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Effect of Age on Egg Quality of Lakenvelder Hens Kept Under Extensive Rearing Conditions

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ABSTRACT

The aim of the study was to evaluate the effect of age of Lakenvelder hens kept under extensive rearing conditions on the quality of eggs obtained. The research material consisted of eggs obtained from Lakenvelder hens. The hens were kept in a barn on the litter with the possibility to use the free-range area and to collect weed. The birds were fed the same feed throughout the study period. From each group, 100 eggs were collected at 36 and 52 weeks of age. A total of 200 eggs were examined for physical and chemical properties. The study showed an increase with age of shell weight and yolk percentage in the egg as well as shape index of shell thickness and yolk percentage in the egg. The quality of protein, total cholesterol and PUFA n-6 content decreased. The results showed that Lakenvelder hens can be kept in extensive rearing conditions obtain good quality table eggs.

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Introduction

Eggs are an important component of the human diet, they contain protein - recognized in terms of amino acid composition as standard, saturated and unsaturated fatty acids, phospholipids, vitamins and minerals (Lesnierowski and Stangierski, 2018). Current research confirms the beneficial effects of EFAs on health, they are exogenous which means they must be supplied with food. Fatty acid profile in egg yolks can be modified by feeding laying hens (Kubiński, 2012). Limitation of egg consumption by consumers is related to the presence of cholesterol in egg yolks especially by consumers with elevated values of this compound in blood. However, exogenous cholesterol supplied with the diet has less influence on the overall level of this compound, the main cause of high levels of this compound is the so-called endogenous cholesterol (Smolenkova and Novikova, 2021). However, it is indicated that the complete elimination of eggs from people's diet causes them to eliminate other valuable nutrients from their diet as well (Sugano and Matsuoka, 2021).

Currently, consumers equate eggs obtained from extensive farming with higher nutritional value and quality (Trziszka et al., 2006). Extensive production is characterized by much lower production due to the lack of control of environmental conditions and its seasonality, which is associated with a much higher price. However, more and more often consumers choose eggs based on their taste properties and also such external characteristics of eggs as shell color, yolk color intensity and egg weight (Rondoni et al., 2020). The welfare of the poultry kept is also important, which according to consumers is achieved in rearing conditions with an access to a free range (Bombik et al., 2015)

Most production traits in laying hens are traits that are polygenically encoded by a large number of low-effect additive genes. Therefore, identification of the genetic background of these traits most often involves detection of chromosomal regions called Quantitative Trait Loci (QTL), so it is important to preserve breeds that are a reservoir of these valuable genes (Sławińska, 2010).

Appropriate selection of hens for extensive rearing should take into account maximization of production performance and egg quality as well as adaptation to local environmental conditions (Sokołowicz et al., 2021). These characteristics are usually possessed by local indigenous breeds. One such breed is the Lakenvelded is breed originated a long time ago and genetic studies have shown its genetic relatedness to other breeds and may be a reservoir of valuable genes (Elferink et al., 2012; Núñez-León et al., 2019). Lakenvelder hens were bred at the turn of the 19th century in northern Westphalia, Germany, as a breed with laying performance, now of importance in amateur breeding as ornamental birds (Verhoef and Rijs, 2006; Jakubowska and Różewicz, 2017). One of the earliest records of the existence of Lakenvelder hens is dated 1797, where hens with the breed's characteristic black and white plumage were found in the Utrecht area and the village of Lakerveld (Schmidt, 2007). The specific location where the breed originated is not clearly explained. Belgium, Germany and the Netherlands are mentioned as the countries of origin, which is related to the breed's border presence. In 1835, hens of this breed were being kept in the Belgian region around Zottegems in what Westphalia is now. Malik (1968) mentions the historical regions of Westphalia and Hannover as the geographical areas where the breed originated. However, Verhoef and Rijs (2006) mentioned the Netherlands - the Utrecht village of Lakerveld - as the area where the breed probably originated, from where the breed may have taken its current name in German, Dutch and also English as Lakenvelder. The feature that distinguishes this breed from many others is undoubtedly the contrasting black and white plumage (Różewicz, 2018). Despite the numerous breeds and color variations, none of them have the characteristic distribution of black and white colors as in Lakenvelder hens. The head and neck as well as the tail have black plumage, while the trunk is white in color (Różewicz and Szablicka, 2020). This breed has good egg hatchability and chick rearing rates. The first eggs from young hens of this breed can be expected around 21 weeks of age. Initially, they have a low egg weight (45-50 g) but with age their egg weight increases (up to 55 g) (Różewicz et al., 2016). Due to their relatively good laying performance, contrasting plumage and willingness to be outdoors, Lakenvelder hens are predisposed to be kept with access to a free-range (Różewicz et al., 2016). The aim of this study was to determine the quality of eggs obtained from Lakenvelder hens kept in free-range housing with regard to age, physical and chemical quality of eggs.

