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THE STORAGE QUALITY OF EGGS from layers fed with the addition of vegetable oils

Abstract. ***The aim of the study was to evaluate the quality of eggs from laying hens fed with vegetable oils, linseed and soybean, in terms of possible changes occurring during the storage. The material for the study consisted of 270 table eggs from hens fed with standard compound feed (C) and with the addition of linseed oil (L) and soybean oil (S) at a dose of 2.5%. On the day of laying, the zootechnical evaluation of egg quality was carried out (30 per group) and the rest of the eggs were placed in transport crates with the blunt end up and stored at 14 °C and 70% humidity. Analyses of their quality were performed after 14 and 28 days of storage. Characteristics of the whole egg (weight, specific gravity, proportion of morphological elements, air cell depth), shell (weight, colour, breaking strength, thickness, density, water conductivity), albumen (height, Haugh units, weight, pH) and yolk (weight, colour, pH) were evaluated.***

Regardless of the oil used, there was no variation between groups in egg weight loss, shell water conductivity or air cell depth during storage. The greatest range of yolk weight change was observed in eggs from groups of hens fed with added oils, which may indicate different permeability of vitelline membranes. Albumen quality traits showed no variation according to laying diet. The lightest shells characterised the eggs from hens in group L and the darkest from group C, while after 28 days of storage, the colour of the shells darkened, which was very evident in eggs from group S. Eggs from this group had the most resistant shells at the end of the experiment. It seems that the addition of linseed or soybean oil to laying feed can modify egg quality characteristics at the time of collection, while it does not significantly affect the storage stability of the raw material.

Keywords: *laying hens, vegetable oils, egg quality, fatty acids, eggs storage*

Fats in poultry nutrition are primarily a source of highly concentrated energy, but they also contribute to improving the physical characteristics of the feed mixture (reduce dustiness, facilitate pelleting) and the efficiency of its use *(Plavnik et al., 1997; Crespo and Esteve-Garcia, 2002; Jahan et al., 2006)*. In the case of laying poultry, the use of dietary fats to modify the quality of the eggs obtained is not without significance, mainly in terms of their lipid profile *(Batkowska et al., 2021)*. Due to the dietary and health importance of fatty acids in the yolk, work is being carried out to obtain a favourable ratio of fatty acids and enrich eggs with particularly biologically valuable lipids. Oilseeds such as rapeseed

(Cherian and Sam, 1991), sunflower and soybean (Milinsk et al., 2003), hemp seed and oil (Goldberg et al., 2012) or linseed (Botsoglou et al., 1998) are most commonly used for this purpose, but additives of animal origin such as fish meal (Navarro et al., 1972) or fish oil (Yalçın et al., 2007) are also used, but their use may affect the sensory qualities of the raw egg material.

As was mentioned previously, vegetable fats are the main high-energy component of animal and human nutrition, and one of the main oilseed crops grown worldwide is soya. Its seeds contain an average of 20 % oil with a positive fatty acid composition, 50 % of which are unsaturated fatty acids, but only a small proportion are n-3 fatty acids (Minkowski et al., 2011). Soybean oil is widely used as an animal feed additive due to its easy availability, relatively low price and high fatty acid content. It can be successfully used in pigs (Dourado et al., 2011) and in slaughtering chickens diet (Tabeidian et al., 2005).

The cold-pressed linseed oil is also very popular. Due to its high PUFA (polyunsaturated fatty acids) content, it is widely used in various industries, including food, pharmaceuticals and animal feed. Due to the presence of α-linolenic acid (ALA) in its composition, it has a quick-drying effect, so it has also found applications in paint and ink production (Demirbas, 2009). Linseed oil even contains more than 40 % fat in its composition, which is a rich source of fatty acids and has the highest ALA content of all oilseeds (Rubilar et al., 2010). Its health-promoting effects have been confirmed, including inhibiting carcinogenesis, preventing heart disease and lowering blood pressure (Katare et al., 2012).

