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The Effect of Dietary Selenium Source on Embryonic Development in Turkeys

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The objective of this study was to determine the effect of dietary selenium source on the growth and development of turkey embryos, and egg hatchability. White broad-breasted BUT Big 6 turkeys (1800 females and 150 males) were placed under optimum management conditions. Turkey diets were supplemented with organic selenium, and in the other with inorganic selenium, in the amount of 0.3 ppm. Eggs intended for incubation and examination were collected in week 2, 10, 18 and 23 of the laying season. The average egg weight was higher ($p \leq 0.05$) in laying hens fed a diet with organic selenium than in layers receiving inorganic selenium. The rate of yolk sac retraction was faster in embryos from the group fed a diet with inorganic selenium, and it reached 0.59 of the complete yolk sac on day 25 of incubation ($p \leq 0.05$). Selenium source had no effect on the hatching rates of fertilized eggs, which reached 79.61% and 79.84% in laying hens fed organic and inorganic selenium, respectively. In the flocks fed diets supplemented with organic selenium, dead embryos were more frequently characterized by problems with protein utilization (19.28%) and delayed pipping (10.83%). Embryo death rates at the first mortality peak were higher in layers fed inorganic selenium than in those receiving organic selenium (15% vs. 13.5%). The second embryo mortality peak occurred earlier (day 26) in laying hens fed inorganic selenium than in those fed organic selenium (day 28).

Key words: Diet, selenium, embryos, hatchability, turkeys.

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Dietary selenium intake affects the selenium content of eggs. An increase in the selenium content of layer diets from 0.3 ppm to 0.5 ppm has been found to increase selenium concentrations per gram of egg weight from 340 ng to 565 ng (BORUTA *et al*. 2007). The selenium content of eggs is also determined by the dietary selenium source (KENYON & SPRING 2003). In layers fed selenium-enriched yeast, the selenium content of eggs increased from 0.3 ppm to 2.7 ppm. A lower increase in the selenium content of eggs, from 0.3 ppm to 0.7 ppm, was noted in laying hens fed inorganic selenium (PAYNE *et al*. 2005). In many bird species, selenium is accumulated in the egg white (0.785-18.98 μg/egg). In quail and chicken eggs, the highest level of selenium ions are found in the yolk (1.35-4.80 μg), while in ostriches in the eggshell (390.2 μg) (GOLUBKINA & PAPAZYAN 2006).

Selenium contained in eggs is known to exert immunostimulatory effects on the lymphoid tissue of the immune system in chickens (SZELESZCZUK *et al*. 2004). An overdose or deficiency of selenium in feed may adversely affect the reproductive performance of birds. A high level of organic selenium increases the number of cracked eggs (PAYNE *et al*. 2005). Excess inorganic selenium contributes to a decrease in yolk weight which is not observed if layers are fed organic selenium. An adequate selenium content of eggs improves the quality of the thick white, thus extending the freshness of eggs (BORUTA *et al*. 2007). Excess selenium is considered embryotoxic. Sulfur amino acid bound selenium is less toxic than inorganic selenium (RZYMOWSKA *et al*. 1985). Yolk injection with sodium selenate, at 0.002 mg/egg, increased embryo mortality in the 48th hour of incubation (SZELESZCZUK *et al*. 2004).

Selenium has been added to bird diets for many years. In turkeys, organic selenium (0.3 ppm) improved egg fertilization rates by 2.5% (DIMITROV *et al*. 2007). In pheasants, a dietary selenium excess (9.3 ppm) contributed to abnormal behavior in laying hens, disrupted embryonic development, reduced egg hatchability and chick quality (LAT-

SHAW *et al*. 2004). In quails, selenium bioplex (0.15-0.25 mg /kg feed) improved egg hatchability (TARASEWICZ *et al*. 2006). Modern turkey nutrition programs are increasingly based upon alternative feed ingredients and mineral supplements, expected to improve both the nutritive value of diets and bird performance.

The objective of this study was to determine the effect of dietary selenium source on the growth and development of turkey embryos, and egg hatchability.

Material and Methods

The study was carried out according to the guidelines of the Ethical Committee (No. 16/BZ/2009, from the 05 January 2009 and number 23/BZ/2009 from the 05 January 2009).

Animals

Two groups (in two replications) of white broad-breasted BUT Big 6 turkeys were placed in four poultry houses, 1800 females and 150 males in each, under optimum management conditions.

