Indian Journal of Animal Sciences **83** (10): 1053–1057, October 2013/Article

Effects of ascorbic acid immersion of eggs on some hatching properties in quail*

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Received: 23 January 2013; Accepted: 27 June 2013

ABSTRACT

This study was undertaken to determine the effects of immersion of hatching quail *(Coturnix coturnix japonica)* eggs into L-ascorbic acid solution on egg white pH, embryonic deaths, and survival of chicks. A total of 5,429 eggs in 9 groups were used. In the first group, immersion into ascorbic acid solution was carried out at the beginning of incubation (BI) (5g/1 min, 5 g/2 min, 10 g/1 min, 10 g/2 min) whereas it was done during incubation (DI) (5g/1 min, 5 g/2 min, 10 g/1 min, 10 g/2 min) in the second group. The third group was used as control group with no treatment. Results indicated that ascorbic acid immersion at BI and DI significantly affected early and middle term embryonic death rates while it was ineffective on late embryonic death rate. pH values measures on day 5 (early term) in BI groups (5g/2 min, 10g/2 min, control) were 8.41, 8.51 and 8.10, respectively, indicating a positive effect of ascorbic acid on egg white pH. Six-week survival of hatched-chicks in BI 10 g/1 min and DI 10g/1 min groups were significantly higher. These results indicated that immersing hatching quail eggs into ascorbic acid solutions may be useful for improving the survival of chicks and for reducing embryonic dead.

Key words: Ascorbic acid, Embryonic mortality, Japanese quail, pH, Viability

People should fulfill 35–40% of their daily protein need from animal proteins for a balanced and healthy nutrition (Seker 2006). As a source of animal protein cattle, sheep, goat, poultry husbandry, and alternative poultry production are commonly practiced (Roenig 1999). The quail has a higher growth rate compared to other poultry species. In quail production, main targets are achieving high fertility rate and the best hatching performance for which optimal conditions should be provided (Seker *et al.* 2004b). Information about factors causing hatching problems and the way they affect these factors is essential for solving problems related to hatching performance (Copur 2004). Attempts were made to improve poor hatching value of eggs by using various chemicals but mainly on hen eggs, and ascorbic acid was commonly used for this purpose (Copur 2004).

Main problems are early, mid-term and late embryonic deaths, poor hatching rate and poor survival of chicks. With increase in temperature loss of CO_2 increases during incubation. As a result of CO_2 loss, pH of egg albumin increases (Burley and Vadehra 1989). In addition to handling hatchery eggs in different ways and with different chemicals, other ways to resolve these problems were investigated.

To increase hatching yield vitamin C (ascorbic acid) was tried by different means and in various amounts (Ingram *et al.* 1997, Shafey 2002, Ipek *et al.* 2004). Ascorbic acid is the acid of choice for this purpose as it is — a weak acid, has higher interaction with egg shell (Shafey 2002), water-soluble and has an antioxidant property. In poultry it can be produced by body cells as well as can be taken via foods. However, animals those are under stress and are high-yielders need additional vitamin C sources (Konca and Yazgan 2002). In order to eliminate the hatchability adverse conditions, vitamin C can be applied by direct immersion to the solutions containing ascorbic acid, or by intra-oval injection (embrex, in ovo) (Elibol *et al.* 2001). Objective of this study was to investigate the effect of ascorbic acid on pH of egg white, embryonic deaths, and survival of chicks in quail eggs.

MATERIALS AND METHODS

This study was conducted in the quail breeding unit of the Department of Zootechny at the Faculty of Veterinary Medicine at Firat University. In the present study fertile eggs were used. The fertile eggs were obtained from the Japanese quails that were 10–12 weeks old and housed 1 male-3 female in 20 × 20 × 25 cm wire cages under a lighting regime of 8 h dark-16 hr light. The eggs were obtained from those laid between 9: 00 AM and 12: 00 AM. In the present study 5, 429 hatching eggs were used.

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The hatching eggs were weighed using a scale with 0.01 g sensitivity and those between 10–11 g were used in the treatment groups. Until 6 weeks of age chicks were fed with concentrated feed containing 20% crude protein and 2800 kcal/kg metabolic energy (Soares *et al.* 2003). The environment temperature of the cages housing hatching chicks were fixed at 26–27 °C. The chicks were grown until 6 weeks of age at 95 cm × 55 cm × 22 cm cages. Two egg sets were used for this study. L-ascorbic acid was used in the study.

