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Effect of *In Ovo* Injection of Vitamin C During Incubation on Hatchability of Chickens and Ducks

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The aim of the investigation was to ascertain the influence of different doses of vitamin C injected at selected dates of incubation into the eggs of broiler breeders and Pekin ducks on hatchability. The injected vitamin C doses were administered into the air cell on the 13th, 15th and 17th days (3 and 6 mg – chickens) and on the 12th and 20th days (4 and 8 mg – ducks) of incubation. In the case of chickens, no significant differences were recorded between the control and experimental groups with regard to hatchability, although the highest value of hatchability from fertilized eggs was determined in the group injected with 6 mg of vitamin C on the 15th day of incubation. On the other hand, in ducks, significant differences were found between the control and experimental groups (4 mg of vitamin C administered on days 12 and 20 and 8 mg of vitamin C injected on day 20 of incubation) regarding hatchability. The value of this trait was higher in the group of eggs injected with ascorbic acid in comparison with the eggs which were not treated. On average, the difference amounted to 32.5 percentage points. Similarly, in the case of the number of dead embryos and unhatched chicks, better results were observed in the above-mentioned experimental groups. In summary, vitamin C injected into chicken eggs failed to influence hatchability. In the case of duck eggs, it was demonstrated that their injection on the 20th day of incubation with selected doses of vitamin C (4 and 8 mg/egg) improved hatchability by decreasing the proportions of dead and unhatched embryos.

Key words: Vitamin C, *in ovo* injection, hatchability, broiler breeders, Peking duck.

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The majority of investigations performed on poultry are carried out on birds during the period of rearing or reproduction and their main concern is most frequently focused on the improvement of rearing or health conditions, optimisation of nutrition etc., so as to achieve better production results. On the other hand, recent advances in technology and techniques as well as the possibilities associated with egg micromanipulation have opened up completely new areas of experimentation on birds which have not hatched, i.e. on embryos. Very frequently, such experiments document the impact of various biologically important compounds (e.g. amino acids and their derivatives, hormones or antibiotics) on the development and biochemical changes of embryos, hatchability, as well as growth and development of chicks after hatching (HENRY & BURKE 1999; MCREYNOLDS *et al.* 2000; HEIBLUM *et al*. 2001; OHTA & KIDD 2001; ZHAI *et al*. 2008; TANGARA *et al*. 2010). Although the methods of egg or embryo injection in the course of embryogenesis require considerable sterility and precision, they have also found a practical application. A good example in this area is the INOVOJEC® system employed in prenatal chick vaccination (MARSH *et al*. 1997).

A number of studies have been conducted in which researchers analysed the impact of *in ovo* administration of various vitamins on hatchability. For instance, it was found that the injection of pantothenic and folic acids into turkey eggs failed to exert a significant effect on chick hatchability (ROBEL 1993 a, b). On the other hand, vitamin C injected into chicken eggs was demonstrated to have a favourable impact on hatchability results, embryo weight at different incubation days and, consequently, on chick body weight after hatching as well as on the reduction of embryo death during incubation (ZAKARIA & AL-ANEZI 1996; ZAKARIA & AL-LATIF 1998; ZAKARIA *et al*. 1998).

Ascorbic acid does not exist in a freshly laid egg and it appears only on the 3rd/4th day of incubation

as a result of endogenous biosynthesis by the developing embryo. However, the manufactured quantities may not be sufficient in conditions of artificial incubation, in particular towards the end of the incubation period when the embryo is most exposed to overheating. This refers, primarily, to ducks and geese but it is also observed during the incubation of broiler chickens. Waterfowl has particularly large vitamin C requirements and therefore is more sensitive to its scarcity. For example one-day old ducklings derived from ducks fed vitamin C supplemented diets had higher numbers of erythrocytes, haemoglobin content and hematocrit value than control group (KONTECKA *et al.* 2006). Hence, *in ovo* administration of vitamin C in the course of embryogenesis may reduce the effects of overheating (thermal stress) and, consequently, improve hatchability.