Materials and Methods

The research material consisted of eggs obtained from 36 and 52 weeks old Lakenvelder hens. The birds were kept in a poultry house (4 m^2 /hen) on litter with access to the free-range area (11 m^2 /hen) in accordance with accepted standards for keeping laying hens. The hens had access to natural light - windows in the poultry house and artificial lighting was used - the poultry house was lit in a diurnal rhythm so that about 1/3 of a day was an uninterrupted period of darkness and there were periods of darkening lasting 1 hour corresponding to dusk. The hens were fed a complete diet, the composition of which is shown in Tables 1, 2 and 3, intended for laying hens according to the Nutritional recommendations and nutritional value of feed, Feeding Standards for Poultry (2005). In addition, the birds had the possibility to supplement the feed with green fodder which grew in the free-range area. The birds had permanent and free access to water in drinkers.

Table 1. Formulation of feed mix used from 21 to 52 weeks of age

Component	Content in %
Winter wheat of the variety Spurge	52.99
Maize	5.00
Peas of the Ezop cultivar	15.00
Linseed cake	8.14
Post-extraction rapeseed meal	4.00
Soybean oil	3.74
Fodder chalk	8.48
Alfalfa meal (20% protein)	1.00
Mineral and vitamin premix[1].	1.00
Calcium phosphate	0.41
Lysine hydroxychloride	0.24
Total	100

[1]The composition of the premix is given in Table 3.

Table 2. Nutritional value of compound feed

Specification	Value
Dry matter (%)	89.00
Metabolic energy (MJ)	11.5
Crude protein (%)	18.00
Crude fibre (%)	3.85
Fat (%)	6.00
Crude ash (%)	11.43
Lysine (%)	0.88
Methionine+cystine (%)	0.92
Tryptophan	0.20
Threonine	0.66

Table 3. vitamin and mineral content of the premix used in the compound feed

Component	Value
Calcium (%)	3.50
Phosphorus (%)	0.58
Sodium (%)	0.18
Chlorine (%)	0.29
Linoleic acid (%)	2.96
Xanthophyll (mg/kg)	18.58
Vitamin A (IE)	12000
Vitamin D (IE)	2000
Vitamin E (mg)	30.00
Vitamin K (mg)	2.00
Vitamin B1 (mg)	4.00
Vitamin B2 (mg)	5.00
Vitamin B6(mg)	3.00
Vitamin B12 (µg)	45
Nicotinic acid (mg)	15.00
Pantothenic acid (mg)	9.00
Folic acid (mg)	1.00
Biotin (µg)	100
Choline chloride (mg)	250
Zinc (mg)	65.00
Iron (mg)	45.00
Manganese (mg)	70.00
Copper (mg)	8.00
Cobalt (mg)	0.45
Iodine (mg)	0.80
Selenium (mg)	0.15

At 36 and 52 weeks of age, 100 eggs (200 in total) were obtained from hens for egg quality analysis.

Eggs from the daily collection were taken for analysis to determine:

- shell colour (% light reflectance)
- egg weight (g),
- height of albumen (mm),
- quality of the thick albumen (HU),
- yolk color (Roche Scale),
- yolk weight (g),
- shell thickness (µm),
- shell weight (g) after drying at 120 °C for 24 h.

On the basis of the results obtained, the following were calculated:

- the percentage of yolk in the egg weight,
- the percentage of protein in egg weight
- the percentage of shell in the egg,
- shape index (ix).

Determination of yolk lipid content by fat extraction using the modified method of Washburn and Nix (1974). From a 0.5 g weight of yolk using a mixture of chloroform and methanol (0.75 ml at a ratio of 2:1), followed by the addition of 2.5 ml of distilled water and centrifugation in a centrifuge for 10 min (2500 rpm/minute), two fluid fractions were visibly stratified in a tube: the water-methanol mixture, which was removed from the tube, and the fat fraction dissolved in chloroform. After filtering the fractions, the filtrate was divided equally into two tubes for the determination of egg lipids. After evaporation of the chloroform, the fat was determined:

- cholesterol
- fatty acids
    - saturated (SFA); C16:0; C18:0
    - monounsaturated (MUFA); C16:1; C18:1
    - polyunsaturated (PUFA) n-3; C18:3; C22:6
    - polyunsaturated (PUFA) n-6; C18:2; C20:3; C20:4.