Treatment groups were group 1 (immersion on day 1 of incubation), groups 2 (immersion of day 10) and group 3 (control). Group 1 was assigned as initial immersion group and 4 different subgroups were set up depending on the immersion time and amount of ascorbic acid in the immersion solution. These were 5 g/1 min, 5 g/2 min, 10 g/1 min, 10 g/2 min. Group 2 was assigned as immersion during incubation and the same subgroups were set up. Group 3 was control group and no treatment was applied to the eggs.

Treatment subgroups are listed below:

Group 1: Beginning of incubation (day 1) L-ascorbic acid 5g/1 lt dH_2O/1 min

Group 2: Beginning of incubation L-ascorbic acid 5g/1 lt dH_2O/2 min

Group 3: Beginning of incubation L-ascorbic acid 10g/1 lt dH_2O/1 min

Group 4: Beginning of incubation L-ascorbic acid 10g/1 lt dH_2O/2 min

Group 5: During incubation (day 10) L-ascorbic acid 5g/1 lt dH_2O/1 min

Group 6: During incubation L-ascorbic acid 5g/1 lt dH_2O/2 min

Group 7: During incubation L-ascorbic acid 10g/1 lt dH_2O/1 min

Group 8: During incubation L-ascorbic acid 10g/1 lt dH_2O/2 min

Group 9: Control (No chemical was applied)

Immersion procedure was carried out in a dark room to protect ascorbic acid from light and at room temperature. Ascorbic acid was resolved in distilled water freshly before each use and care was taken to immerse eggs completely in the solution during the immersion period. The eggs were taken out from the immersion solution and placed on an egg cartoons for drying prior to placing in the incubator.

For pH measurements in both groups, 30 eggs were randomly selected on each on day 5 (early period), day 10 (middle period) and day 12–13 (late period). The eggs were broken and the pH of the egg albumin was measured by direct immersion of the pH probe. Likewise, 30 eggs in control group were broken before incubation and pH of egg albumins were measured. With all these pH measurements, whether use of ascorbic acid caused any change in egg white pH was determined.

The resulting data at the end of the study were used to evaluate the effect of immersion, immersion content and immersion time on the hatching outputs (early, middle and late embryonic deaths) and survival rate within the 6 weeks of age. Survival rate was calculated using the below equation (Konca and Yazgan 2002).

Survival within 6 weeks of age (%) = (Numbers of live animals/ numbers of animals per replicate) ×100

The data obtained about the hatching properties were analyzed using SPSS 11.5 (2002) software. The data were analyzed using Logistical Regression Analysis to determine the effect of solution content and immersion time on the hatching properties. For comparison of pH values between the groups, variance analysis followed by Duncan test was used while Chi-Square test was used for analysis of survival data.

RESULTS AND DISCUSSION

Compared to control group, early embryonic death rates increased 3.88 times in BI 5g/1 min group, 2.97 times in DI

Table 1. Embryonic deaths (%) in quail eggs immersed in ascorbic acid solution and results of logistic regression analysis

Groups	Embryo deaths							Logistic analysis					
								Early term		Middle term		Late term	
	Numbers of eggs	Early Term	Middle Term	Late Term	Early Term %	Middle Term %	Late Term %	P value	Odds	P value	Odds rate	P value	Odds rate
BI 5 g/1min	578	10	20	56	1.73	3.46	9.68	0.000	3.88	0.031	1.85	0.841	9.61
BI 5 g/2 min	505	21	15	34	4.15	2.97	6.73	0.104	1.57	0.014	2.17	0.118	1.42
BI 10 g/1 min	526	25	30	46	4.75	5.70	8.74	0.236	1.37	0.713	1.09	0.730	1.07
BI 10 g/2 min	570	22	24	79	3.85	4.21	13.85	0.054	1.70	0.127	1.51	0.017	0.64
DI 5 g/1 min	668	15	15	50	2.24	2.24	7.48	0.000	2.97	0.001	2.89	0.238	1.27
DI 5 g/2 min	668	7	10	49	1.04	1.49	7.33	0.000	6.45	0.000	4.37	0.200	1.30
DI10 g/1 min	668	25	30	50	3.74	4.49	7.48	0.033	1.75	0.174	1.41	0.238	1.27
DI10 g/2 min	668	19	39	75	2.99	5.83	11.22	0.003	2.33	0.773	1.07	0.277	0.81
Control	578	37	36	54	6.40	6.22	9.34						

BI, Beginning of incubation; DI, during incubation.

