieu C., Buyse J., Muylaert K., Foubert I. (2012). Dietary enrichment of eggs with omega-3 fatty acids: A review. Food Res. Int., 48: 961–969.

Franczyk-Żarów M., Szymczyk B., Kostogrys R.B. (2019). Effects of dietary conjugated linoleic acid and selected vegetable oils or vitamin E on fatty acid composition of hen egg yolks. Ann. Anim. Sci., 19: 173–178.

Givens D.I., Gibbs R.A. (2008). Current intakes of EPA and DHA in European populations and the potential of animal-derived foods to increase them. Proc. Nutr. Soc., 67: 273–280.

Gonzalez-Esquerra R., Leeson S. (2000). Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile, and sensory quality of eggs. Poultry Sci., 79: 1597–1602.

Haberecht S., Wilkinson S., Roberts J., Wu S., Swick R. (2018). Unlocking the potential health and growth benefits of macroscopic algae for poultry. World's Poult. Sci. J., 74: 5–20.

Herber S.M., Van Elswyk M.E. (1996). Dietary marine algae promotes efficient deposition of *n-3* fatty acids for the production of enriched shell eggs. Poultry Sci., 75: 1501–1507.

James M.J., Gibson R.A., Cleland L.G. (2000). Dietary polyunsaturated fatty acids and inflammatory mediator production. Amer. J. Clin. Nutr., 71: 343s–348s.

Janssen W.M.M.A. (1989). European Table of Energy Values for Poultry Feedstuffs. 3rd ed. Working Group No. 2 of the European Branch, World's Poultry Science Association, Beekbergen, The Netherlands.

Josling G.C., Hugo A., Fair M.D., de Witt F.H. (2019). Long term effect of dietary lipid saturation on eggshell quality and bone characteristics of laying hens. Poultry Sci., 98: 3593–3601.

Keegan J.D., Currie D., Knox A., Moran C.A. (2019). Heterotrophic *Aurantiochytrium* sp. supplementation to layer diets sustainably increases the omega-3 concentration of eggs. Brit. Poult. Sci, 60: 570–578.

Krawczyk J., Sokołowicz Z., Świątkiewicz S., Sosin-Bzducha E. (2013). Effect of outdoor access and increased amounts of local feed materials in the diets of hens covered by the gene-pool protection programme for farm animals in Poland on quality of eggs during peak egg production. Ann. Anim. Sci., 13: 327–339.

Lawlor J.B., Gaudette N., Dickson T., House J.D. (2010). Fatty acid profile and sensory characteristics of table eggs from laying hens fed diets containing microencapsulated fish oil. Anim. Feed Sci. Technol., 156: 97–103.

Lemahieu C., Bruneel C., Termote-Verhalle R., Muylaert K., Buyse J., Foubert I. (2013). Impact of feed supplementation with different omega-3 rich microalgae species on enrichment of eggs of laying hens. Food Chem., 141: 4051–4059.

Lemahieu C., Bruneel C., Termote-Verhalle R., Muylaert K., Buyse J., Foubert I. (2014). Effect of different microalgal *n-3* PUFA supplementation doses on yolk color and *n-3* LC-PUFA enrichment in the egg. Algal Res., 6: 119–123.

Lemahieu C., Bruneel C., Ryckebosch E., Muylaert K., Buyse J., Foubert I. (2015). Impact of different omega-3 polyunsaturated fatty acid (*n-3* PUFA) sources (flaxseed, *Isochrysis galbana*, fish oil and DHA Gold) on *n-3* LC-PUFA enrichment (efficiency) in the egg yolk. J. Funct. Foods, 19: 821–827.

Liu W.C., Kim I.H. (2018). Acid composition of pork, carcass traits and meat quality in finishing pigs. Ann. Anim. Sci., 18: 143–154.

Lum K.K., Kim J., Lei X.G. (2013). Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. J. Anim. Sci. Biotechnol., 4: 53.

Moran C.A., Morlacchini M., Keegan J.D., Fusconi G. (2019). Increasing the omega-3 content of hen's eggs through dietary supplementation with *Aurantiochytrium limacinum* microalgae: effect of inclusion rate on the temporal pattern of docosahexaenoic acid enrichment, efficiency of transfer, and egg characteristics. J. Appl. Poult. Res., 28: 329–338.

Mori T.A. (2014). Dietary *n-3* PUFA and CVD: a review of the evidence. Proc. Nutr. Soc., 73: 57–64.

Park J.H., Upadhaya S.D., Kim I.H. (2015). Effect of dietary marine microalgae (*Schizochytrium*) powder on egg production, blood lipid profiles, egg quality, and fatty acid composition of egg yolk in layers. Asian-Austral. J. Anim.. Sci., 28: 391–395.

Parpinello G.P., Meluzzi A., Sirri F., Tallarico N., Versari A. (2006). Sensory evaluation of egg products and eggs laid from hens fed diets with different fatty acid composition and supplemented with antioxidants. Food Res. Int., 39: 47–52.

Riediger N.D., Othman R.A., Suh M., Moghadasian M.H. (2009). A systemic review of the roles of *n-3* fatty acids in health and disease. J. Amer. Diet. Assoc., 109: 668–679.

Rizzi L., Bochocchio D., Bargellini A., Parazza P., Simiolo M. (2009). Effects of dietary microalgae, other lipid sources, inorganic selenium and iodine on yolk *n-3* fatty acid composition, selenium content and quality of eggs in laying hens. J. Sci. Food Agricult., 89: 1775–1781.

Schmitz G., Ecker J. (2008). The opposing effects of *n-3* and n-fatty acids. Progr. Lipid Res., 47: 147–155.

Selim S., Hussein E., Abou-Elkhair R. (2018). Effect of *Spirulina platensis* as a feed additive on laying performance, egg quality and hepatoprotective activity of laying hens. Europ. Poult. Sci., 82, doi: 10.1399/eps.2018.227.

Simopoulos A.P. (2004). Omega-6/omega-3 essential fatty acid ratio and chronic diseases. Food Rev. Int., 20: 77–90.

Simopoulos A.P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med., 233: 674–688.

Simopoulos A.P. (2011). Importance of the omega-6/omega-3 balance in health and disease: evolutionary aspects of diet. World Rev. Nutr. Diet., 102: 10–21.

Stadnik J., Czech A., Ognik K. (2018). Effect of soybean or linseed oil with RRR-d-α-tocopherol or dl-α-tocopherol acetate on quality characteristics and fatty acid profile of turkey meat. Ann. Anim. Sci., 18: 991–1005.

Świątkiewicz S., Arczewska-Wlosek A., Józefiak D. (2015 a). The relationship between dietary fat sources and immune response in poultry and pigs: An updated review. Livest. Sci., 180: 237–246.

Świątkiewicz S., Arczewska-Włosek A., Józefiak D. (2015 b). Application of microalgae biomass in poultry nutrition. World. Poult. Sci. J., 71: 663–672.

Van Elswyk M.E. (1997). Nutritional and physiological effects of flax seed in diets for laying fowl. World. Poult. Sci. J., 53: 253–264.

Yashodhara B.M., Umakanth S., Pappachan J.M., Bhat S.K., Kamath R., Choo B.H. (2009). Omega-3 fatty acids: a comprehensive review of their role in health and disease. Postgr. Med. J., 85: 84–90.

Zduńczyk Z., Jankowski J. (2013). Poultry meat as functional food: modification of the fatty acid profile – a review. Ann. Anim. Sci., 13: 463–480.

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