Indian Journal of Animal Sciences **84** (1): 18–21, January 2014/Article

In ovo injection effect of sodium nitrite on hatchability, day-old chick quality, organ weights and body antioxidant status of newly hatched chicks

ELMIRA MOHAMADI[1], MOHSEN DANESHYAR[2] and FARHAD FAROKHI ARDABILI[3]

Urmia University, Urmia, Iran

Received: 17 April 2013; Accepted: 29 September 2013

ABSTRACT

This experiment was conducted to investigate the effect of sodium nitrite injection per egg on day 7 of incubation on chick quality, hatchability, chick weights and some blood antioxidant metabolites in chicken embryo. Fertile eggs (308) were injected by 4 different levels of 0, 0.042, 0.084 and 0.168 mg per egg sodium nitrite at day 7 of incubation. At hatch, no effect of sodium nitrite injection was indicated on hatchability and mortality, chick quality and organ weights. However chick weight of sodium nitrite injected eggs were lower than those of control chicks. No significant differences were observed between the treatments for activity of blood superoxide dismutase and glutathione peroxidase. Blood malondialdehyde content of chicks from 0.084 mg sodium nitrite injected eggs was greater than control and 0.042 mg sodium nitrite injected ones. Furthermore, lower blood total antioxidant capacity was observed for chicks of 0.042 and 0.168 mg sodium nitrite injected eggs as compared to those of control eggs. It was concluded that *In ovo* injection of sodium nitrite does not exert negative effects on 1–day chick quality, hatchability and mortality, but it decreased the both chick weights and blood antioxidant status.

Key words: Blood metabolites, Chick quality, Malondialdehyde, Nitrite, Total antioxidant capacity

Nitrate, a naturally occurring ion and part of the nitrogen cycle, is implicated in methemoglobinemia and a number of currently inconclusive health problems (Gupta *et al*. 2000). In blood, nitrite undergoes a coupled oxidation reaction with oxyhemoglobin to produce methemoglobin. Unlike hemoglobin, methemoglobin cannot exchange oxygen. Increased levels of blood nitrite and methemoglobin increase these deleterious components subsequently in the fetuses (Gruener *et al*. 1973). Chromosome mutation, developmental problem and even fetus mortality have been reported by nitrite toxicity and its related methemoglobinemia in hamster (Inui *et al*. 1979), cattle (Winter and Hokanson 1964) and heifers (Page *et al.* 1990). Although there is some information regarding the negative effects of nitrite on offspring of most animals, no information is available about its effect on chicken. So this experiment was conducted to investigate the effect of 0, 0.042, 0.084 and 0.168 mg sodium nitrite injection/egg on day 7 of incubation on chick quality, hatchability, chick weights and some blood antioxidant metabolites in chicken embryo.

Present address: [1]M Sc Student (mohammadi766@yahoo.com), [2]Assistant Professor (m.daneshyar@urmia.ac.ir), [3]Asistant Professor (f.farrokhi@urmia.ac.ir), Department of Animal Science, Faculty of Agriculture.

MATERIALS AND METHODS

Fertile eggs (308, average weight of 55.91 g) from the same broiler breeder flock were put in an incubator and incubated under standard conditions (temperature of 37.7ºC and relative humidity of 65% for incubator and temperature of 37.2°C and relative humidity of 75% for hatchery). The maximum contaminant limit (MCL) for nitrite in drinking water is 3 mg/liter on the basis global standards, if suppose daily water consumption is of 3 liter for a pregnant woman (average body weight 80 kg), the nitrite MCL equals to 0.11 mg/kg. By using these human calculations for an egg (with average egg weight of 55 g), nitrite MCL of 0.006 mg was obtained. The nitrite contribution to sodium nitrite is 67% and thus 0.009 mg sodium nitrite per egg is needed. For 9 days, 0.081 mg was calculated. Hence 4 levels of 0.0, 0.042, 0.084 and 0.168 mg of sodium nitrite were injected to the eggs of each group in this experiment. At day 7 of incubation, eggs were randomly divided into 4 groups, each containing 77 eggs: distillated water injected eggs as a solvent served (zero nitrite, ZN), 0.042 mg sodium nitrite injected eggs (low nitrite, LN), and 0.084 mg sodium nitrite injected eggs (medium nitrite, MN) and 0.168 mg sodium nitrite injected eggs (high nitrite, HN). The eggs were injected with 100 µl of distillated water (containing no,