5g/1min, 6.45 times in DI 5g/2min group, 1.75 times in DI 10g/1min group, and 2.33 times in DI 10g/2 min group (Table 1). These increases were significant (P<0.05, P<0.01 and P<0.001).

Middle term embryonic death rates also increased significantly (P<0.05, P<0.01 and P<0.001) compared to control groups; 1.85 times in BI 5g/1 min group, 2.17 times in BI 5g/2min group, 2.89 times in DI 5g/1min group, and 4.37 times in DI 5g/2min group.

Compared to control group, late-embryonic death rates significantly (P<0.05) increased (0.64 times) in B. I 10g/2 min group. The differences between control group and other treatment groups were not significant (P>0.05).

Egg albumin pH (Table 2) in BI was significantly affected from treating with ascorbic acid. Immersion in ascorbic acid resulted in a significant increase (P<0.001) in the pH of egg whites compared to control group. In DI day 10, there were a significant difference in pH between the groups (P<0.001). pH values in DI day 12–13 (last period) were found as 7.66 in 10g/1min group, 7.65 in 10 g/2 min group and 7.30 in control group and the differences were significant (P<0.001).

Compared to the control group, survival rates increased 2.12 times in BI 10 g/1 min and 1.57 times in DI 10g/1 min (Table 3) and these increases were significant (P<0.01, P<0.001). The survival rates in other groups and control were not significant (P>0.05).

In the present study, early embryonic death rates in BI 5 g/1 min, DI 5 g/1 min, 5 g/2 min and 10 g/2 min groups differed significantly compared to control and other groups (P<0.01 and P<0.001). These differences showed a positive effect. For instance, early embryonic death rate in control groups was 6.40% while it was 1.04% in DI 5g/2 min group which was 6.45 times lower. There were differences between the groups treated with ascorbic acid. Results indicated that ascorbic acid treatment resulted in a decrease in early embryonic death rate. In addition, early embryonic death rates of the present study are similar to 1.50% reported by Shafey (2002) with ascorbic acid treated broiler eggs, 1.92% by Seker *et al.* (2004a) in no ascorbic acid eggs, 3.10% by Mani *et al.* (2008). However, our early embryonic death rates were lower than 17.77% reported by Khurshid *et al.* (2004), 20.25% by Farooq *et al.* (2001).

Table 2. pH values of egg whites in ascorbic acid treated quail eggs (n=30)

Groups	BI Day 5 (Early Term) $\bar{x} \pm S_{\bar{x}}$	Groups	DI Day 10 (Middle Term) $\bar{x} \pm S_{\bar{x}}$	DI Days 12-13 (Late Term) $\bar{x} \pm S_{\bar{x}}$
BI 5 g/1min	8.59^{ab}	DI 5 g/	7.98^{a}	7.46^{ab}
	±0.07	1 min	±0.07	±0.06
BI 5 g/2 min	8.41^{b}	DI 5 g/	7.78^{b}	7.52^{ab}
	±0.07	2 min	±0.08	±0.07
BI 10 g/1 min	8.59^{ab}	DI10 g/	7.72^{b}	7.66^{a}
	±0.11	1 min	±0.08	±0.13
BI 10 g/2 min	8.51^{b}	DI 10 g/	7.87^{ab}	7.65^{a}
	±0.10	2 min	±0.07	±0.11
Control	8.10^{c}	Control	7.70^{b}	7.30^{b}
	±0.13		±0.07	±0.11
P	***	P	***	***

***P<0.001. a,b,c, Means with the same superscripts are significantly different (P<0.001). BI, Beginning of incubation. DI, during incubation.

Table 3. Up to 6-week survival (%) of chicks hatched from quail eggs treated with ascorbic acid and the results of Logistic regression

Groups	Survival			Logistic regression		
	n	Hatched chicks	Surviving chicks	%	P value	Odds rate
BI 5 g/1min	578	370	281	75.94	0.237	1.223
BI 5 g/2 min	505	364	278	76.37	0.19	1.252
BI 10 g/1 min	526	343	290	84.54	0	2.119
BI 10 g/2 min	570	419	297	70.88	0.714	0.943
DI 5 g/1 min	668	455	341	74.94	0.36	1.159
DI 5 g/2 min	668	477	368	77.14	0.096	1.308
DI 10 g/1 min	668	455	365	80.21	0.007	1.571
DI 10 g/2 min	668	423	305	72.1	0.994	1.001
Control	578	351	253	72.07		

BI, Beginning of incubation; DI, during incubation.

