Incubation Parameters in the Transylvanian Naked Neck Breed Following Supplementation with Vitamin E and Selenium

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Abstract
The importance of the Transylvanian Naked Neck breed is highlighted not only from an economic standpoint but also from a cultural perspective, due to its adaptability to maintenance conditions and its robustness. The two nutrients, vitamin E and selenium, support the biological processes involved in reproduction and organism development, making them essential. Introducing these nutrients in the context of bird egg incubation can positively influence incubation parameters and hatch rates. The biological material consisted of birds from the Transylvanian Naked Neck breed, which were divided into two groups. The experimental group received a supplement of vitamin E and selenium in their drinking water. Supplementation with vitamin E and selenium improved egg weight, hatch percentage, and chick weight at hatching.

Keywords: incubation parameters, selenium, Transylvanian Naked Neck, vitamin E.

1. Introduction

Due to its low production cost, superior nutritional value, excellent digestibility, taste characteristics and high slaughter yield, poultry meat has an increasing share in human nutrition [1]. Eggs, along with milk, are the only complete foods.
The Transylvanian Naked Neck breed was first attested at the beginning of the 20th century, becoming a symbol of genetic diversity in poultry farming [2]. In 1930, Hungary established a breeding program to perfect the breed, bringing it into line with the standards of other breeds bred for economic purposes [3]. Due to its unique appearance, this breed is easily recognizable. The black variety has a black beak, shanks, and toes. Regardless of plumage colour, the skin is white in all varieties. The plumage is quite sparse and sits tightly against the body [4]. Raised in a semi-intensive system, the Transylvanian Naked Neck breed can provide, at least partially, the biological material necessary for the organic production of poultry meat and eggs, a sector in which Romania has favourable perspectives [5].
Selenium is an essential trace element that is present in small amounts in the body and maintains its normal functioning. Selenium is a component of antioxidant enzymes, such as glutathione peroxidase, which protect cells against oxidative damage caused by free radicals [6], but it is also involved in the metabolism of thyroid hormones [7]. Decreased sperm quality, fertility rate and embryo viability can be caused by selenium deficiencies, and administration of this trace element can improve sperm quality, fertility rate and embryo viability [8]. Selenium promotes the reduction of hydrogen peroxide and lipid hydroperoxides at the molecular level through glutathione peroxidase, protecting cell membranes and organelles against oxidative stress [9]. The

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important role of vitamin E in the functioning of the immune system is well known and contributes to maintaining the structural and functional integrity of cell membranes [10]. Vitamin E influences embryo viability, egg quality and the functioning of the immune system, contributing to maintaining the structural and functional integrity of cell membranes [10]. The synergy between selenium and vitamin E contributes to the body's antioxidant defence, selenium through glutathione peroxidase, and vitamin E, through greater action on diseases and good vitality due to the synergistic effect between the two that protects embryos from oxidative stress [9].

2. Materials and methods

The biological material used in the present experiment consisted of a group of 10 birds of the Transylvanian Naked Neck breed, which was divided into 2 batches according to the experimental design (Table 1). The shelter where the birds were kept had an area of 8 m^2, divided into two equal compartments. The lighting was natural, with the birds having access to pasture. The selenium and vitamin E supplement was introduced to the experimental group in the drinking water (2 millilitres per 1 Liter of water). Eggs were collected daily, marked for batch identification. The incubation process was carried out in the Cleo 5 incubator. The objectives of the experiment were: performing the external examination and internal study of the eggs, monitoring the incubation parameters (fecundity percentage, percentage of live embryos at the first candling, percentage of live embryos at the second candling, hatching percentage) and establishing the quality of the resulting one-day-old chicks. The external examination and internal study of the eggs was performed on eggs collected from the chickens in the studied batches, in two stages: at the beginning of the experiment (before administering the supplement), on a number of 10 eggs at LM and 10 eggs at LE and after a period of 30 days, in which the chickens in the experimental batch received the Neovita supplement. The groups were fed a mixture of cereals and Deuka feed (Table 2). The quality of the one-day-old chicks resulting from the incubation process was determined by weighing the viable chicks resulting from the incubation process. The data obtained from the experiment were statistically processed using the SPSS software.