0.042, 0.084 and 0.168 mg sodium nitrite) by a syringe (insulin syringe, 1 cc) going into the air sac. Before injection, the top of the eggs was sterilized with 70% ethanol, and a small hole was made with a needle of 22 G. After injection, the holes were sealed up with a lacquer and returned to the incubator afterwards (Daneshyar *et al*. 2010). Day 7 of incubation was chosen as a suitable injection time, because this time is a critical developmental stage of the embryo (Daneshyar *et al*. 2010, Willier 1954). Sodium nitrite was injected in the air cell, since the embryo lies between the yolk and air cell at that moment. On day 18 of incubation, eggs were candled and those with evidence of living embryos were transferred to hatchery baskets. At hatch (day 21), unhatched eggs were opened to identify the infertile and dead embryos. Dead embryos were classified as early (those that died before injection) and late dead embryos (those that died after injection). Age of mortality was determined according to Hamburger-Hamilton Criteria (Hamburger and Hamilton 1951). Hatching percentage was calculated as number of hatched chicks to fertile eggs multiplied by 100. At hatch, 30 chicks from each group were used for chick quality parameters assessment according a standard method (Tona *et al.* 2003). Furthermore, all chicks were weighed at hatch and 10 chicks of each group were randomly selected and sacrificed by decapitation to take the blood samples. Moreover all of these scarified birds were dissected and liver and heart weights were determined. Blood samples were collected into anticoagulant tubes (EDTA). Half of these blood samples were centrifuged at 5000 rpm for 5 minute to collection the plasma samples. These plasma samples were stored at –20 °C along with another half of the blood samples until further analysis. The blood samples were used for determination of hematocrit, and glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities. The plasma samples were used for determination of total cholesterol, triglyceride, malondialdehyde (MDA), total protein and total antioxidant capacity (TAC) content. Blood SOD enzyme activity was determined by spectrophotometric kit and blood glutathione peroxidase (GPX) activity by spectrophotometric kit. The MDA concentration was measured using MDA reaction with thiobarbituric acid followed by extraction with butanol. The experiment was approved in the Department of Animal Science, Faculty of Agriculture, Urmia University. The data were subjected to GLM procedure of SAS statistical package based a completely randomized design. The Duncan multiple comparison test was used to comparison of means at significance level of 0.05. Data at significance level of 0.05 was used to assess the normality of the distribution of chick quality and organs weight. Normalized data in the case of abnormality were used for statistical analysis. Chi-square test was used for comparison of hatchability, dead embryos and infertile eggs of different groups.

RESULTS AND DISCUSSION

In ovo injection effect of sodium nitrite on hatchability and dead embryos is depicted in Table 1. None of the sodium nitrite levels (0, 0.042, 0.084 and 0.168 mg) affected the hatchability and the number of dead embryos (P>0.05). There was no difference in occurrence of severe anomalies between chicks of the different treatment as shown by chick's quality parameters in Table 2. Moreover no significant differences indicated between the treatments for quality parameters (chicks with score 100, average score and average score of chicks <100) (P>0.05). The eggs (Table 3) with the same weights were used in recent experiment (P>0.05). Moreover there were no significant differences between the treatments

Table 1. Hatchability and embryo mortality of injected eggs before (dead 7) or after injection (dead upwards) with 0 (ZN), 0.042 (LN), 0.084 (MN) and 0.168 mg per egg (HN) sodium nitrite

	ZN	LN	MN	HN
Hatchability	71/75 *(94%)	69/75* (92%)	65/71* (91%)	67/72* (93%)
Dead 7	2	1	3	2
Dead upward	2	5	3	2

* The number of sound eggs (from 77 fertile eggs put in the incubation for each group) after removing the cracked eggs due to injection on day 7 of incubation.

