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EFFECT OF HIGH DOSE OF RIBOFLAVIN INJECTED IN OVO ON HATCHABILITY AND GAIN OF BROILER CHICKEN

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The aim of this work was to examine consequence of riboflavin in ovo injection on the chicken embryo development and chickens production parameters. The aim of the experiment was to investigate the influence of high doses of riboflavin (0, 300, 1,500 and 3,000 μg/egg, n= 1,200 eggs from the broiler breeder flock of Ross 308 line) injected in ovo on 0. or 6. day of incubation for hatching and growth during the fattening. It was proved that in ovo injection of riboflavin before the beginning of incubation caused mortality increase of embryos in the early stages of embryogenesis. 3,000 μg/egg riboflavin application decreased chickens fattening rates. Summarizing the data presented here suggest that riboflavin in ovo injection affect hatching and some aspects of chicken metabolism. Further studies are needed to explain the role of riboflavin in chicken embryogenesis.

Key words: CHICKEN EMBRYO, EMBRYOGENESIS, RIBOFLAVIN, IN OVO, BODY WEIGHT

Riboflavin plays a key role in the metabolism of the organism as a source of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) [Murray *et al.* 2008]. At adult birds deficiency of this vitamin is very rare. Riboflavin deficiency usually occurs in chickens at the age of 2–4 weeks. In this case, growth retardation, progressive wasting and weakness with preserved appetite are observed. Toes become bent or make a fist. The skin is rough and cracking on fingers. Leg muscles disappear and the wings are lowered. Because of that riboflavin is common fodder additive, which should adjust effects of broiler chicken fattening [Borzemska 1984, Olkowski and Classen 1998, Mazurkiewicz 2005].

When riboflavin level in chicken egg is lover than 60 μg then embryo mortality increase and problems with hatching are observed [Borzemska 1984, Squires and Naber 1993, Villamide and Fraga 1999, Mazurkiewicz 2005]. Especially form of vitamin B2 depression is the one caused by RBP gene mutation which encourages hens to deposit riboflavin in their eggs. Effects of riboflavin deficiency during embryogenesis are revealed only after the 10^{th} day of incubation: the hypoglycemia and the process of accumulation of substrate oxidation of fatty acids begin. Embryo death occurs suddenly, about 13^{th} day of incubation, and is preceded only by short-lived, lasting about 1 hour disturbance of the heart rate [Borzemska 1984, Lee and White 1996]. The effect of both: the RBP gene and other reasons of riboflavin deficiency in chicken egg can be abolished by *in ovo* injection of riboflavin. Supplemented embryos survive and develop normally, indicating that the unbound riboflavin in the egg protein can also be used during development. After hatching chicks take riboflavin from the feed and develop normally [Lee and White 1996]. In the available literature there is lack of data describing excess of riboflavin on embryonic metabolism, therefore, the aim of the present study was to examine the consequence of *in ovo* injection of riboflavin before incubation or on 6^{th} day of embryogenesis (E 6) on chick embryo development and later body gain of these chickens.

Materials and methods

In the experiment 1,200 eggs from a flock Ross308 were taken. Eggs were randomly divided into eight groups (n = 150 eggs) due to the injection time: before the start of incubation (E 0) or E 6, and the dose of riboflavin: 0,0, 300, 1,500, 3,000 μg per egg dissolved in 50μl 0.7% NaCl. Before injection eggshells were disinfected with 70 % ethanol, followed by big end of egg 5 mm wide hole was made. Riboflavin solution was injected into egg with Minilab dispenser, which to avoid decomposition of riboflavin by light was wrapped with tinfoil. Riboflavin solution was

administered by entering through a hole in the egg shell the pipette tip with a capacity of 100 ml, to not incubated eggs vitamin was given under the inner shell membrane, whereas in E6 under chorioallantoic membrane considering not to damage the blood vessels. After the manipulation holes were sealed with Parafilm ® tape.