Both oils can be successfully used in laying hen nutrition, contributing to positive changes in the raw material in terms of modification of the lipid profile with constant cholesterol levels and maintaining consumer acceptability (Batkowska et al., 2021). However, questions arise as to whether the change in some quality traits due to supplementation will translate into negative changes in eggs during raw material ageing. Therefore, the aim of this study was to assess the quality of eggs from laying hens fed with feed supplemented with vegetable, linseed and soybean oils, in terms of possible changes occurring during storage.

Material and methods. The material for the study consisted of table eggs collected one day from laying hens kept on litter in compliance with the principles of zoohygiene and welfare (Council Directive 1999/74/EC). During the subsequent stages of rearing, commercial balanced feed mixtures adapted to the age and physiological state of the birds were used. At 24 weeks of age, the birds were divided into three groups, with five replications in each. The experimental factor was applied as a feed additive in the form of 2.5% soybean oil (group S) and linseed oil (group L). The control group (C) consisted of birds fed a fullstaff feed mixture without added oils. The chemical composition of the feed was previously presented in Batkowska et al. (2021).

After 5 weeks of use of experimental diets (29th week of the life of the birds), 90 eggs from each group (270 eggs in total) were taken randomly, numbered individually, laid in transport crates with the blunt end up and placed at 14 °C and 70% humidity (typical storage conditions). Changes in egg weight were monitored in 7-day intervals, and complete analyses of egg quality were performed at the beginning of the study and then after 14 and 28 days of storage according to the scheme (Table 1).