Table 2. Effect of different sodium nitrite levels of 0 (ZN), 0.042 (LN), 0.084 (MN) and 0.168 mg per egg (HN) on chick quality score

Quality parameters	ZN	LN	MN	HN
Chicks with score 100	28/30	23/30	17/30	20/30
Average score (n=30)	99.13	98.4	96.2	98.4
Average score of chicks with score <100	87	93.14	84.15	95.2

Table 3. Effect of different sodium nitrite levels of 0 (ZN), 0.042 (LN), 0.084 (MN) and 0.168 mg per egg (HN) on egg weight, chick weight, liver and heart weights at hatch

Treatment	Egg weight (g)	Chick weight (g)	Liver weight (g)	Heart weight (g)
ZN	56.22	41.42 [a]	2.08	1.39
LN	54.93	39.91 [b]	2.08	1.37
MN	56.00	40.35 [b]	2.10	1.39
HN	56.50	40.10 [b]	2.07	1.38
Pooled SEM	0.39	0.16	0.01	0.01
P-value	0.50	0.004	0.95	0.91
N vs control P-value	0.37	0.0004	0.88	0.81

Mean values with different letters at the same column differ significantly (P < 0.05).

for liver and heart weights at hatch (P>0.05). But chick weight affected by nitrite injection and all the sodium nitrite injected chicks had lower body weights at hatch (P<0.05). *In ovo* injection of 0.042, 0.084 and 0.168 mg sodium nitrite at day 7 of incubation negatively did influence the day-old chick weight.

This is the first research regarding the negative effects of nitrite on chicken embryo and no report has been reported in this area already. Consistent with our results on hatchability of embryo mortality, no effects of water nitrite on hatching or embryo viability have been observed in crab (*Cyprinus carpio* L, Kroupova *et al*. 2010). Negative effects of nitrite were reported in other animals. Experimental data on reproductive toxicity of both nitrite and nitrate were reviewed (Fan *et al.* 1987) and no evidence of teratogenic effects was reported but the possible induced abortion effect of nitrates and nitrites indicated in experimental animals (Fan *et al*. 1996). Orally administration of 0.5 mg/day sodium nitrite to pregnant mice at beginning the first day of gestation and continuing up to the 14th, 16th, or 18th day of gestation have shown no significant changes on litter size, gross anatomical defects, weight, number of resorption sites, proportion of fetal deaths and the occurrence of skeletal abnormalities (Globus and Samuel 1978) but an increase in the production of red blood cells in offspring of treated mice has been indicated that related to methemoglobinemia in the fetus by nitrite. In hamsters, administration 500 mg/kg sodium nitrite on 11th or 12th day of pregnancy caused the mutation (Inui *et al*. 1979).