Eggs were incubated in a setter Petersime ® S576 (E1–E18, T = 37.8 ± 0,1°C, RH = 50 % ± 1 %) and hatcher Petersime ® H192 (E19–E21, T = 37,2 ± 0.1 °C and RH = 55–70 %).
Hatching process was controlled from 464 hour of incubation. In every 2 hours the number of eggs piped and hatched was checked. Tables of piping and hatching for cumulative hatching percentages were made based on the obtained data. Based on this, synchronization processes were compared for all groups according to the method described by Lis [2007].

From the chicks hatched from each experimental group were chosen randomly 20 healthy chicks (10 each sex), each labelled with an individual wing stamp and earmarked for fattening. Fattening chickens last 42 days in the production conditions of rearing with unlimited access to water and feed. Birds were fed with concentrates for broiler chickens (Tab. 1). Weighting birds were carried out every seven days on electronic scale accurate to 1g.

Table 1

Content of protein and energy in feed for broiler chickens

Type of concentrate	Protein [%]	Energy [kcal.]
Brojler starter	min. 22,0	min. 3050
Brojler grower	min. 20,0	min. 3180
Brojler finiszer	min. 19,0	min. 3220

The hatchability and embryopathological results were statistically analysed by z-test, the results of fattening were analyzed by two-way ANOVA followed by Tukey's multiple range test. The course of hatching processes was analyzed by two-way ANOVA followed by Kruskal Wallis test. The statistical analyses were performed using Sigma-Stat 2.03 (SPSS Science Software Ltd., USA) while the figures were prepared using Grapher 7,0 (Golden Software Inc., USA).

Results and discussion

Injection *in ovo* of riboflavin solution, before incubation, regardless of the dose ($P > 0,05$) caused a rapid mortality of embryos at 60–70 % during the first six days of incubation. Hatchability in the control group was 22,8 % and was significantly higher ($P \leq 0,05$) by 12,7, 15,3 and 13,2 % compared to the groups, which are respectively 300, 1,500 and 3,000 µg of riboflavin in the egg ($P > 0,05$, Tab. 2).

Table 2

Effect of riboflavin injected *in ovo* before incubation on hatchability (per cent of fertilized eggs)

Riboflavin dose	n	Mortalized date [day of incubation]					Hatched chicks
		1–3	4–6	7–8	9–18	19–21	
		[per cent of fertilized eggs]					
0 µg/jajo	136	$47,1^{A}$	$13,2^{B}$	$5,9^{C}$	$8,8^{BC}$	$2,2^{D}$	$22,8^{E}$
300 µg/jajo	138	$52,2^{A}$	$17,4^{BE}$	$8,7^{BC}$	$8,0^{BC}$	$3,6^{CD}$	$10,1^{BC}$
1,500 µg/jajo	134	$56,7^{A}$	$18,7^{BE}$	$6,7^{C}$	$8,2^{BC}$	$2,2^{D}$	$7,5^{BC}$
3,000 µg/jajo	136	$54,4^{A}$	$16,9^{B}$	$8,8^{BC}$	$7,4^{BC}$	$2,9^{D}$	$9,6^{BC}$

Note: ABCD — Values marked with different letters differ significantly ($P \leq 0,05$)

However, injection of riboflavin solution on E 6 caused a rapid death of embryos at the level of 30–40 % irrespective of the dose ($P > 0,05$) over approximately 48 hours after manipulation ($P \leq 0,05$). Hatchability in the control group was 45,7 % and was higher compared to the group which was given 300 µg of riboflavin per egg by 6,0 % ($P > 0,05$) and by 12,3 and 14,8 % ($P \leq 0,05$), to the group which were respectively injected with 1,500 and 3,000 µg of riboflavin per egg (Tab. 3).