Cholesterol was determined by thin layer chromatography on Silica gel 60F. The extracted yolk fat was dissolved in 0.5 ml of diethyl ether, after which 0.01 ml of the solution was spotted onto a Silica gel 60F plate. The plates were placed in a chamber with vehicle (80 ml hexane + 20 ml ether + 2ml formic acid) for 1.5 hours. The individual lipid fractions, from the dried plates, were transferred to tubes and mixed with 2ml of methanol. The content of phospholipids, triglycerides and cholesterol was calculated from the extinction read on a UV WIS spectrophotometer (wavelength 190-320µm).

Separation of fatty acids was performed by gas chromatography on a Hewlett Packard HP 6890 GC apparatus. To the tube with the extracted fat, 1 ml of sodium methanolate was added and heated in a thermostat (37°C) until complete dissolution of the fat. Then, 1 ml of isooctane was added and the whole was made up with saturated NaCL solution. From the mixture thus formed, a separated, clear layer containing fatty acid methyl esters was taken. The extracted mixture of esters was separated on a gas chromatograph. The separation of fatty acids was performed on a capillary column Ultra 2 Crosslink 5% Ph Me Silicone length 25m x 0.32mm x 0.52µm film, from Hewlett Packard. An FID flame ionization detector was used and helium was used as the carrier gas. The chromatograms were read by comparison with Sigma standards and the fatty acid content was given as a percentage of the total fatty acids determined.

The obtained results were statistically elaborated by means of the analysis of variance calculated by the least squares method using the computer program Statist.

Results and Discussion

Young Lakenvelder hens laid their first eggs at 21 weeks of age. The initial weight of the eggs laid was low but the weight increased as the hens grew older. At 39 weeks of age the weight of the hens started to stabilise (Table 4). Sokolowicz et al. (2019) indicate that egg weights increased with the age of laying hens. Several studies confirm that the age of hens has a tremendous impact on external and internal egg quality characteristics (Inca et al., 2020) . As research Ogbu (2023) shows, the age and breed of laying hens have a significant impact on egg quality. The lower weight of eggs obtained from Lakenvelder hens. May be due to the lack of selection for this trait within the breed. Nys (2000) also draws attention to genetic determinants related to egg weight and the high heritability of this trait (h^2 = 0.4 - 0.6). Egg weight is influenced by the housing system of the hens. Vakili et al. (2021) found a significantly lower egg weight obtained from hens from housing with access to the free range. Proper, balanced nutrition is also important. This applies especially to feed energy and lysine content. As shown by Scappaticcio et al. (2021), the lysine content should be 0.68 - 0.76%. In this study, the rate was higher, so it should not affect egg weight.

The Lakenvelder hens have a light shell colour which is associated with high light reflectance (Table 5). Shell colour is genetically determined by the production of three main pigments: protoporphyrin, biliverdin and biliverdin-zinc chelate (Zheng et al., 2014). Protoporphyrin is the main pigment of brown and light brown eggshells, while white eggshells lack protoporphyrin. Lakenvelder hens are characterized by white to slightly creamy eggshells (Różewicz and Szablicka, 2020). The shell weight increased with the age of the hens. The value recorded in our study at 36 weeks of age was significantly lower than that found by Lewko et al. (2020) in breeds of Leghorn (H-22), Yellow leg partridge (Ż-33), Greenleg partridge (Z-11), Rhode Island Red (R-11), Sussex (S-66), Rhode Island White (A-33) hens that were of similar age, where the value ranged from 5.95 to 6.88. Anderson et al. (2004), found varying proportion of shell in hens of different strains of Leghorn hens indicating that this trait is genetically determined. The lower shell weight results in a lower percentage of egg weight at the same time. However, it is usually thinner and increases the susceptibility to breakage. In Lakenvelder hens the percentage of egg weight increased with age but the value of this trait was lower than in the Leghorn breed (Anderson et al. 2004). Shell thickness was also lower (Table 5) than in other breeds, where the value is 0.47 in Leghorn (Anderson et al. 2004) but was comparable to Green leg partridge (Lewko et al. 2020) and Asseal - 0.35mm (Kumar et al., 2021). Shell weight and thickness depend on the age of the hens, which was also confirmed by our own study.

In older hens the percentage of shell in the egg and its thickness increases (Rodriguez-Navarro et al., 2002; Lewko et al., 2020), which was also found in our own study. The egg shape index increased highly significantly with the age of the hens. Egg shape index is a trait that may vary depending on the breed. Kumar et al. (2021) found differences in egg shape index in Asseal and Kadaknath breeds. The egg shape index values recorded were above 74% which was higher than the value recorded in our study in Lakenvelder hens.