Feeding regime

The nutritional value of feed corresponded to the nutrient requirements of turkeys. Diets for laying hens contained 11.7-12.1 MJ metabolizable energy and 16-19% total protein, depending on the stage of the laying period. Diets for turkey-males contained 13.4 MJ metabolizable energy and 10% total protein (JANKOWSKI 2005). In two houses, turkey diets were supplemented with organic selenium, and in the other two houses – with inorganic selenium, in the amount of 0.3 ppm. Selenium was added to feed from week 29 to the end of the laying season.

Analysis of egg quality and embryonic development

In week 2, 10, 18 and 23 of the laying season, 40 eggs with normal structure were collected from each house on the same day (a total of 640 eggs). Eggs were weighed (with an accuracy of 0.1 g) and were stored for four days under standard microclimate conditions before incubation. Eggs were incubated in a single-chamber Pas Reform incubator at a temperature of 37.5-37.7°C and relative humidity of 65%. Eggs were turned over every hour.

Live embryos were evaluated in the 72nd hour and on day 12, 20 and 25 of incubation. At each sampling date, 115, 152, 145 and 108 live embryos were obtained from 160 eggs, respectively, following the elimination of unfertilized eggs and eggs containing dead embryos (DZIACZKOWSKA & FARUGA 1983). In the 72nd hour of incubation, the diameter of the area vasculosa (mm) was measured at the borderline from the head region to the tail region at the bifurcation of the anterior and posterior vitelline veins. Older embryos were chilled at 2°C for two hours prior to analysis. After that, the eggs were cracked, and embryos with the yolk sacs were removed, dried on filter paper and weighed without the sacs. In the eggs collected on day 25 of incubation, the yolk sacs were not separated from the embryos due to the advanced retraction of the yolk sac into the abdominal cavity at this stage of development. Embryo weight was expressed as the percentage of egg weight before incubation. On day 25 of incubation, yolk sac absorption was estimated using a scale from 0 to 1, where 1 denoted complete absorption of the yolk sac and 0 denoted that yolk sac absorption had not yet begun. Absorption of half of the yolk sac volume was indicated as 0.5 on the scale. If more or less than half of the yolk sac was absorbed, the scale refers to the decimal fraction value (i.e. 3/4 denoted absorption of the yolk sac marked as 0.75). In week 23 of the laying season, embryos incubated for 25 days were not analyzed for organizational reasons.

Egg hatchability

In selected weeks of the laying period, eggs were incubated in Petersime incubators. Four batches of eggs were analyzed: in week 2 of the laying period, a batch comprised 8392 and 7856 eggs from groups fed diets supplemented with organic and inorganic selenium, respectively, in week 10-11766 and 11654 eggs, respectively. In week 18 the number of eggs in the groups fed diets supplemented with organic selenium was 7329, whereas 5702 eggs came from the groups fed diets supplemented with inorganic selenium. In week 23, 6108 eggs originated from the "organic groups" and 5035 from the "inorganic groups". The number of unfertilized eggs and eggs containing dead embryos, and the number of weak, disabled poults and hatched turkeys were expressed in percentage terms in relation to the set eggs and fertilized eggs.

Analysis of dead embryos and unhatched poultry

Unfertilized eggs, eggs containing dead embryos and unhatched turkeys, collected from the hatchery waste, were subjected to an embryopathological analysis (BORZEMSKA 2005). Each egg was broken open to determine the actual number of infertile eggs and eggs containing dead embryos, and to indicate the day of death of each embryo. Embryo mortality curves were plotted using a previously developed method (MRÓZ *et al.* 2010).

The following pathological lesions of embryos were determined: hyperemia and hydration of the body and fetal membranes, abnormal position, problems with protein utilization, congenital disorders (monsters), unabsorbed yolk sac. Problems with protein utilization was assessed visually. Until the 21st day of incubation, embryos utilize all proteins. The presence of proteins after the 21st day of incubation indicates a developmental disorder (DZIACZKOWSKA & FARUGA 1983). The percentage of live, unhatched embryos was also determined. The percentage of embryos with a given pathological defect was calculated in relation to all analyzed eggs containing embryos and unhatched poults (after candling and at the end of incubation).

Statistical analysis

Experimental data were verified statistically by a two-way analysis of variance. Differences between means in groups were determined by Duncan's test.

Results

Laying hens fed a diet with organic selenium laid bigger eggs ($p \leq 0.05$, Table 1). Egg weight was also affected by the stage of the laying period. In week 18, egg weight exceeded the optimal value of 95 g in both groups. The interaction between selenium source and the stage of the laying period had no effect on egg weight in week 2 and 23 of the laying season.