The objective of this study was to determine the effect of different doses of vitamin C injected into eggs at selected dates during the embryogenesis of broilers and ducks on hatchability results.

Material and Methods

Two experiments were designed in which eggs of the following poultry species were applied: Ross 308 broiler breeders (1500 eggs) and Pekin ducks (1200 eggs) from intensive rearing systems. Three hatchings were conducted for the examined bird species (500 and 400 eggs in each for broiler breeders and ducks, respectively). In the case of chickens egg incubation was carried out in a single-stage Jamesway Company hatching machine, whereas duck eggs were incubated in a Petersime Company apparatus. The incubation process was conducted in accordance with the commonly accepted procedures applying optimal hatching parameters for individual bird species.

Procedures

Broiler breeders

For each hatching, on the 7th day of incubation after candling, 294 eggs of broiler breeders with live embryos were selected and 1 control group (I) and 6 experimental groups (II-VII) with 42 eggs each were established. Through a hole in the eggshell, 3 or 6 mg of vitamin C dissolved in 0.1 ml of physiological saline were injected into the air cell depending on the group: on day 13 of incubation – groups II and III, on day 15 – groups IV and V and on day 17 – groups VI and VII.

Ducks

For each hatching, on the 10th day of incubation after candling, 275 duck eggs with live embryos were selected and arranged in the following groups of 55 eggs: control – I and 4 experimental groups (II-V). Through a hole in the eggshell, 4 or 8 mg of vitamin C dissolved in 0.1 ml of physiological saline were injected into the air cell depending on the group: on day 12 of incubation – groups II and III and on day 20 of incubation – groups IV and V.

In ovo injection

Prior to drilling a hole in the eggshell using a microdrill with a prothetic sleeve, eggs from the experimental groups were candled in order to recognize the outline of the air cell. Next, the site where the hole was to be made was thoroughly disinfected (75 % ethyl alcohol) and swabbed with a small amount of antibiotic (Gentamicin - 80 mg/2ml). After puncturing the eggshell, a freshly prepared solution of crystalline vitamin C (*acidum ascorbicum* – Polfa Kraków) was gently injected into the air cell with the assistance of a micropipette. Then the hole was sealed with liquid paraffin and eggs were placed back in the incubator.

We consulted a previous study (SEEMAN 1991) for the selection of vitamin C doses according to egg size and the amount of ascorbic acid endogenously produced by chicks in pre- and postnatal life. The term of injection of vitamin C was chosen deliberately in the middle and final periods of incubation, when embryo overheating can be expected.

Data recording

Chicks were removed at 21 and 28 days of incubation for broiler breeders and ducks, respectively. Healthy and crippled chicks were counted. All remaining eggs were examined and classified as eggs with unhatched chicks and dead embryos. Hatchability was measured as the number of healthy chicks hatched as a percentage of injected eggs with living embryos.

Statistical analysis

The obtained results were examined statistically using the SAS v. 9.1 statistical package. The significance of differences between groups regarding hatchability results was verified by two-way ANOVA and statistical differences were established at the level of $P \leq 0.05$.

Results

Table 1 presents the effects of the introduction of vitamin C into chicken eggs. It is clear from these results that no statistically significant differences were observed between the control group and experimental groups with regard to hatchability from fertilized eggs, although the value of this trait was found to be the highest in the group of eggs injected with 6 mg of vitamin C on the 15th day of incubation, whereas eggs which were injected with 3 and 6 mg of ascorbic acid on the 17th day of incubation showed the lowest values for this trait (97.1 against 90.3 %). There were no significant differences between control and experimental groups in the case of the number of dead embryos and unhatched chicks. However, eggs injected with various doses of vitamin C on the 17th day of incubation as well as eggs treated with 3 mg of ascorbic acid on the 15th day of incubation were characterised by the highest value of this trait, on average amounting to 9.5 %. No crippled chicks were found in any of the groups.