Table 3

Effect of riboflavin injected in ovo at 6th day of incubation on hatchability (per cent of fertilized eggs)

Riboflavin dose	n	Mortalized date (day of incubation)					Hatched chicks
		1–3	4–6	7–8	9–18	19–21	
		(per cent of fertilized eggs)					
0 µg/jajo	136	$5,8^{A}$	$2,9^{B}$	$25,4^{CD}$	$15,9^{D}$	$4,3^{AB}$	$45,7^{F}$
300 µg/jajo	138	$6,6^{A}$	$2,2^{B}$	$32,4^{C}$	$13,2^{D}$	$5,9^{A}$	$39,7^{EF}$
1,500 µg/jajo	134	$5,8^{A}$	$3,6^{AB}$	$36,5^{C}$	$16,1^{D}$	$6,6^{A}$	$31,4^{CE}$
3,000 µg/jajo	136	$8,6^{A}$	$1,4^{B}$	$37,4^{C}$	$15,1^{D}$	$6,5^{A}$	$30,9^{CE}$

Note: ABCD — Values marked with different letters differ significantly (P≤0,05)

The riboflavin effected on the time of hatching chicks (P ≤ 0,05), although there was no dependance on dose (P > 0,05). Peak of piping chicks from eggs after administration of 0 µg pre egg at 0 and 6 day of incubation was respectively at 480 and 484 hour of incubation (P > 0,05), while in the groups after administration of 3,000 µg of riboflavin per egg was observed at 476 and 488 hour of incubation (P > 0,05). However, after administration of 300 and 1,500 µg of riboflavin per egg, peak was found in 488 hour of incubation period irrespective of the injection term (P > 0,05, Tab. 4). Peak of hatching chicks from eggs after administration of 0 µg per egg at 0 and 6 day incubation was respectively at 502 and 496 hour of incubation (P > 0,05), while after injection of 300, 1,500 and 3,000 µg of riboflavin per egg hatching peak on E0 fell respectively 501, 502 and 498 hour of incubation (P > 0,05), in the case of injection on E 6 was observed, respectively at 492, 502 and 502 (P > 0.05; Tabl. 4).

Table 4

Effect of riboflavin dose and injection date on course of hatching process

Injection date	Riboflavine dose	n	median	min	max	mean	±SD
		szt.	(incubation hour)				
Pipping							
before incubation	0 µg/egg	38	480	464	502	482,1	±7,90
	300 µg/egg	17	488	472	496	485,1	±8,23
	1,500 µg/egg	28	488	476	496	488,0	±6,15
	3,000 µg/egg	16	476	472	502	481,7	±12,42
6^{th} day incubation	0 µg/egg	39	484	468	504	484,7	±7,28
	300 µg/egg	29	488	472	496	486,5	±6,88
	1,500 µg/egg	20	488	472	502	488,0	±8,39
	3,000 µg/egg	21	488	472	502	484,9	±7,76
Hatching							
before incubation	0 µg/egg	31	502	476	502	496,6	±7,32
	300 µg/egg	14	501	488	506	499,0	±8,72
	1,500 µg/egg	10	502	488	502	498,4	±5,15
	3,000 µg/egg	14	498	492	502	497,5	±4,43
6^{th} day incubation	0 µg/egg	13	496	472	502	495,1	±8,11
	300 µg/egg	33	492	484	506	495,8	±6,79
	1,500 µg/egg	24	502	488	502	499,1	±4,21
	3,000 µg/egg	13	502	476	502	496,2	±8,14

Because of very low hatchability in groups, which were injected *in ovo* before the incubation (Tab. 3), rearing chickens in these groups were not performed.

Start from second week of rearing, sex of chicks had a statistically significant effect on their weight (P ≤ 0,05). Roosters were heavier than the hens for about 5–18 % in second week of fattening for about 12–30 % at 6^{th} week of fattening, cognitively to the test group (P> 0,05) (Fig. 1 and Fig. 2). Based on this, the analysis of body weight of chickens in the following weeks of fattening was carried out separately for each sex.

Hens' body weight increased significantly in subsequent weeks (P ≤ 0,05) regardless to the dose of riboflavin injected *in ovo* on E6 (P > 0,05). For groups after injection of 0, 300, 1,500 and 3,000 µg riboflavin per egg, average body mass of 1 day-old female chick was respectively 48.6 ± 2,21 g, 47.5 ± 3,23 g, 46,1 g and 49,2 ± 3,18 ± 4,71 g (P > 0,05). Hens' weight from checked groups increased in 1^{st} week of fattening by 210–242 % (P > 0,05) in the 2^{nd} week by 249–278 % (P > 0,05) in the 3^{rd} week by 206–223 % in the 4^{th} week by 175–180 % (P > 0,05). In 5^{th} week of