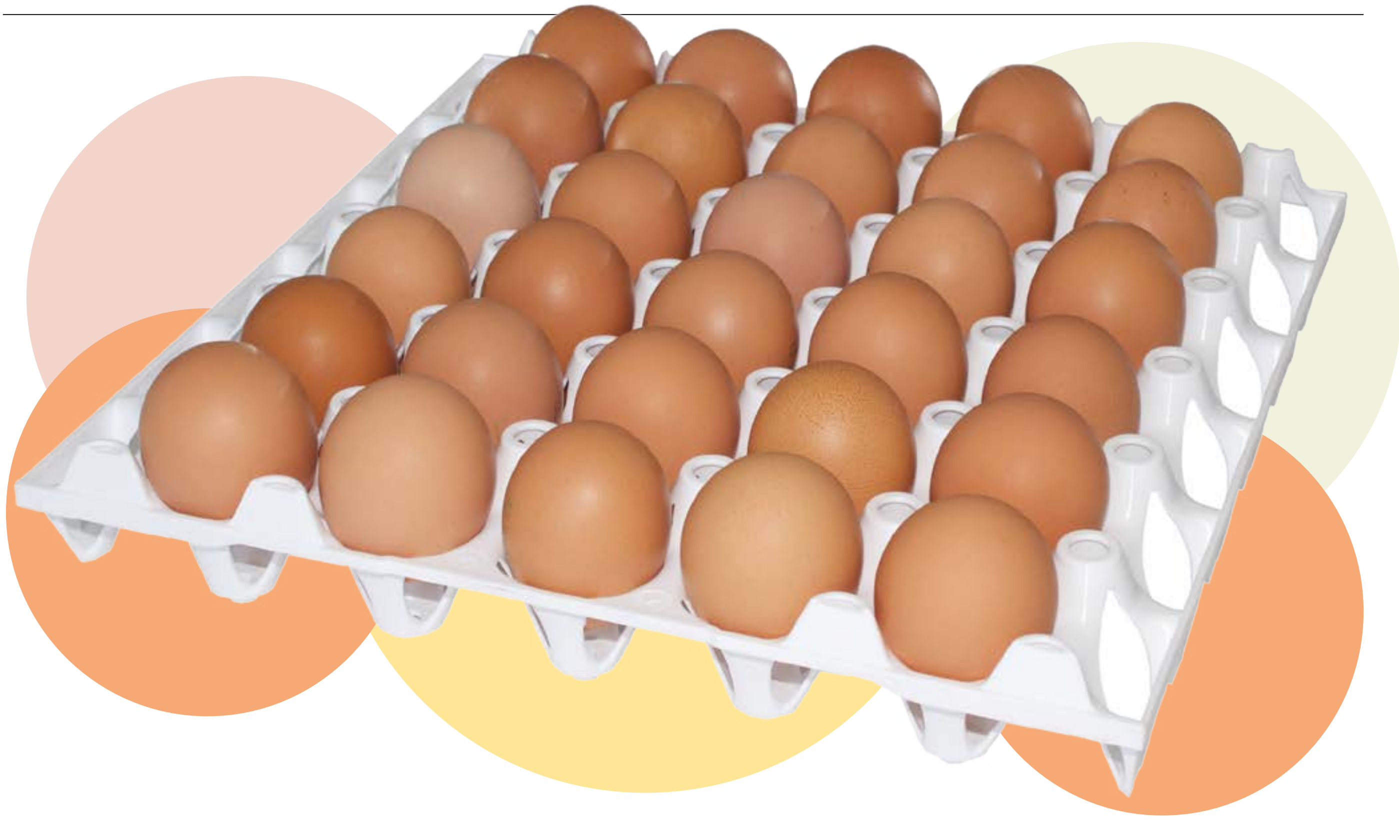
Table 1. The design of the experiment

Time (days)	Group C (control)	Group L (linen oil)	Group S (soya oil)	Total
0	30	30	30	90
14	30	30	30	90
28	30	30	30	90
Total	90	90	90	270

An EQM (Egg Quality Measurement, TSS®) analytical kit and an Instron Mini 55 tester were used to assess egg quality. The following traits were recorded:

1. Characteristics of the whole egg, such as:

- weight (using an electronic balance with an accuracy of 0.01 g);
- weight loss (%);
- shell water conductivity (according to the formula of Ar et al., 1974);
- air cell depth (using an egg lamp "Ovolux" and then measured with a scaled template);



- the proportion of morphological elements (based on the ratio of the weight of particular morphological elements to the weight of the whole egg).

2. Characteristics of the yolk:

- weight (by electronic balance with an accuracy of 0,01 g);
- colour (16-points Roche scale, DSM®);
- pH (ph-meter with a combined glass electrode).

3. Albumen characteristics:

- weight (from the difference in the weight of the whole egg and of the yolk and shell);
- height (of the EQM sensor through its contact with the surface of the dense albumen);
- Haugh units *(Williams, 1992)*;
- pH (ph-meter with a combined glass electrode).

4. Shell characteristics:

- colour (as a percentage of reflected light);
- strength (Instron Mini 55);
- weight (using an electronic scale with an accuracy of 0.01 g);
- thickness measured at the 'equator' of the egg (using a micromillimetre screw);
- density (according to a formula proposed by *Shafey, 2002)*.

Data were statistically analysed using the SPSS 24.0 package *(IBM Corp., 2016)*. The normality of the data distribution was verified using the Shapiro-Wilk test. Differences between groups were assessed using a one-way analysis of variance with Tukey's multiple comparisons tests. A significance level of $p \leq 0.05$ was adopted.

Results and discussion. *Table 2* shows the changes in the quality characteristics of whole eggs during storage depending on the type of oil added to the layers' feed. It was found that the highest loss of egg weight during the first 14 days of the experiment was characteristic of the eggs obtained from birds fed feed with linseed oil, and the lowest from the control group. Slightly different results were observed between days 14 and 21 of egg storage when the highest result was recorded in eggs from hens in group S and the lowest in eggs obtained from hens in group L. No significant changes were observed on subsequent days.

The highest percentage of weight loss was observed in eggs from hens fed with linseed oil (6.16%), while eggs from the control group and hens fed with soybean oil (5.86 vs. 5.88%) showed this change at almost the same level. Egg weight loss is an integral change during raw material ageing due to water evaporation and diffusion occurring in the eggs. Many works indicate that this change occurs independently of the protective factors used *(Drabik et al., 2018; Pires et al., 2019)*, the time or temperature of raw material storage *(Brodacki et al., 2019)*.