In addition, we observed higher blood MDA and lower TAC in chicks of sodium nitrite injected eggs especially at higher doses (Table 4). There were no significant differences between the treatments for blood activity of GPX and SOD (P>0.05). Sodium nitrite injection affected the blood MDA and TAC contents (P<0.05). At hatch MN chicks had the higher blood MDA as compared to ZN and LN ones (P<0.05). Although blood MDA content of HN birds was numerically higher than those of ZN and LN ones, it was not significant. Blood TAC of LN and HN chicks was lower than that of ZN chicks (P<0.05). However it was not significant, MN chicks had numerically lower blood TAC than ZN ones. In orthogonal comparisons, sodium nitrite injection decreased the blood TAC compared to control (P<0.05). This phenomenon is possibly due to hypoxia which has happened in embryos of sodium nitrite injected eggs. Hypoxia and ischemia are associated with increased free radical production and membrane lipid peroxidation and suggesting the potential contributor of oxidative stress to the cellular injury associated with hypoxia and ischemia (Horakova *et al*. 1998). Studies showed that intermittent hypoxia in rats increases the oxidative stress and deficits the spatial learning (Row *et al.* 2002). Nitrite in toxic doses induces the hemic hypoxia, which is mainly due to higher methemoglobin formation (Gladwin *et al*. 2004, Ger *et al*. 1996). Increased levels of blood methemoglobin and nitrite in rat and subsequently in the fetuses have reported by consumption of 2.5–50 mg/kg sodium nitrite in drinking water or through intraperitoneal injection (Gruener *et al*. 1973). In the other experiment on rat increased lipid peroxidation (measured by MDA formation) in abdominal aortic wall tissue, brain tissue, and serum during chronic hypoxia (maintained at 15% O_2 for 2 wk) were indicated (Yoshikawa *et al*. 1982). In other experiment on adaptation to high-altitude hypoxia, an increase in lipid hydroperoxide of rat heart was recorded (Kagan 1988). Furthermore, the increased lipid peroxidation and depleted intracellular GSH contents of the brain, heart and liver of sodium nitrite (60 mg/kg) fed rats (Padmaja *et al.* 1998). Peroxide production was reported in the other ischemic condition. Higher MDA production in brain and blood of stroke-prone spontaneously hypertensive rats (for which evidence of regional ischemia were present) were reported (Meerson *et al*. 1982). Also increases in content MDA in experimentally induced canine heart infarcts and in ischemic rat heart (Meerson *et al.* 1982).

Table 4. Effect of different sodium nitrite levels of 0 (ZN), 0.042 (LN), 0.084 (MN) and 0.168 mg per egg (HN) on blood total antioxidant capacity (TAC), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities of newly hatched chicks

Treatment	TAC (mmol/L)	MDA (nmol/Ml)	SOD (U/gr Hb)	GPX (U/gr Hb)
ZN	0.74 [a]	51.52	1516.6	2.00[b]
LN	0.60[bc]	51.45	1617.8	1.67 [b]
MN	0.70[ab]	51.56	1449.4	3.90[a]
HN	0.57 [c]	50.20	1577.9	2.80[ab]
Pooled SEM	0.02	1.36	47.52	0.28
P-value	0.02	0.98	0.67	0.01
N vs Control P-value	0.01	0.90	0.80	0.13

Mean values with different letters at the same column differ significantly (P < 0.05).

Based on the results of recent experiment, injection of nitrite sodium to air sac of fertile eggs does not change the one-day chick quality, hatchability and mortality. But it induces the hypoxia, diminishes the body TAC and increases the body MDA, and hence cause the decreased newly chick weight.

ACKNOWLEDGEMENTS

The authors appreciate their thank to Mr Amir Mansor Vatankhah (Department of Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran) for his helps in determination of blood antioxidants indices.

REFERENCES

Daneshyar M, Geuns M C J, Buyse G J, Willemsen H, Decuypere

E, Everaert N, Kermanshahi H and Ansari Z. 2010. Evaluation of steviol injection on chicken embryos: Effects on post-hatch development, proportional organ weights, plasma thyroid hormons and metabolites. *Journal of Poultry Science* **47**: 71–76.

Fan A M, Willhite C C and Book S A. 1987. Evaluation of the nitrate drinking water standard with reference to infant methemoglobinemia and potential reproductive toxicity. *Regulatory Toxicology and Pharmacolology* **7**: 135–48.

Fan A M and Steinberg V E. 1996. Health implications of nitrate and nitrite in drinking water: an update on methemoglobinemia occurrence and reproductive and developmental toxicity. *Regulatory Toxicology and Pharmacolology* **23**: 35–43.