The size of the area vasculosa was similar in both groups. Selenium source had no effect on the first 72 hours of embryo and blood vessel development. The development of the area vasculosa was affected by the stage of the laying period. The area vasculosa diameter was smaller in week 2 than in other weeks of the laying period ($p \leq 0.05$), indicating that the development of the hemopoietic system and the cardiovascular system was slower at the beginning of the laying season.

Table 1

Evaluation of egg weight and live embryos

Specification	N	Egg weight before incubation (g)	Area vasculosa diameter, day 3 of incubation (mm)	Embryo body weight without yolk sac, day 12 of incubation (%)	Embryo body weight without yolk sac, day 20 of incubation, (%)	Embryo body weight with yolk sac, day 25 of incubation (%)	Yolk sac absorption, day 25 of incubation (0-1 scale)
Selenium							
organic	320	94.95	15.05	3.26	27.00	73.45	0.52*
inorganic	320	93.01*	14.52	3.25	27.57	72.89	0.59
Laying weeks							
2	160	83.73[a]	12.25[a]	3.46[c]	29.55[b]	73.11[b]	0.54
10	160	94.26[b]	15.59[b]	2.97[a]	26.76[a]	75.28[c]	0.53
18	160	97.73[c]	16.22[b]	3.39[c]	26.05[a]	71.01[a]	0.60
23	160	100.20[d]	15.19[b]	3.22[b]	26.49[a]	xxx	xxx
Selenium x Laying weeks							
organic x 2	80	83.94[a]	12.13	3.52	29.73	73.78	0.55
organic x 10	80	95.86[c]	16.35	2.99	26.60	75.32	0.47
organic x 18	80	99.47[d]	16.48	3.30	25.05	71.05	0.52
organicx 23	80	100.54[d]	15.35	3.27	26.31	xxx	xxx
inorganic x 2	80	83.52[a]	12.38	3.41	29.36	72.51	0.53
inorganic x 10	80	92.67[b]	14.88	2.94	26.92	75.22	0.60
inorganic x 18	80	96.00[c]	15.93	3.48	27.10	70.98	0.68
inorganic x 23	80	99.86[d]	15.04	3.18	26.71	xxx	xxx
SEM		0.35	0.25	0.03	0.24	0.24	0.01
p-value							
Selenium		<0.05	0.223	0.739	0.158	0.176	<0.05
Laying weeks		<0.05	<0.05	<0.05	<0.05	<0.05	0.275
Selenium x Laying weeks		<0.05	0.539	0.290	0.238	0.287	0.089

* $p \leq 0.05$

a-d – means within a column with different letters are significantly different ($p \leq 0.05$)

N – number of samples

There were no significant differences in embryonic body weight between the groups on day 12, 20 and 25 of incubation. The largest embryos ($p \leq 0.05$) were noted in week 2 and 10 of the laying period (Table 1). Yolk sac absorption reached 0.52 to 0.60. The process was slower ($p \leq 0.05$) in the eggs of hens fed organic selenium (Table 1). Higher egg weight in the selenium-supplemented group could slow down the process of yolk sac retraction.

Dietary selenium source had no influence on egg fertilization rates and other hatchability parameters (Table 2). The percentage of unfertilized eggs, embryos that died between day 15 and 28 of incubation and hatching rates were affected by the week of the reproduction period ($p \leq 0.05$). The lowest egg hatchability was observed in week 23 of the laying season (Table 2). An analysis of dead embryos (Table 3) revealed a correlation between dietary selenium source and pathomorphological changes. In the group of turkeys receiving organic selenium, a higher number of embryos did not manage to utilize protein until day 21 of in-

Table 2

Egg hatchability, %

Specification	N	Unfertilized eggs	Embryos that died until day 14 of incubation	Embryos that died between day 15 and 28 of incubation	Weak and disabled poults	Hatching rates of eggs set	Hatching rates of fertilized eggs
Selenium							
organic	8	2.23	7.08	6.50	6.44	78.06	79.61
inorganic	8	2.43	7.58	6.34	6.21	77.92	79.84
Laying weeks							
2	4	1.51^{a}	8.74	4.79^{a}	5.35^{a}	80.02^{a}	81.01^{a}
10	4	1.66^{a}	6.98	5.11^{a}	6.08^{b}	80.59^{a}	81.78^{a}
18	4	2.43^{a}	6.55	6.95^{b}	6.55^{b}	78.00^{a}	79.94^{a}
23	4	3.72^{b}	7.05	8.82^{c}	7.31^{b}	73.35^{b}	76.18^{b}
SEM		0.27	0.31	0.44	0.28	0.80	0.288
p-value							
Selenium		0.597	0.377	0.663	0.566	0.871	0.318
Laying weeks		<0.05	0.085	<0.05	<0.05	<0.05	<0.05
Selenium x Laying weeks		0.530	0.602	0.650	0.458	0.755	0.585

Explanations as in Table 1.