Middle term embryonic death rates found in DI 5g/1min and 2 min was significantly different at various levels (P<0.01 and P<0.001) from control group and other treatment groups. This difference was positive. For instance, mid-term embryonic death rate in control groups was 6.22% while it was 1.49% in DI 5g/2 min group indicating a lot lower rate. It was found that different periods of immersion in ascorbic acid solution caused a decrease in mid-term embryonic death rates. When Mid-term embryonic death rates (Table 1) are compared to those reported by other researchers on no ascorbic acid treated eggs, it was found that our findings were similar to 1.60% reported by Elibol *et al.* (2001), 1.92 by Seker (2006), 2.25% by Altan *et al.* (1995) while they were lower from 18.80% reported by Mani *et al.* (2008).

Another positive effect of ascorbic acid was seen on late embryonic death rate. Late embryonic death rate found in BI 5g/1min group increased 0.64 times compared to control group and this increase was significant (P<0.01 and P<0.001). The differences between other groups were not significant (P>0.05). Similarly, our results indicated that ascorbic acid application resulted in a lower late-term embryonic death rate. Zakaria *et al.* (1998) and Shafey (2002) reported that they found a higher late embryonic death rate in ascorbic acid treated broiler eggs than those in control eggs. Late embryonic death rates found in this study are higher than 3.10% by Mani *et al.* (2008), 3.60% by Farooq *et al.* (2001), 5.11% by Ipek *et al.* (2004) similar to 6.90% by Elibol *et al.*

(2001) and 14.63% by Khurshid *et al.* (2004).

It is thought that these results could be explained by anti-stress effect of ascorbic acid as well as its CO_2 lowering effect. Similar approaches are supported by some reports (Zakaria *et al.* 1998, Elibol *et al.* 2001, Ipek *et al.* 2004).

The egg white pH values was 7.98 in DI day 10 5g/1 min group and 7.87 in 10g/2 min group. These values are similar to those of 8.50 in non-ascorbic acid treated eggs by Brake and Pardeu (1998), 8.51 by Shafey (2002) in ascorbic acid-treated broiler eggs, 8.55 by Silversides and Budgell (2004) in non-ascorbic acid treated hen eggs, 8.56 by Benton and Brake (2000), 8.65 by Lapao *et al.* (1999) and 9.05 by Ahn *et al.* (1999).

In general, it was reported that decrease of egg white pH in ascorbic acid treated eggs during incubation was an advantage (Romanoff 1944, Shafey 2002).

Another positive effect of ascorbic acid application in the current study was on survival rate. Survival rate until 6 weeks of age in BI 10g/1min and DI 10g/1min groups were significantly ($P<0.01$, $P<0.001$) different from those found in control and other groups. For example, survival rate of control group was 72.07% while it was 84.54% in BI 10g/1 min group. Our findings about survival rate was similar to 70.88% by Testik *et al.* (1993), 72% by Chidananda *et al.* (1986) and 76.88% by Cerit and Altinel (1998).

When considered with pH, the increase of survival rate can be a sign of the effect of ascorbic acid solution.

In summary, immersing hatching eggs into ascorbic acid solution resulted in superior findings in terms of egg white pH, embryonic death rate, and survival rates compared to control group. Therefore, ascorbic acid may have positive effect on hatching parameters.

REFERENCES

Ahn D U, Sell J L, Jo C, Chamruspollert M and Jeffrey M. 1999. Effect of dietary conjugated linoleic acid on the quality characteristics of chicken eggs during refrigerated storage. *Poultry Science* **78**: 922–28.

Altan O, Oguz I and Settar P. 1995. Effects of egg weight and specific gravity of the hatchability characteristics in Japanese quail. *Turkish Journal of Agriculture and Forestry* **19**: 219–22.

Benton C E and Brake J. 2000. Effects of atmospheric ammonia on albumen height and pH of fresh broiler breeder eggs. *Poultry Science* **79**: 1562–65.

Brake J and Pardeu S L. 1998. Role of ascorbic acid in poultry nutrition. *Proceedings 10th European Poultry Conference.* Pp. 63–67.