fattening weight of the hens has increased by 135–144 % (P > 0,05) and in compare to the previous week was in the group injected with 0μg 1525,9 ± 120,47 g what was lower by 0,5 g (P > 0,05) than the group treated with 300 of riboflavin, and higher by 120,9 g (P > 0,05) and 79,2 g (P ≤ 0,05) than in the groups treated with 1500 and 3000 μg of riboflavin respectively. In the last week of fattening hens body weight increased to 134–149 % in the control group and reached 2113,.0 ± 106,80 g, while the groups which received riboflavin *in ovo* at dose: 300 μg — 2043,3 ± 392,46 g; 1500 μg — 2081,6 ± 190,46 g, 3,000 μg — 2148,3 ± 352,50 g (P > 0,05, Fig. 1).

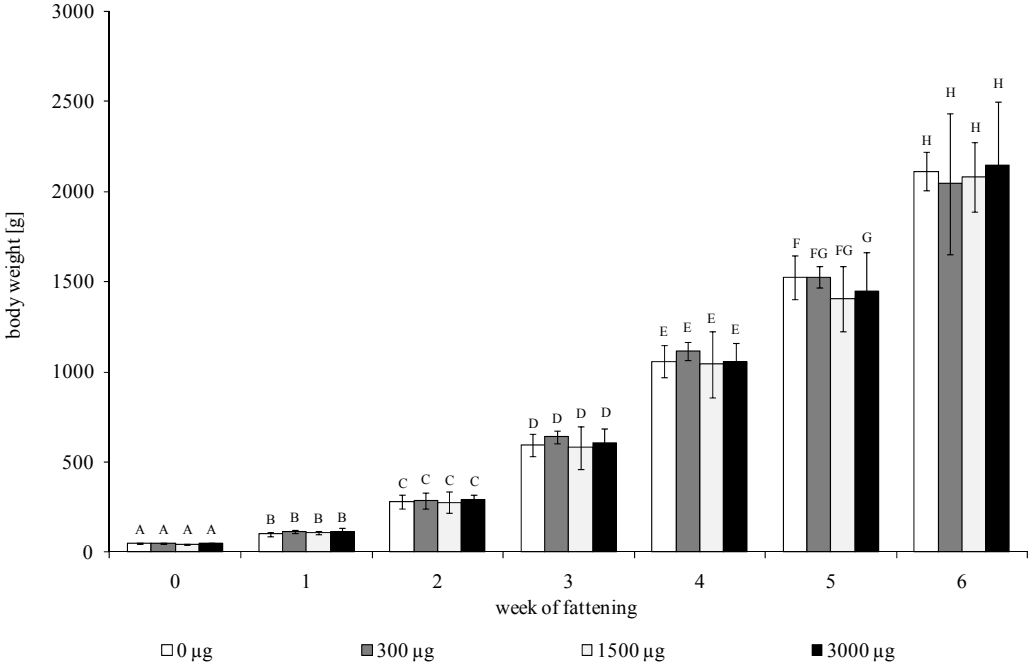

Fig. 1. Effect of riboflavin injected in ovo in 6th day of incubation on growth of hen broiler chicken body weight in following weeks of fattening.

Note: ABCDEFGH — Values marked with different letters differ significantly (P≤0,05)