The weight of albumen the test eggs was higher at 36th week of age than at 52nd weeks of age, but the difference was not significant. The share of albumen in the test eggs was higher at 52nd weeks of age but the difference was not significant. Albumen quality expressed in Haugh units was significantly lower at 52nd week of age (Table 6). Calik (2011) also found a decrease in the quality of albumen with the age of laying hens. Also genotype has an influence on this quality in laying hens (Andres et al., 2008; Sokołowicz et al. 2019).

Table 4. Weight of eggs depending on hen age

Week of life	Average egg weight	Standard error
21	42.25	1.53
22	42.50	1.42
23	43.40	1.18
24	43.37	1.14
25	44.48	1.13
26	45.95	1.32
27	50.38	1.07
28	51.71	1.31
29	52.66	1.19
30	53.18	1.20
31	53.58	1.24
32	53.95	1.18
33	54.03	1.44
34	54.40	1.32
35	54.75	1.24
36	55.35	1.04
37	55.28	1.10
38	56.16	1.00
39	56.67	0.88
40	56.28	0.92
41	56.30	1.28
42	56.98	0.96
43	57.02	0.56
44	57.00	0.33
45	57.04	0.29
46	57.00	0.40
47	57.05	0.44
48	57.11	0.32
49	57.00	0.33
50	57.05	0.12
51	57.07	0.22
52	57.00	0.15

Table 5. Characteristics of shells

Item	Value of trait		Standard error
	36th week of life	52nd week of life	
Shell colour	61.9	61.2	0.56
Shell weight (g)	4.77^{A}	5.34^{B}	0.12
Share in egg weight (%)	8.20^{A}	8.92^{B}	0.25
Shell Shape Index (%)	72.56^{A}	73.28^{B}	0.27
Shell thickness (mm)	0.30^{A}	0.33^{B}	0.01

A, B – values in rows with different letters differ significantly at P≤0.01; a, b – values in rows with different letters differ significantly at P≤0.05

Table 6. Characteristics of protein

Item	Weight of protein [g]		Standard error
	36th week of life	52nd week of life	
Weight of protein	36.70	36.48	1.24
Share of (%)protein in egg weight	62.87	64.00	0.76
Quality of protein (Haugh units)	79.66^{A}	72.43^{B}	3.81

A, B – values in rows with different letters differ significantly at P≤0.01; a, b – values in rows with different letters differ significantly at P≤0.05

Table 7. Characteristics of the yolk

Item	Yolk colour [RCF]		Standard error
	36th week of life	52nd week of life	
Yolk colour (RCF)	8.7	8.6	0.50
Yolk weight (g)	16.83	17.12	0.58
Share of (%) yolk in egg weight	28.92^{A}	29.21^{B}	0.71

A, B – values in rows with different letters differ significantly at P≤0.01; a, b – values in rows with different letters differ significantly at P≤0.05

Table 8. Fatty acid profile of yolk

Item	Value		Standard error
	36th week of life	52nd week of life	
C14:0	0.34	0.33	0.016
C14:1	0.07	0.07	0.009
C16:0	23.06	22.94	0.49
C16:1	2.71	3.63	0.238
C18:0	8.84	7.93	0.231
C18:1	45.57	45.37	0.759
C18:2	11.93	11.52	0.886
C18:3	0.66	0.61	0.066
C20:4	1.74^{a}	1.86^{b}	0.090
C22:6	1.90	1.94	0.117

A, B – values in rows with different letters differ significantly at P≤0.01; a, b – values in rows with different letters differ significantly at P≤0.05

Table 9. Lipid profile of egg yolk (g/100g)

Item	Value		Standard Error
	36th week of life	52nd week of life	
SFA (14:0; 16:0; 18:0)	32.29	32.73	0.498
MUFA(14:1; 16:1; 17:1; 18;1; 20:1)	49.25	50,43	0.893
PUFA	15.39	13,49	1.025
PUFA n-3 (18:3; 22:6)	2,26	2,43	0.148
PUFA n-6 (18:2; 20:4)	13.13^{a}	11,06^{b}	0.942
Cholesterol (mg/g zółtka)	8.70^{A}	8.40^{B}	0.03

A, B – values in rows with different letters differ significantly at P≤0.01; a, b – values in rows with different letters differ significantly at P≤0.05

In yolk quality studies, there were no significant differences in colour intensity or yolk weight, but there was a significant increase in the percentage of yolk in egg weight (Table 7). The percentage of yolk is a breed trait. The increase in yolk weight in eggs with age was confirmed by Calik (2011). Anders et al. (2008) found a higher proportion of yolk in eggs of Polish bantams which may be related to the need to provide the developing embryo with sufficient nutrients.