Burley R W and Vadehra D V. 1989. The egg shell and shell membranes: properties and synthesis. *The Avian Egg: Chemistry and Biology*. John Wiley and Sons Inc. USA

Cerit H and Altinel A. 1998. Genetic and phenotypic parameters of various traits in the Japanese quail (Coturnix coturnix japonica). *Journal of the Faculty of Veterinary Medicine Istanbul University* **24**: 111–36.

Chidananda B L Prathapkumar K S, Sreenwasaiah P V, Lokanath G R and Ramappa B S. 1986. Comparative performance of Japanese quail reared in cages and deep litter. 1. Body weight, feed efficiency and mortality. *Journal of Poultry Science* **20**: 162–4.

Copur G. 2004. Problems in hatchery of parent-stock breeding. *Journal of Animal Production* **45**: 31–5.

Elibol O, Turkoglu M, Akan M and Erol H. 2001. Effects of ascorbic acid injection during incubation on the hatchability of large broiler eggs. *Turkish Journal of Veterinary and Animal Sciences* **25**: 245–48.

Farooq M, Aneela K, Durrani FR, Muqarrab AK, Chand N and Khurshid A. 2001. Egg and shell weight, hatching and production performance of Japanese broiler quails. *Poultry Science* **17**: 289–93.

Ingram D R, Deao C E, Floyd S A and Pittman S T. 1997. Effects of in-ovo injection of ascorbic acid on broiler hatchability and body weight. *Poultry Science 86th Annual Meeting* Abstract **76**, Supll 1.

Ipek A, Sahan U and Yilmaz B. 2004. The effect of in ovo ascorbic acid and glucose injection in broiler breeder eggs on hatchability and chick weight. *Archiv Fur Geflugelkunde* **68**: 132–35.

Khurshid A, Farooq M, Durrani F R, Sabiland K and Mazoor A. 2004. Hatching performance of Japanese quails. *Livestock Research for Rural Development* **16**: 1–5.

Konca Y and Yazgan O. 2002. Heat stress and vitamin C in laying hens. *Journal of Animal Production* **43**: 16–25.

Lapao C, Gama L T and Chaveiro M S. 1999. Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability. *Poultry Science* **78**: 640–45.

Mani A U, Garndawa I I and Usman B A. 2008. Effects of pre-incubation storage on the hatchability of quail (*Coturnix coturnix japonica*) eggs in the Sahel region of Nigeria. *International Poultry Science* **7**: 350–54.

Roenig W P. 1999. World Poultry Consumption Symposium: Muscle growth and development keynote address. *Poultry Science* **78**: 722–28.

Seker I. 2006. The effects of the different factors on fertility and hatchability of hatching eggs in quails. *Journal of the Faculty of Veterinary Medicine Yuzuncu Yil University* **14**: 42–46.

Seker I, Kul S and Bayraktar M. 2004a. Effects of parental age and hatching egg weight of Japanese quails on hatchability and chick weight. *International Journal of Poultry Science* **3**: 259–65.

Seker I, Kul S, Bayraktar M, Ekmen F and Yildirim O. 2004b. Effects of egg storage period and parental age on hatchability results in hatching eggs in Japanese Quail (Coturnix coturnix japonica). *Journal of the Faculty of Veterinary Medicine Uludag University* **23**: 59–64.

Shafey T M. 2002. Eggshell conductance, embryonic growth, hatchability and embryonic mortality of broiler breeder eggs dipped into ascorbic acid solution. *British Poultry Science* **43**: 135–40.

Silversides F G and Budgell K. 2004. The relationships among measures of egg albumen height, pH and whipping volume. *Poultry Science* **83**: 1619–23.

SPSS. 2002. *SPSS for Windows Release* 11.5. Standard Version. Copyright SPSS Inc.

Soares R da T R N, Fonseca J B, Santos A S de O dos and Mercandante M B. 2003. Protein requirement of Japanese quail (Coturnix coturnix japonica) during rearing and laying periods. *Revista Brasileira de Ciência Avícola* **5**: 153–6.

Testik A, Uluocak N and Sarica M. 1993. Some production characteristics of Japanese Quails (*Coturnix coturnix japonica*) in different genotypes. *Turkish Journal of Veterinary and Animal Sciences* **17**: 167–73.

Zakaria A H, Latif A A and Al-Anezi M A. 1998. Effect of ascorbic acid on embryonic development, hatch time and growth of extended delayed placement of broiler chickens. *Archiv Fur Geflugelkunde* **62**: 11–5.