Table 3

Evaluation of dead embryos, %

Specification	N	No lesions	Hyperemia	Hydration	Abnormal position	Problems with protein utilization	Congenital disorders	Unabsorbed yolk sac	Live unhatched poults
Selenium									
organic	8	52.11	15.80	18.19	4.67	19.28	3.49	23.04	10.83
inorganic	8	47.62	21.19	21.78	4.42	18.04*	3.89	23.95	4.66*
Laying weeks									
2	4	53.03^{a}	22.83	36.88^{a}	1.32^{a}	11.28^{a}	2.58	14.81^{a}	8.49^{ab}
10	4	37.71^{b}	13.47	23.45^{b}	4.59^{ab}	24.54^{b}	3.77	34.12^{c}	13.74^{a}
18	4	51.91^{a}	20.53	8.93^{c}	5.35^{b}	20.39^{b}	4.93	19.61^{a}	4.13^{b}
23	4	56.82^{a}	17.24	10.68^{c}	6.93^{b}	18.43^{b}	3.46	25.44^{bc}	4.62^{a}
SEM		2.16	1.80	3.07	0.70	1.55	0.44	2.26	1.49
p-value									
Selenium		0.069	0.161	0.140	0.826	<0.05	0.640	0.745	<0.05
Laying weeks		<0.05	0.317	<0.05	<0.05	<0.05	0.325	<0.05	<0.05
Selenium x Laying weeks		0.432	0.660	0.719	0.585	0.227	0.269	0.204	0.333

Explanations as in Table 1.

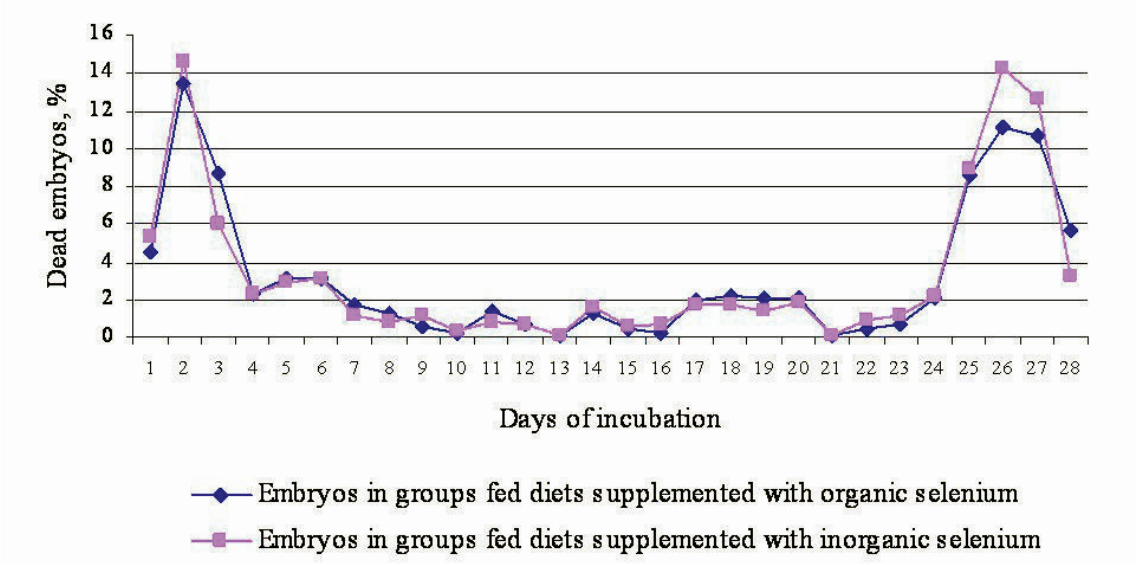

Fig. 1. Embryo mortality distribution.

cubation, which contributed to delayed hatching (Table 3). The number of embryos with pathological defects was affected by the stage of the laying season. The lowest percentage of dead embryos with no visible lesions was observed in week 10. The highest number of hydrated embryos was noted in week 2 and 10 (Table 3). Abnormal position and problems with protein utilization were reported more frequently towards the end of the laying season. The number of live unhatched poults was higher at the initial stage of the laying period.

