

Influence of Sex and Dietary Ascorbic Acid on Nitrite-Induced¹ Methemoglobinemia in Japanese Quail (34646)

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(Introduced by M. L. Scott)

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Increased attention is being given to the potential ecological hazards associated with high nitrate concentrations in food sources and water supplies. The toxicity of nitrates in animals and man is primarily due to its reduction to nitrite prior to, or during, ingestion with the accompanying conversion of hemoglobin into methemoglobin (1). Methemoglobinemia is the condition in which the ferrous ion of hemoglobin is oxidized to ferric ion, thereby impairing the capacity of the blood to transport oxygen (1).

Sell *et al.* (2) observed reduced feed consumption and increased methemoglobin concentrations of the blood within 8 hr after feeding male chicks diets containing 0.4% nitrite.

The study reported here was designed to determine the effects of dietary nitrite and ascorbic acid upon the formation of methemoglobin in adult male and female Japanese quail (*Coturnix coturnix* Japonica) and upon nitrite deposition in the egg. Injection, or in some instances, oral administration of vitamin C has been shown to reduce methemoglobin levels (3). The Japanese quail was chosen as the experimental animal for use in these studies because of their limited space requirements and their relatively rapid maturation.

Experimental Methods. Japanese quail were obtained from the Department of Poultry Science, Cornell University and were placed in a commercial brood unit at one day of age. They were divided into 4 groups with 13 quail chicks in each group and were fed a purified diet consisting of: glucose,

53.89; isolated soybean protein, 30.00; corn oil, 5.00; cellulose, 3.00; DL-methionine, 0.75; glycine, 0.30; and required vitamins and minerals, 7.06 (4). At 3 weeks of age, 0.5% L-ascorbic acid was added at the expense of glucose in the diets of two of the groups. Since female Japanese quail begin to lay eggs at about 5 weeks of age, the dietary calcium was then increased to 2.4% substituted for glucose in all groups. At 15 weeks of age, one group of quail fed the ascorbic acid and one group fed the basal diet without the additional dietary ascorbic acid were given 0.5% dietary nitrite as NaNO₂ for 1 week.

Methemoglobin and hemoglobin concentrations of blood obtained by heart puncture from 4 to 160 hr after the nitrite regimen commenced were determined by the modified (5) method of Evelyn and Malloy (6). Egg nitrite deposition was determined by the method developed by Diven *et al.* (7).

Results. When nitrite was fed for 1 week to the 15-week-old Japanese quail, body weights of both males and females were depressed (Fig. 1). Food intake was concomi-

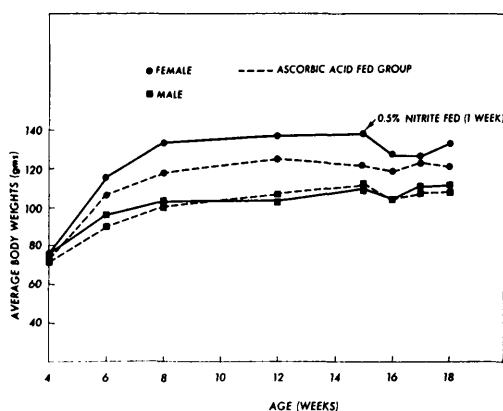


FIG. 1. Growth curves of male and female Japanese quail fed ascorbic acid and nitrite.

¹ Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1741.

TABLE I. Hemoglobin and Methemoglobin Concentration of Male and Female Japanese Quail as Influenced by Dietary Ascorbic Acid and Nitrite.

Sex	Ascorbic ^a acid fed	Nitrite ^a fed	Hemoglobin ^b	Methemoglobin ^b	
			(g/100 ml)	(g/100 ml)	(% of hemoglobin)
Male	0	0	13.28 ± 0.89 ^c	0.51 ± 0.09	4.15 ± 0.27 (5) ^d
	0	+	11.28 ± 0.81	2.09 ± 0.38	15.16 ± 2.99 (6)
	+	0	12.23 ± 0.30	0.52 ± 0.03	4.27 ± 0.26 (5)
	+	+	12.13 ± 0.94	1.60 ± 0.41	13.17 ± 2.89 (7)
Female	0	0	10.75 ± 1.48	1.03 ± 0.03	10.07 ± 0.79 (3)
	0	+	10.26 ± 0.49	2.68 ± 0.80	26.54 ± 7.86 (6)
	+	0	10.36 ± 0.18	1.54 ± 0.20	14.80 ± 1.81 (5)
	+	+	9.90 ± 0.41	1.89 ± 0.26	19.33 ± 2.79 (5)

^a Ascorbic acid added to diets (+) at 3 weeks of age. Nitrite fed for 7 days at 15 weeks of age.