The highest shell water conductivity on day 14 of the study was recorded in eggs obtained from hens fed with linseed oil and the lowest in soybean one. On the other hand, on day 21 of the study, the highest value of this indicator was in eggs from S group, while the lowest value was in eggs from group L hens. In the last week of the study (from day 21 to 28), no significant changes were observed, eggs were losing about 5 mg of water per day regardless of the laying hens' diet. The water conductivity of the shell is influenced by many factors. Among the most important is the age of the flock and the laying phase or a result of the flock's moulting *(Meir and Ar, 2008)*. It should be mentioned that, in terms of egg storage, temperature and time are also important factors *(Drabik et al., 2021)*.

After 28 days of the study, statistically significant changes in air cell depth were observed due to the variation in hens' feeding from which the eggs were obtained. The deepest cells were recorded in eggs from birds fed with the linseed oil (5.6 mm), and the smallest in eggs from the control group (5.21 mm), which may confirm observations made previously regarding weight loss or shell water conductivity. The change in air cell depth is closely related to the loss of water from the egg during storage. It is one of the basic parameters for the quality assessment of raw materials in accordance with Commission Regulation (EC) No 589/2008, which sets 6 mm as the limit for table eggs. In the present study, this value was not exceeded in any of the groups. It has also been shown that the rate of air cell deepening can also be influenced by the age of the birds, their housing system and the initial egg weight *(Batkowska et al., 2014; Batkowska et al., 2016)*.

The change in the egg morphological elements' proportions is related to its weight loss during storage and the already described phenomena of water diffusion within its contents *(Drabik et al., 2018; Brodacki et al., 2019)*. The lack of feeding effect on changes in the proportions of particular elements in eggs observed in our study is therefore a positive observation, as it did not significantly affect the stability of these traits during egg storage.

Table 3 shows the changes in egg content quality traits during storage concerning the addition of oil to the laying feed. After 28 days of the experiment, there were no significant differences between the groups in terms of yolk weight, the yolk of the control group showed the least change in this indicator, it was 4.79%, while in the experimental groups, it was 7.44 and 5.37% in groups L and S, respectively. This may indicate the different permeability of the vitelline mem-

Table 2. The changes of the whole eggs traits during egg storage depending on the vegetable oil feed additive for laying hens

Trait		Time (days)	Group					
			C		L		S	
			$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Weight (g)		0	52.86	2.784	51.86	2.383	52.42	2.898
		7	52.20	2.753	51.11	2.393	51.69	2.890
		14	51.39b	2.714	50.18a	2.374	50.95ab	2.835
		21	50.65b	2.683	49.52a	2.394	50.15ab	2.783
		28	49.77	2.635	48.67	2.376	49.34	2.808
Weight loss (%)		0-7	1.26a	0.245	1.45b	0.548	1.40ab	0.335
		7-14	1.55a	0.275	1.82b	0.626	1.41a	0.336
		14-21	1.42ab	0.277	1.32a	0.573	1.57b	0.355
		21-28	1.75	0.870	1.72	0.732	1.63	0.462
		total	5.86	1.073	6.16	1.303	5.88	0.874
Shell permeability (mg H_2O/day)		0-7	1.08	0.219	1.22	0.449	1.19	0.280
		7-14	1.32a	0.245	1.51b	0.517	1.19a	0.301
		14-21	1.19ab	0.238	1.08a	0.444	1.31b	0.306
		21-28	1.45	0.760	1.39	0.596	1.32	0.352
		total	5.04	1.006	5.20	1.105	5.01	0.763
Air cell depth (mm)		7	1.38	0.464	1.18	0.582	1.30	0.434
		14	2.97	0.544	3.02	0.566	3.03	0.337
		21	4.58	0.609	4.81	0.859	4.77	0.516
		28	5.25a	0.654	5.60b	0.960	5.51ab	0.607
Proportion (%) of	yolk	0	26.04	1.982	26.35	2.139	25.40	3.645
		14	27.79a	2.037	28.95b	2.151	27.82a	2.436
		28	14.40	0.883	14.38	0.986	13.96	2.880
	albumen	0	60.71	2.222	60.99	2.106	61.85	3.785
		14	59.03	2.066	59.43	5.751	59.47	2.718
		28	57.53	1.883	57.18	2.029	59.02	6.896
	shell	0	13.25b	0.854	12.66a	1.566	12.75a	0.792
		14	13.18	0.723	12.60	1.848	12.70	1.850
		28	13.54	0.724	13.24	0.719	13.18	2.092