The experimental groups differed with respect to the embryo mortality distribution. Embryo death rates at the first mortality peak were higher in layers fed inorganic selenium than in those receiving organic selenium. The second embryo mortality peak occurred earlier (day 26) in laying hens fed diets supplemented with inorganic selenium (Fig. 1). Embryo death rates at mortality peaks did not exceed 15%. In the interval between mortality peaks, embryo death rates were low in both groups, thus indicating that the nutritional factor did not significantly effect the embryo mortality distribution at that stage of incubation.

Discussion

The present experiment confirms previous findings regarding the undesirable increase in turkey egg weight during the laying season (MRÓZ & ORŁOWSKA 2009) which was enhanced by organic selenium supplementation. This correlation has also been observed in laying hens by PAYNE *et al*. (2005). UTTERBACK *et al*. (2005) reported no differences in eggs weight from 90 Hy-Line hens fed a diet supplemented with sodium selenite or *Se* yeast. A different effect was noted by PAPPAS *et al.* (2005). Reduction in egg weight due to the addition of fish oil to breeder hen diets was not as pronounced as when organic selenium was also added to the diet.

The size of the area vasculosa was similar to that reported by other authors. The development of the area vasculosa was found to be slower at the beginning of the laying season compared with later stages (ORŁOWSKA *et al.* 2006). There is no information directly concerning the influence of selenium on the development of the area vasculosa and the blood circulation system of turkey embryos. MOUSA *et. al.* (2007), using the chick chorioallantoic membrane (CAM) model, examined the effect and mechanism of angiogenesis of arsenite at a low level and its potential reversal by various selenium-derived compounds. The authors noted that the pro-angiogenesis effect of arsenite was blocked by various selenium-containing compounds at 10 μmoles of either dimethyl selonone or diphenyl selonone blocked b-FGF-induced angiogenesis. 10 μmoles of either sodium selenite or *Se*-methyl selenocysteine inhibited arsenite-induced angiogenesis or b-FGF-induced angiogenesis. Selenous acid dissolved in saline (doses ranging from 0.0007 to 0.05 mg/egg) was injected into chick eggs. Microscopic studies of the chick embryo heart showed ventricular septal defects and thin ventricular walls. The reported experiments indicate that selenium poisoning induces changes in the embryonic heart (KHAN & GILANI 1980).

KHAN and GILANI (1980) also observed a reduced body size under the influence of selenium. PATON *et al.* (2002) studied the effect of dietary source

(organic or inorganic) and level of *Se* on chick embryos. Leghorn laying hens were fed a diet supplied by sodium selenite or *Se*-enriched yeast. The authors did not detect significant changes on embryo and extra-embryonic weights. We found a similar effect on turkey embryos. BURKE (1994) studied embryonic body weight in Hybrid and Nicholas turkeys and found that it increased 7.5- to 7.6-fold from day 12 to 20 of incubation. In our study, embryonic body weight increased over this period 7.6- to 9.0-fold which most probably resulted from the genetically-determined growth rate of birds. In an experiment conducted by CROOM *et al.* (2006), the average embryonic body weight on day 20 of incubation reached 25.36 g, compared with 27.00-29.55 g in the present study. Differences in embryo weight on the same day of incubation may be due to considerable differences in hatching egg weight. As shown by our findings and previous research, the growth weight and body weight of embryos are influenced by egg weight and hen age (CHRISTENSEN *et al.* 2000; RICKLEFS & STARCK 1998).

The lowest number of embryos with structural abnormalities and developmental defects was noted in week 10 of the laying season. This period was characterized by low embryo mortality and the highest hatch rates, supporting the findings of other authors (BORZEMSKA 2005; MRÓZ 2010; ORŁOWSKA *et al.* 2006). The number of embryos with pathological defects was similar in both groups, with the exception of problems with protein utilization. The 11 day chick embryos demonstrated gross deformations including twisted limbs and neck, everted viscera, edema, etopic heart, and body hemorrhage under the influence of selenium (KHAN & GILANI 1980). Approximately 10 % of the chicks from pheasant hens fed a diet with high selenium content had deformed beaks and abnormal eyes. Many of the chicks that died in the shell had deformities (LATSHAW *et al.* 2004).

The mortality peaks reported in this experiment were lower than those noted in previous studies (MRÓZ 2010; MRÓZ *et al.* 2007). According to the cited authors, mortality peaks are affected by egg quality. Embryo death rates were low in the interval between mortality peaks, suggesting that the nutritional factor (organic and inorganic selenium administered at 0.3 ppm) had no embryotoxic effects (BORZEMSKA 1978; BORZEMSKA 1984; KENYON & SPRING 2003; RICKLEFS & STARCK 1998; RZYMOWSKA *et al.* 1985).

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