Table 1. Experimental Design

Item	LM	LE
Number of birds	1 rooster + 4 hens	1 rooster + 4 hens
Pre-experimental period	7 days (26.04.2024 – 02.05.2024)	7 days (26.04.2024 – 02.05.2024)
Feeding	Standard feed	
Nutritional supplement administered in drinking water	-	Neovita 2 ml/l water
Experimental period	15 days (03.05.2024 - 17.05.2024)	15 days (03.05.2024 - 17.05.2024)
Feeding	Standard feed	
Nutritional supplement administered in drinking water	-	Neovita 2 ml/l water
Neovita **Supplement Recommendations:** Neovita is a nutritional supplement recommended for correcting vitamin E deficiencies, manifested by encephalomalacia, muscular dystrophy, exudative diathesis, fertility issues, reduced hatchability rates in cattle, sheep, goats, poultry, and pigs. **Composition per millilitre:** Vitamin E: 25 mg Sodium selenite: 0.25 mg Excipients: up to 1 ml		

Table 2. Description of the characteristics of DEUKA feed

Composition:
wheat, corn, extruded soybean meal (steam fermented), extruded sunflower meal, calcium carbonate, monocalcium phosphate, vegetable fatty acids, sodium chloride (coccidiostat free)
***Analytical constituents*:**
crude protein 18.00 %, crude fat 2.80 %, crude fiber 3.00 %, crude ash 5.40 %, lysine 0.80 %, methionine 0.40 %, calcium 1.00 %, phosphorus 0.60 %, sodium 0.15 %, energy 12.00 MJ ME/kg
***Additives per kg**:*
10000 I.U. vitamin A (E 672) / 5000 I.U. Vitamin D (E 671) as vitamin D3 / 50 mg vitamin E (3a700) as rac-alpha-tocopheryl acetate 25 mg iron (E 1) from ferrous sulphate / 0.6 mg iodine (E 2) from calcium iodate, anhydrous / 7 mg of copper (E 4) from copper sulphate (ÎI), pentahydrate / 80 mg of manganese (E 5) from manganese oxide (ÎI) / 60 mg of zinc (E 6) from zinc sulphate, monohydrate / 0.2 mg selenium (E 8) from sodium selenite 300 PPU 6-phytase (EC 3. 1.3.26) (4a12) / 1500 VU endo-1,3(4)- beta-glucanase EC 3.2.1.6 / 1100 VU endo-1,4-beta-xylanase EC 3.2.1.8 (4a1604i) 3.5 mg of canthaxanthin (E 161g), 2 mg of lutein (E161b).

3. Results and discussion

In Table 3, we may observe that the eggs from the experimental group had an average weight of 54.47 g, without statistically significant differences compared to the weight of the eggs produced by the control group. The weight of the yolk and egg white was higher in the experimental group, with values of 19.90 g and 31.23 g, respectively, compared to the control group which had values of 18.65 g and 26.25 g, but the differences were not statistically significant. Regarding the shell weight, an increase can be observed in the eggs produced by the hens in the control group (6.70 g), and the eggs of the experimental group had a weight approximately 3.7% lower. This difference is also non-significant ($p>0.05$).

Following the statistical analysis of data on egg weight, weight and proportion of their components (Table 4), as well as measuring the diameters (Table 5) of eggs produced by Transylvanian Naked Neck hens, we observed the following: administration of the Neovita supplement in the drinking water of the chickens in the experimental group determined a significant increase in the average weight of the eggs (55.00 g, $p<0.05$). However, the weight and proportion of egg components were not influenced by the supplement.

Table 3. Mean and dispersion indices of egg weight and egg components at the beginning of the experiment (23.04.2024)

Item		Mean	SD	SEM	Value	
					Minimum	Maximum
Egg weight (g)	LM	52.95[a]	2.029	1.014	50.80	55.20
	LE	54.47[a]	5.157	1.719	48.70	65.50
Yolk weight (g)	LM	18.65[a]	0.636	0.450	18.20	19.10
	LE	19.90[a]	1.359	0.680	18.40	21.10
Albumen weight (g)	LM	26.25[a]	0.778	0.550	25.70	26.80
	LE	31.23[a]	4.377	2.189	27.00	36.30
Shell weight (g)	LM	6.70[a]	0.283	0.200	6.50	6.90
	LE	6.45[a]	1.207	0.604	5.50	8.10

[a-a] $p>0.05$; [a-b] $p < 0.05$

Hens in the experimental group produced eggs with higher values for large diameter (5.72 cm), small diameter (4.28 cm) and absolute format index (1.34), compared to eggs in the control group, which recorded 5.53 cm, 4.15 cm and 1.33, respectively. The differences observed were small and statistically non-significant. A non-significant difference was also noted in the case of the format index expressed in relative values. However, this indicator was slightly higher in the control group (75.15%) compared to the experimental group (74.94%).