The determined values of the fatty acid profile did not show any changes, except for arachidonic acid (C20:4), which significantly affected as PUFA n-6 (Table 8 and 9). The cholesterol content of the eggs changed significantly by 0.3 mg/g yolk between the egg study periods (Table 9.)

Krawczyk and Cywa-Benko (2004) in their study on six laying hen lines showed differences in cholesterol content depending on the genotype, the age and the type of feed used. The researchers used three types of feed, including one with an addition of a herbal preparation, which had a significant effect on lowering yolk cholesterol levels. Differences were also found between cholesterol content and the type of feed administered and the age of laying hens. In our own study in Lkenvelder hens, the effect of hen age on the increase of cholesterol in eggs was also confirmed. Calik (2011) also found that cholesterol levels in egg yolk are influenced by factors such as the origin of the hens, the age of the layers, the housing system and the type of feed provided. The author evaluating the average cholesterol content in egg yolks of laying hen breeds and comparing the pedigrees within the breeds studied (Island Red (K-44, K-66), Rhode Island White (A-22, A-88), Barred Rock (P-11), New Hampshire (N-11)), found no statistically significant differences. Przybylski (2012) indicates very little difference between breed and yolk cholesterol content in both the native Green leg breed and general purpose breeds, but compared to other domestic bird species, hens have lower cholesterol levels per gram of yolk. According to Jamróz and Hawałej (1994), the significantly higher level of cholesterol in egg yolks from young hens may be due to a lower yolk weight, as yolk weight increases with age and its cholesterol concentration decreases. An increase in yolk proportion and a decrease in cholesterol content with age was also found in our study. The feeding of laying hens is also a factor influencing cholesterol content. Yalçyn et al. (1994) in five feeding groups of laying hens with different fibre content in the feed, found that in the group with the highest fibre content in the feed, the lowest yolk cholesterol content was determined. In our study, the level of fibre may have been a differentiating factor and influenced the cholesterol content of yolks, because the birds had the opportunity to take in green fodder, which is also a source of fibre. The birds were allowed to take it at will depending on their individual needs. Ayerza and Coates (2000) used Argentine chia seeds as a nutritional supplement in two groups of hens, they showed a positive effect of this plant

on significantly reducing cholesterol levels in egg yolks. Cholesterol levels in the yolk are also influenced by genetic factors. Kumar et al. (2021) found differences in cholesterol content of yolk of two breeds: Aseal and Kadaknath. The value recorded in Aseal breed (9.38) was much higher than that recorded in Lkenvelder hens in their study, while the value determined at 52 weeks of age of Lakenvelder hens was similar to that recorded in Kadaknath breed (8.38).

The fatty acid profile of eggs can be modified by feeding the laying hens. Yalçyn et al. (1994) showed the effect of the addition of fish oil as well as linseed in laying hen feeding, they obtained an increase in the content of essential unsaturated fatty acids, while decreasing the amount of saturated palmitic and stearic acids. The feed on which the Lakenvelder hens were fed contained linseed cake, which may have influenced the fatty acid profile. Pita et al. (2006) in a study on the use of two additives, rapeseed oil and flax, found that the addition of flax significantly increased the content of polyunsaturated acids - PUFAs - in the eggs. The researchers also found that the addition of vitamin E as an antioxidant reduces the content of saturated fatty acids, which has a beneficial effect on the nutritional value of the eggs obtained. In the studied groups of hens, no additives were used to intentionally affect the fatty acid profile. However, the hens had sources of various unsaturated fats in their feed in the form of linseed cake, poektrak soybean meal and rapeseed meal. Smulikowska (2006) reports that currently post-extraction rapeseed meal and linseed meal are used as protein additives added to feed for laying hens, which can cause a slight increase in essential fatty acids in eggs. The fatty acid profile is also influenced by genetic factors. Different breeds of laying hens have different fatty acid profiles (Sokołowicz et al., 2019; Gomułka et al. 2021)

Conclusions

As Lakenvelder hens grow older, the weight of the eggs they lay increases, with a maximum weight of 57 g. Egg quality changed with age. The study showed an increase with age in shell weight and percentage in the egg and in shell shape index shell thickness and yolk percentage in the egg. The quality of protein, total cholesterol and PUFA n-6 content decreased. The results showed that Lakenvelder hens can be kept in extensive breeding to obtain good quality table eggs.

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