C – eggs from the control group of hens (fed without additive of oil); L – eggs from hens fed with linen oil additive; S - eggs from hens fed with soya oil additive; $\bar{x}$ – mean; SD – standard deviation; a,b - means differ significantly at P≤0.05

Table 3. The changes of the egg content traits during egg storage depending on the vegetable oil feed additive for laying hens

Trait	Time (days)	Group					
		C		L		S	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Yolk							
weight (g)	0	13.71b	1.006	13.31ab	1.374	13.21a	0.911
	14	14.26	1.133	14.31	1.221	14.07	1.060
	28	14.40	0.883	14.38	0.986	13.96	2.880
colour (pts)	0	9.09a	1.367	9.68ab	1.269	9.83b	2.052
	14	8.93	0.672	9.08	1.639	8.82	2.652
	28	8.97	1.262	9.74	2.496	9.57	2.670
pH	0	6.30	0.284	6.22	0.139	6.25	0.130
	14	6.28	0.111	6.33	0.117	6.25	0.108
		6.47	0.222	6.45	0.135	6.54	0.320
Albumen							
weight (g)	0	32.02b	2.302	30.84a	2.617	31.65ab	2.727
	14	30.32b	1.879	28.67a	6.451	30.26b	3.699
	28	28.69ab	2.105	27.84a	2.039	29.11b	3.623
height (mm)	0	6.37	1.448	6.43	1.218	6.02	0.962
	14	3.60	0.930	3.73	0.781	3.75	0.676
	28	3.10	0.820	3.12	0.717	3.19	0.650
haugh units	0	80.69	10.109	82.24	7.608	79.38	7.505
	14	57.76	9.263	61.01	8.730	60.09	8.154
	28	52.52	10.769	53.93	9.462	54.47	8.831
pH	0	8.75	0.141	8.81	0.120	8.74	0.179
	14	9.25b	0.070	9.20a	0.049	9.20a	0.077
	28	9.21b	0.051	9.19ab	0.049	9.16a	0.044

C – eggs from the control group of hens (fed without additive of oil); L – eggs from hens fed with linen oil additive; S – eggs from hens fed with soya oil additive; $\bar{x}$ – mean; SD – standard deviation; a,b – means differ significantly at P≤0.05

branes depending on the feed additive used. Yolk weight increases during storage due to the diffusion of water from the albumen into the yolk (Menezes et al., 2012). Although the yolk is the most valuable morphological element of the egg, the increase in its weight during storage is not a positive change. The lack of significant differences in this respect indicates that there was no negative effect of the oils used in the laying diet on the raw material stability during storage. At day 14, the highest proportion of yolk in the egg weight from L-group hens was recorded, with lower values for C and S-groups; however, these groups did not differ significantly from each other; after 28 days, this ratio was also equalised between groups.

The control group. The storage time of the raw material influenced the change of this parameter; after 28 days of storage, the yolk colour intensity decreased, mostly in the control group, but the differences between groups were not statistically confirmed. The initial colour difference is an important observation, since yolk colour depends on the carotenoid content of the feed. Therefore, darker yolks are observed in eggs from birds kept with access to green feed *(Gornowicz et al., 2013)* or when synthetic pigments are present in the feed *(Spada et al., 2016)*. In our study, the only modification was the addition of oil without the use of additional dye sources. With storage time, yolk pH increases regardless of the experimental diet used, but no differences were found in the yolk pH of eggs from various groups at different study periods, suggesting that there is no influence of the feed composition on this quality parameter of table eggs.