Gladwin M T, Crawford J H and Patel R P. 2004. The biochemistry of nitric oxide, nitrite, and hemoglobin: role in blood flow regulation. *Free Radical Biology and Medicine* **36**: 707–17.

Ger J, Kao H, Shih T S and Deng J F. 1996. Fatal toxic methemoglobinemia due to occupational exposure to methyl nitrite. *Clinical Medicine Journal* **57**: S78.

Globus M and Samuel D. 1978. Effect of maternally administered sodium nitrite on hepatic erythropoiesis in fetal CD–1 mice. *Teratology* **18**: 367–78.

Gruener N, Shuval H I, Behroozi K, Cohen S and Shechte R H. 1973. Methemoglobinemia induced by trans placental passage of nitrites in rats. *Bulletin Environmental Contamination and Toxicology* **9**: 44–48.

Gupta R C, Gupta A B, Seth A K, Bassin J K and Gupta A. 2000. Recurrent acute respiratory infections in areas with high nitrate concentrations in drinking water. *Environmental Health Perspectives* **108**: 363–66.

Hamburger V and Hamilton H L. 1951. A series of normal stages in the ability development of the chick embryo. *Journal of Morphology* **88**: 49–92.

Hill M J. 1999. Nitrate toxicity: myth or reality? *British Journal of Nutrition* **81**: 343–44.

Horakova L, Stole S, Chromikova Z, Pekarova A and Derkova L. 1998. Mechanisms of hippocampal reoxygenation injury. *Molecular and Chemical Neuropathology* **33**: 223–36.

Inui N, Nishi Y, Taketomi M and Mori M. 1979. Trans placentalaction of sodium nitrite on embryonic cells of Syrian golden hamster. *Mutatation Research* **66**: 149–58.

Kagan V E. 1988. *Lipid Peroxidation in Biomembranes*. Boca. Raton, FL, CRC.

Kroupova H, Prokes M, Macova S, Penaz M, Barus V, Novotny L and Machova J. 2010. Effect of nitrite on early-life stages of common crap (*Cyprinus carpio* L.). *Environmental Toxicology Chemistry* **29**: 535–40.

Meerson F Z, Kagan V E, Kozlov Y P, Belkina L M and Arkhipenko Y V. 1982. The role of lipid peroxidation in pathogenesis of ischemic damage and the antioxidant protection of the heart. *Basic Research in Cardiology* **77**: 465–85.

Padmaja S, Squadrito G L and Pryor W A. 1998. Inactivation of glutathione peroxidase by peroxy nitrite. *Archives of Biochemistry and Biophysics* **349**: 1–6.

Page R D, Gilson W D, Guthri E D, Mertens D R and Hatch R C. 1990. Serum progesterone and milk production and composition in dairy cows fed two concentrations of nitrate. *Veterinary and Human Toxicolology* **32**: 27–31.

Row B W, Kheirandish L, Neville J J and Gozal D. 2002. Impaired spatial learning and hyper activity in developing rats exposed to intermittent hypoxia. *Pediatric Research* **52**: 449–53.

SAS Institute. 2002. *SAS Users Guide: Statistics*. SAS Institute Inc., Cary, NC.

Tona K, Bamelis F, De Ketelaere B, Bruggeman V, Moraes V M B, Buyse J, Onagbesan O, Winter A J and Hokanson J F. 1964. Effects of long-term feeding of nitrate, nitrite, or hydroxylamine on pregnant dairy heifers. *American Journal of Veterinary Research* **25**: 353–61.

Willier B H. 1954. Phases in embryonic development. *Journal of Cellular and Comparative Physiology* **43**: 307–17.

Yoshikawa T Y, Furukawa S, Wakamatsu H, Takemura H and Kondo M. 1982. Experimental hypoxia and lipid peroxidation in rats. *Biochemical Medicine* **27**: 207–13.