Table 4. Mean and dispersion indices of egg weight and egg components after supplement administration *(21.05.2024)*

Item		Mean	SD	SEM	Value	
					Minimum	Maximum
Egg weight (g)	LM	51.49[a]	6.021	3.01	44.47	56.86
	LE	55.00[b]	3.353	1.369	50.78	58.99
Yolk weight (g)	LM	19.55[a]	1.625	0.812	17.91	21.73
	LE	19.06[a]	0.964	0.393	17.5	20.05
Albumen weight (g)	LM	25.89[a]	6.396	3.198	19.99	31.45
	LE	29.43[a]	3.401	1.389	25.40	34.72
Shell weight (g)	LM	6.05[a]	0.887	0.443	4.81	6.78
	LE	6.51[a]	0.617	0.252	5.33	7.15

[a-a] p> 0.05; [a-b] p<0.05;

Table 5. Mean and dispersion indices of egg diameters (large and small) and format index (absolute and relative values)

Item		Mean	SD	SEM	Value	
					Minimum	Maximum
Large diameter (cm)	LM	5.53[a]	0.206	0.103	5.30	5.80
	LE	5.72[a]	0.075	0.030	5.60	5.80
Small diameter (cm)	LM	4.15[a]	0.129	0.064	4.00	4.30
	LE	4.28[a]	0.098	0.040	4.20	4.40
Format index (absolute value)	LM	1.33[a]	0.041	0.020	1.28	1.38
	LE	1.34[a]	0.032	0.013	1.30	1.38
Format index (relative value, %)	LM	75.15[a]	2.391	1.196	72.41	78.18
	LE	74.94[a]	1.937	0.790	72.41	77.19

[a-a] p>0.05; [a-b] p < 0.05

From the analysis of data regarding the incubation process (Table 6), it was revealed that the best hatching results, of 66.67%, were obtained in the experimental group. No clear (unfertilized) eggs were recorded in the analysed batches. The number of eggs with dead embryos at the first candling was higher in the control group (7 pcs., 35%) compared to the experimental group (6 pcs., 28.57%). Also, at the second candling, the number of eggs with dead embryos was higher in the control group (2 pcs.) compared to the experimental group (1 pc.). During the hatching period, the control batch recorded additional losses (3 pcs.) due to eggs with dead chicks in the shell. These losses, together with those caused by the elimination of eggs with dead embryos at the first and second candling, led to a hatching rate of only 40% for the control group, compared to 66.67% for the experimental group. The difference between the two batches was statistically significant (p<0.05), statistical significance also being present in the case of the number of eggs with dead chicks in the shell.

Table 6. Results of the incubation process

Item*	LM		LE	
	pcs.	%	pcs.	%
Incubated eggs	20	100	21	100
OL (clear eggs)	0	0	0	0
EM I (eggs with embryos deceased at the first candling)	7[a]	35	6[a]	28.57
EM II (eggs with embryos deceased at the second candling)	2[a]	10	1[a]	4.76
OPMC (eggs with chicks deceased in shell)	3[a]	15	0[b]	0
PN (non-viable chicks)	0	0	0	0
PE (hatched chicks)	8[a]	40	14[b]	66.67

[a-a] p>0.05; [a-b] p < 0.05

Another objective of the experiment was to evaluate the quality of the chicks obtained following the incubation process. The quality of the chicks was assessed based on criteria such as liveliness, the ability to quickly adapt to the new living environment and a measurable parameter – weight at hatching, determined by weighing the chicks from the two batches analysed.
The results of the weighing, statistically processed, are presented in Table 7, providing a clear picture of the differences between the two batches.

Table 7. Mean and Dispersion Indices for Chick Body Weight at Hatching

Item		LM	LE
N		8	13
Mean (g)		**37.50^{a}**	**38.80^{b}**
Standard deviation		2.07	2.92
Standard error of the mean		0.732	0.81
Value	Minimum (g)	34.00	35.00
	Maximum (g)	41.00	43.00
Coefficient of variation (%)		5.52	7.53

$^{a-a}$ $p>0.05$; $^{a-b}$ $p < 0.05$

The average weight of the chicks in the control group was 37.50 ± 2.07 g, approximately 3.35% lower than that observed in the chicks in the experimental group (38.80 ± 2.92 g). The difference between the two average weights was statistically significant ($p<0.05$). The chicks in the experimental group presented wider weight variation limits, between 35.00 and 43.00 g, with a coefficient of variability of 7.53.
Supplementation with selenium or vitamin E under heat stress conditions did not result in significant changes in egg production or weight, consistent with the observations of Puthpongsiriporn et al., (2001) [11], who reported that vitamin E does not influence egg number or weight during short-term cyclic heat stress. However, further research [12, 13, 14] suggests that vitamin E may reduce the negative effects of heat stress on egg production, increasing egg production. Regarding egg weight, most studies [11, 13, 15,] did not show significant effects, except for the observations of Ciftci et al., (2005) [14], who reported an increase in weight with vitamin E supplementation.

4. Conclusions

The weight of eggs produced was significantly influenced by the administration of selenium and vitamin E supplements.
The components of the egg (white, yolk, shell) and their weight did not register significant changes following the administration of the Neovita supplement.
Egg dimensions (large diameter, small diameter) and format index (absolute value) were not significantly influenced by the supplement.
Administration of the Neovita supplement, based on selenium and vitamin E, contributed to improving the hatchability percentage by increasing the hatchability index.
The average weight of chicks at hatching was significantly higher in the group that benefited from Neovita supplementation in the drinking water.

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