The results of duck egg injections with vitamin C are shown in Table 2. Significant differences were

Table 1

Effect of *in ovo* injection of vitamin C on hatchability results in broiler breeders

Group*		Hatchability from fertilized eggs (%)	Dead embryos and unhatched chicks (%)
I – control	mean	95.9 abc	4.1 ab
	SEM	2.02	2.27
II – 3 mg vitamin C (13th day)	mean	93.8 abc	6.2 ab
	SEM	1.65	2.17
III – 6 mg vitamin C (13th day)	mean	95.6 ab	4.4 ab
	SEM	1.74	1.76
IV – 3 mg vitamin C (15th day)	mean	91.0 bc	9.0 ab
	SEM	2.16	3.45
V – 6 mg vitamin C (15th day)	mean	97.1 a	2.9 b
	SEM	1.51	1.52
VI – 3 mg vitamin C (17th day)	mean	89.7 c	10.3 a
	SEM	4.35	5.41
VII – 6 mg vitamin C (17th day)	mean	90.9 bc	9.1 ab
	SEM	2.81	3.9

Mean values designated in columns with different letters differ significantly at the level $P \leq 0.05$.
*(n = 126 eggs in each group).

Table 2

Effect of *in ovo* injection of vitamin C on hatchability results in Pekin ducks

Group*		Hatchability from fertilized eggs (%)	Dead embryos and unhatched chicks (%)	Crippled chicks (%)
I – control	mean	37.4 c	60.9 a	1.7 a
	SEM	6.24	4.59	1.67
II – 4 mg vitamin C (12th day)	mean	65.2 ab	34.8 bc	0.0 a
	SEM	8.94	8.9	–
III – 8 mg vitamin C (12th day)	mean	47.9 bc	50.4 ab	1.7 a
	SEM	6.95	5.44	1.67
IV – 4 mg vitamin C (20th day)	mean	69.8 ab	30.2 bc	0.0 a
	SEM	10.24	10.21	–
V – 8 mg vitamin C (20th day)	mean	74.6 a	25.4 c	0.0 a
	SEM	7.44	6.45	–

Mean values designated in columns with different letters differ significantly at the level $P \leq 0.05$.

*(n = 165 eggs in each group).

observed in the proportion of chicks hatched from fertilized eggs between the control group and experimental groups II and IV (4 mg of vitamin C applied on days 12 and 20 of incubation) and group V (8 mg of vitamin C applied on day 20 of incubation). The value of this trait was higher in experimental groups in comparison with the control and this difference, on average, amounted to 32.5 %. Similarly, better results were recorded in the above-mentioned experimental groups in the case of the number of dead embryos and unhatched chicks (30.1 against 60.9 %). On the other hand, there were no differences between groups with respect to the proportion of crippled chicks.

Discussion

Our investigation failed to demonstrate a significant impact of the *in ovo* introduction of vitamin C on the hatchability of chickens from fertilized eggs, although the best results were recorded following the injection of 6 mg of ascorbic acid on days 13 and 15 of incubation. On the other hand, ZAKARIA and AL-ANEZI (1996) observed the best hatchability results for chickens when 3 mg/egg of vitamin C were injected on day 15 of incubation. The difference between this group and the control was statistically significant and reached 7.5%. Similarly, in the case of the proportion of dead chicks as well as chicks culled after hatching, better ($\bar{x} = 0.7$ %) results than in the control group ($\bar{x} = 3.8$ %) were obtained when eggs were injected with 3 mg of ascorbic acid on the 15th day of incubation. However, this was not statistically confirmed. A favourable influence on hatchability of injection of 3 mg/egg of vitamin C into broiler breeder eggs on day 13 of incubation was also reported by IPEK *et al*. (2004). Contrary to the reports of ZAKARIA and AL-ANEZI (1996), in our experiments, the highest percentage of dead embryos and unhatched chicks was observed in the group in which 3 mg/egg of vitamin C were injected both on days 15 and 17 of incubation ($\bar{x} = 9.7$ %). On the other hand, the results of trials performed by the above researchers as well as by ZAKARIA and AL-LATIF (1998) showed that premature and excessively high doses of ascorbic acid introduced *in ovo* can lead to the deterioration of chick hatchability. For instance, it was found that when 3 mg/egg of vitamin C was injected on day 7 of incubation and 12 mg/egg – on day 15, hatchability was worse in comparison with the control group (by 19.3 and 38.3 %, respectively). The above-mentioned researchers put forward the hypothesis that the most optimal time for the introduction of vitamin C into chicken eggs with the aim of improving hatchability is the period between the 11th and 15th day of incubation. This hypothesis was partially confirmed by the results of our investigation.