Roosters body weight increased significantly in subsequent weeks (P ≤ 0,05) regardless to the dose of riboflavin injected *in ovo* on E6 (P> 0,05). For groups after injection at the dose of 0, 300, 1,500 and 3,000 μg of riboflavin average weight of 1 day-old male chicks was respectively 48,6 ± 2,21 g, 47,5 ± 3,23 g, 46,1 g and 49,2 ± 3,18 ± 4,71 g (P > 0,05). Riboflavin injected *in ovo* had an impact on the increases' rate for P = 0,08 and was associated with lower body weight of roosters injected in ovo with 3,000 μg of riboflavin (P ≤ 0,05). Weight of roosters from examined groups increased in the 1st fattening week by 231–253 % (P > 0,05) in the 2nd week by 265–284 % (P>0,05) in the 3rd week for 205–231 % in the 4th week of 175–186 % (P>0,05). In 5th week of fattening weight of roosters increased by 140–152 % (P>0,05) compared to the previous week and was in a group injected with 0μg of riboflavin per egg 1866,0±140,50 g which was higher by 109,7 g (P>0,05) than the group treated with 300 μg of riboflavin, and 11 g higher (P>0,05) and 254,3 g (P≤0,05) than in the groups treated respectively with 1,500 and 3,000 μg of riboflavin per egg. In the last week of fattening roosters had increased their body weight by 142–150 % reaching in the control group 2657,0±207,70 g, while the groups which received riboflavin in ovo at the dose: 300 μg — 2523,8±200,90 g; 1,500 μg -2675,0±233,33 g, 3,000 μg — 2410,0±457,13 g. Roosters' body weight in control group and a group treated with 3,000 μg of riboflavin differed significantly (P≤0,05, Fig. 2).

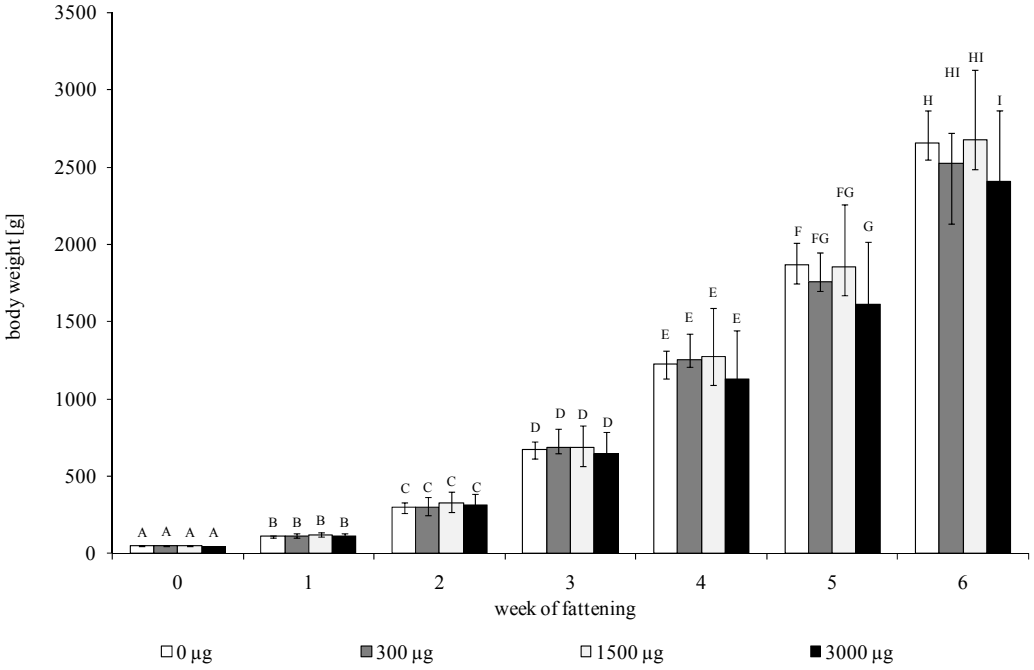

Fig. 2. Effect of riboflavin injected in ovo in 6th day of incubation on growth of rooster broiler chicken body weight in following weeks of fattening.

Note: ABCDEFGHI — Values marked with different letters differ significantly (P≤0,05)

In the held experiment injection of riboflavin solution in both terms: before the incubation (E 0) and in the six day of incubation (E 6) caused a rapid mortality of embryos up to 60 %, within 2–3 days after the manipulation. Comparing the level of the early embryo mortality of control and experimental groups should be noted, that the main cause of early death of embryos was disturbance in the homeostasis of the embryo as result of the manipulation. This is confirmed by observations of Brüggemann *et al* (2003), which shows that the sensitivity of chick embryos to *in ovo* manipulation in the early stages of embryogenesis is very high but gradually decreases with the progress of chick development. However hatchability of chicks in the control group was higher by 10–15 % than hatchability of chicks injected *in ovo* with high doses of riboflavin (300–3,000 µg/egg) shows some embryotoxic action of this substance. It is interesting because of that in the literature are described mainly the cases of embryonic death due to deficiency of riboflavin. Characteristic is also the time of death which occurs at the 10th–13th day of incubation. It is believed that the immediate cause of death of embryos is a disturbance of oxidation of fatty acids and the occurrence of hypoglycaemia [White *et al* 1996].