Significant differences in albumen weight were found between the groups on the first day of the study *(Table 3)*. Egg albumen from the control group had the highest weight while the lowest was from hens fed with a linseed oil supplemented diet, while no similar increase in weight was recorded in other papers *(Grobas et al., 2001; Rowghani et al., 2007)*. On day 28, the greatest decrease in egg albumen weight was observed from hens in the C group, here the change amounted to 11.4%. Albumen weight decreased at the lowest rate in eggs from hens fed with soybean oil – 8.0%. Concerning such albumen quality traits as the proportion of its weight in the whole egg, height or correlation with it Haugh units showed no significant variation independently of the experimental diet used.

After 28 days of storage, it was found that eggs from the control group had the highest albumen pH value with the significantly lowest result obtained for eggs from birds fed with soybean oil. Eggs from L group birds were not considerably different from the other groups included in the study. Alkalinisation of egg contents is a natural qualitative change in the raw material occurring during storage; in the yolk, it occurs by diffusion of water from the albumen, while in the albumen it is related to the release of carbon dioxide through dissociation of carbonic acid *(Monira et al., 2003)*. Studies indicate that the use of high concentrations of carbon dioxide in the storage atmosphere can effectively reduce these changes *(Rocculi et al., 2009)*.

Table 4 presents the changes in eggshell quality traits during storage concerning the addition of oil to the laying hens' feed. The type of additive used contributed significantly to eggshell colour. It was found that the lightest shells had eggs from birds fed with linseed oil additive and the darkest from the control ones. After 28 days of trial, the colour of eggshells darkened and it was found that they were still brightest in group L, with no differences between groups C and S. Shell colour is mainly genetically determined *(Roberts, 2004)* but it appears that it can be modified slightly by nutrition *(Park et al., 2009)*, but this does not affect the quality of eggs during storage.

The diet did not significantly affect the initial eggshell strength, but after 28 days of the study, it was shown that eggs from group S had the most resistant shell in contrast to eggs from laying hens fed with linseed oil, which had the weakest shell. Shell strength is one of the most important elements of egg quality, as it largely determines the amount of raw material loss at all stages of harvesting and distribution. The most important factors influencing shell strength include the age of the laying hen *(Zita et al., 2009)*, while for nutritional factors, the important role of calcium and vitamins in shaping this element has been shown *(Lichovnikova, 2007)*.

In terms of studies related to the use of vegetable oils, their influence on eggshell quality parameters was also reported. *Jiang et al. (2014)* confirmed a significant effect of the amount of soybean oil used on the shell quality of chicken eggs, with too high oil content (1.9 vs. 7 and 10%) limiting the shell thickness and strength. The eggshell weight on the first day of the study was highest in the control group while it was the lowest in eggs from hens fed with linseed oil. The same relationship was observed on day 14 of the study, but no significant differences were observed after 28 days. This confirms the previously described relationship between groups in terms of egg weight. The highest proportion of shell in the egg weight on the 1st day of the study was shown by eggs from the C group, while the lowest was in the L egg group, this result also differed slightly from that of eggs from layers fed with soybean oil, no differences were observed at later periods.

Table 4. The changes of the eggshell traits during egg storage depending on the vegetable oil feed additive for laying hens

Trait	Time (days)	Group					
		C		L		S	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
colour (%)	0	44.40a	8.768	47.73b	8.843	46.17ab	8.840
	14	44.05	8.167	47.28	6.987	46.53	9.146
	28	42.50a	7.347	46.24b	6.364	42.00a	9.202
resistance (N)	0	48.80	10.703	46.79	10.913	45.90	8.343
	14	52.87b	10.058	45.76a	12.080	48.84b	9.944
	28	50.52ab	8.564	46.48a	9.538	52.80b	10.034
weight (g)	0	6.98b	0.512	6.40a	0.840	6.52a	0.531
	14	6.77b	0.454	6.24a	0.958	6.44ab	1.021
	28	6.75	0.474	6.44	0.468	6.50	1.047
thickness (mm)	0	0.329b	0.033	0.312a	0.034	0.318ab	0.034
	14	0.334	0.034	0.341	0.084	0.343	0.085
	28	0.320	0.039	0.311	0.035	0.335	0.082
density (g/cm^3)	0	3.20	0.259	3.18	0.436	3.17	0.381
	14	3.11	0.241	2.95	0.458	3.00	0.492
	28	3.32	0.453	3.31	0.393	3.15	0.523