Hatchability results for ducks can vary greatly (from 20 to over 90 %) and depend on many factors including age of the flock, environmental and incubation conditions (GOHER *et al*. 1983; BRAUN *et al*. 2002; EL-HANOUN & MOSSAD 2008). Low hatchability results for duck eggs in the control group in our experiment could have been caused by the fact that eggs used for the experiments were laid in June (at the end of the reproductive period) at a time of high air temperatures which may have also affected the biological value of the eggs. For example, KOKOSZYŃSKI *et al*. (2007) showed that the value of Haugh units and yolk quality deteriorated at the end of the first laying period of ducks. According to MUAMBI *et al*. and SERGEEV (after RÓŻYCKA & WĘŻYK 1985), a significant improvement in hatchability depends on, among other factors, the quality of the egg content which includes the white and yolk. KONTECKA *et al*. (2005) found that reproduction ducks fed diets supplemented with 500 mg vitamin C per 1 kg complete diet were characterised by better hatching of ducklings from set and fertilized eggs in comparison with the control group. It is quite possible that these results could be attributed to the anti-stress action of vitamin C during the final period of reproduction. On the other hand NOWACZEWSKI and KONTECKA (2005) showed a decreasing trend in the number of hatched crippled chicks in comparison to the control in pheasants fed a complete diet supplemented with vitamin C.

In the case of duck eggs, a favourable influence of the introduction of ascorbic acid on hatchability during incubation was demonstrated. The greatest improvement in hatchability was achieved when 6 mg/egg of vitamin C were injected into eggs on the 20th day of incubation, i.e. about 8 days before hatching. GHONIM *et al*. (2009) carried out a trial on Muscovy ducks in which they analysed the impact of the introduction of 3 mg ascorbic acid on day 14 of egg incubation. These researchers demonstrated a statistically significant improvement in hatchability following treatment with vitamin C in comparison with the control group (71.6 against 65.8 %). A similar, significantly favourable impact of the introduction of the above-mentioned dose of ascorbic acid in eggs of Muscovy ducks was demonstrated by the proportion of dead embryos. The value of this trait in the control and experimental groups was determined at 34.2 and 28.4 %, respectively. A positive influence of ascorbic acid injection into duck eggs (Domayti) was also observed by Tag El-Din *et al*. (after GHONIM *et al*. 2009). They introduced *in ovo* 3 mg/egg vitamin C directly before placing the eggs in the incubator (day 0) and observed improvement of hatchability in this group in comparison with the control. The advantageous impact of ascorbic acid

injected into duck eggs is attributed to its modifying influence on adrenal gland metabolism inhibiting the synthesis of 21-hydroxylase and 11-beta hydroxylase, i.e. enzymes which take active part in corticosterone production (TULLET 1990). This hormone, in turn, is quite active in gluconeogenesis, supporting the production of energy during the period of adaptation to new conditions (thermal stress, especially during the later period of incubation). However, if the level of this hormone in blood is excessively high for too long, it may exert a cytotoxic effect on the organism reducing its health condition (deteriorating hatchability) and, during the post-natal period, production results.

On the basis of the performed investigations, it is not possible to conclude unequivocally that vitamin C injected into broiler breeder eggs on the selected dates of incubation exerted a favourable impact on hatchability. In the case of duck eggs, it was demonstrated that injection on the 20th day of incubation of particular doses of vitamin C (4 and 8 mg/egg) improved hatchability and, hence, the proportion of dead embryos and unhatched chicks. This may indicate insufficient endogenous synthesis of ascorbic acid by duck embryos accompanied by a simultaneous, increased demand of these birds for vitamin C, especially towards the final period of incubation, when there is a risk of thermal stress.

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