One of the symptoms of riboflavin deficiency in animals is the inhibition of growth, and therefore also for the rearing and poultry farming supplementation of this compound in the feed is widespread [Baker *et al* 1999,. In these studies, administration of riboflavin to the eggs did not cause changes in weight of embryos. However, analyzing the results of fattening chickens weaker results in the roosters' fattening from group which received 3,000 µg of riboflavin were observed. It is interesting that the results of work held by Olkowski and Classen. [1998] have shown greater increases in broilers treated with feed enriched with exogenous riboflavin than in birds fed unsupplemented dose. However this effect was apparent only in the first fattening period (1–21 days of life), and the final body weight of chickens at 42nd day in both groups did not differ significantly. Based on this it can be assumed that when the chicken's B_2 vitamin demand is covered by feed the additional supplementation of poultry with high doses of riboflavin have no economic justification.

Conclusions

The high doses of riboflavin injected *in ovo* can cause death embryos during the first week of incubation. Moreover, the chicken supplemented with high dose riboflavin during embryogenesis

did not grow better than unsupplemented ones. However riboflavin *in ovo* injection affect hatching and some aspects of chicken metabolism and further studies seem to be needed to explain the role of riboflavin in chicken embryogenesis.

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ВПЛИВ ВЕЛИКИХ ДОЗ РИБОФЛАВІНУ, ЯКИЙ ВВОДИТЬСЯ *IN OVO,* НА НЕСУЧІСТЬ КУРЕЙ ТА ВИВЕДЕННЯ КУРЧАТ-БРОЙЛЕРІВ

Р е з ю м е

Мета роботи — дослідити наслідки введення рибофлавіну *in ovo* на розвиток ембріонів курчат та показники їх продуктивності. Визначено його вплив у великих дозах (0, 300, 1,500 and 3,000 μg/яйце n = 1,200 яєць курчат-бройлерів лінії Росс 308) на виведення курчат (0 чи 6 день інкубації) і їх ріст під час вигодовування. Доведено, що введення рибофлавіну до початку інкубації спричинило зростання загибелі ембріонів на ранніх стадіях ембріогенезу. Використання 3,000 μg/яйце рибофлавіну знизило показники відгодівлі. Підсумовуючи наведені дані, припускаємо, що введення рибофлавіну *in ovo* впливає на вивід курчат і деякі аспекти метаболізму. Необхідні подальші дослідження, щоб пояснити роль рибофлавіну у ембріогенезі курчат.

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ВЛИЯНИЕ БОЛЬШИХ ДОЗ РИБОФЛАВИНА, КОТОРЫЙ ВВОДИТСЯ IN OVO, НА НЕСУЧЕСТЬ КУР И ВЫВЕДЕНИЕ ЦЫПЛЯТ-БРОЙЛЕРОВ

А н н о т а ц и я

Целью этой работы было исследовать последствия введения рибофлавина in ovo на развитие эмбрионов цыплят и показатели их производительности. Целью опыта было определить влияние больших доз рибофлавина (0, 300, 1,500 and 3,000 μg/яйцо n= 1,200 яиц цыплят-бройлеров линии Росс 308) которым его вводили in ovo на 0. или 6. день инкубации на вывод на вывод цыплят и их рост во время выкармливания. Доказано, что введение рибофлавина к началу инкубации повлекло рост гибели эмбрионов на ранних стадиях эмбриогенеза. Использование 3,000 μg/яйцо рибофлавина привело к ухудшению откормных показателей. Подытоживая приведенные в статье данные можно допустить, что введение рибофлавина in ovo влияет на вывод цыплят и некоторые аспекты метаболизма. Необходимы последующие исследования, чтобы объяснить роль рибофлавина в эмбриогенезе цыплят.

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