C – eggs from the control group of hens (fed without additive of oil); L – eggs from hens fed with linen oil additive; S - eggs from hens fed with soya oil additive; $\bar{x}$ *– mean; SD – standard deviation; a,b – means differ significantly at* $P \le 0.05$

The highest eggshell thickness on the day of collection was recorded in the control group and the lowest in eggs from hens fed with a linseed oil additive. The addition of vegetable oils to the feed had no significant effect on shell thickness contrary to the study by *Grobas et al. (2001)*, where a remarkable reduction was observed after supplementation with various types of vegetable oils.

CONCLUSIONS

1. Regardless of the type of vegetable oil used (linseed oil vs. soybean oil) in the laying feed, there was no variation between groups in terms of egg weight loss during storage, and air cell depth after 28 days in none of the groups exceeded value that would discredit the eggs as fresh.
2. The range of yolk weight change was greater in the groups fed with added oils, which may indicate different vitelline membrane permeability depending on the additive used.
3. In the case of mealbumen traits the vegetable oil diet used affected only the weight and pH of this element after 28 days of storage.
4. Eggs from laying hens fed soybean oil supplemented feed had the most resistant shells in contrast to eggs from birds receiving linseed oil supplementation, which showed the lowest value for this trait.
5. It appears that the addition of linseed or soybean oil to laying feed can modify egg quality characteristics at the time of egg production, while it does not significantly affect the storage stability of the raw material. ■

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Вплив терміну зберігання на якість яєць від курей-несучок, які отримували комбікорм з додаванням рослинних олій

Анотація. ***Метою проведеного дослідження було оцінити якість яєць курей-несучок, яким згодовували рослинні олії, льняну та соєву, з погляду можливих змін, що відбуваються під час зберігання. Матеріалом для дослідження були 270 харчових яєць від курей, яких годували стандартним комбікормом (С) та з додаванням лляної (Л) і соєвої (S) олії в дозі 2,5%. У день знесення проводили зоотехнічну оцінку якості яєць (по 30 штук на групу), а решту яєць поміщали в транспортні ящики тупим кінцем догори і зберігали при температурі 14 °С та вологості 70%. Аналіз якості яєць проводили через 14 та 28 діб зберігання. Оцінювали характеристики цілого яйця (маса, питома вага, співвідношення морфологічних елементів, величина повітряної камери), шкаралупи (маса, колір, міцність на розрив, товщина, щільність, водопровідність), білка (висота, одиниці Хау, маса, pH) та жовтка (маса, колір, pH). Незалежно від використовуваної олії, не було ніяких відмінностей між групами у втраті маси яєць, водопровідності шкаралупи або величині повітряної комери під час зберігання. Найбільший діапазон зміни маси жовтка спостерігали в яйцях від груп курей, яким додавали олії, що може свідчити про різну проникність вітелінових мембран. Показники якості білка не змінювалися залежно від раціону курей-несучок. Найсвітліша шкаралупа характеризувала яйця курей групи L, а найтемніша – групи С, тоді як після 28 днів зберігання колір шкаралупи потемнів, що було дуже помітно в яйцях групи S. Яйця з цієї групи мали найбільш міцну шкаралупу в кінці експерименту. Схоже, що додавання лляної або соєвої олії до корму для курей-несучок може змінювати якісні характеристики яєць під час збору, в той час як на стабільність сировини при зберіганні це суттєво не впливає.***

Ключові слова: *кури-несучки, рослинні олії, якість яєць, жирні кислоти, зберігання яєць*

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