^b Values obtained during week of nitrite regimen.

^c Mean ± SE.

^d Number of birds determined in each group.

tantly depressed from a daily intake of 16–17 g/birds to 11–12 g. Quail not fed the nitrite regimen maintained their weight with an average of 2–5-g increase during this period.

The blood hemoglobin and methemoglobin values of male and female quail fed ascorbic acid and nitrite, and those of the controls, are presented in Table I. Hemoglobin values for females were lower and the methemoglobin blood levels were higher than those in the male quail. Analysis of variance of the data show that the methemoglobin values of either control or nitrite fed females were significantly ($p < 0.05$) increased, as percentage of the total hemoglobin content, compared to the male quail fed the same diet. Without nitrite feeding, female quail exhibited a twofold increase of blood methemoglobin compared to males. When nitrite was added to the diet, a significant increased ($p < 0.05$) level of blood methemoglobin occurred in all of the animals. This level remained relatively constant from 4 to 160 hr after initiation of nitrite intake. Dietary ascorbic acid had no significant ($p > 0.05$) effect on blood methemoglobin in either sex regardless of nitrite intake.

Nitrite first appeared in the quail eggs about 10 hr after nitrite feeding started and continued at reduced levels up to 140 hr after nitrite intake ceased. Nitrites (as μg nitrite-nitrogen/g of egg) in control eggs averaged

0.29 ± 0.02 as compared to 0.33 ± 0.06 in eggs produced from quail hens fed ascorbic acid. Ascorbic acid has no significant effect on nitrite deposition in eggs of nitrite fed quail.

Discussion. Methemoglobin is normally present in the blood of animals and man (1). Von Issekutz (8) observed that cats normally have a methemoglobin concentration ranging between 4 and 17% of the total hemoglobin level in the blood. These levels are quite similar to the range of methemoglobin values of the Japanese quail in this study, dependent on the sex, not treated with nitrite. Sex hormones have a marked effect on methemoglobinemia, since there was approximately a twofold increase in methemoglobin blood levels of the female quail as compared with the similarly treated males. The observation of Tanaka and Rosenberg (9) that androgens increase hemoglobin blood levels in chickens, offers a possible explanation for the higher blood hemoglobin levels in the male Japanese quail than in the females.

In agreement with Sell *et al.* (2), results using male chicks, nitrite intake reduced body weights and food consumption concurrently with the development of a methemoglobinemia in male and female Japanese quail. The methemoglobinemia together with nitrite deposition into eggs, were not affected by dietary ascorbic acid, although this vitamin has been used therapeutically for congen-

ital methemoglobinemia (3). These results are in agreement with Kilgore *et al.* (10) who reported that guinea pigs fed diets low in ascorbic acid did not show increased susceptibility to nitrite toxicosis.

Summary. Japanese quail fed diets containing 0.5% nitrite developed a methemoglobinemia together with reduced food consumption and depressed body weights. Nitrite also was deposited in eggs, and continued to be present for about 140 hr after nitrite feeding stopped. Female Japanese quail, either controls or nitrite treated, were observed to have less blood hemoglobin, with approximately a twofold increase in blood methemoglobin than males from the equivalent treatments. Ascorbic acid added at 0.5% to the diet had no effect on methemoglobinemia or egg nitrite deposition.

I am grateful to the Department of Poultry Science, Cornell University, Ithaca for the experimen-

tal animals, and to Mrs. Margaret Minute for her assistance in the laboratory.

1. Bodansky, O., *Pharmacol. Rev.* 3, 144 (1951).
2. Sell, J. L., Roberts, W. K., and Waddell, D. G., *Poultry Sci.* 42, 1474 (1963).
3. Carnrick, M., Polis, B. D., and Klein, T., *Arch. Intern. Med.* 78, 296 (1946).
4. Howes, J. R., and Beane, W. L., *Feedstuffs* 38, 18 (1966).
5. Hawk, P. B., Oser, B. L., and Summerson, W. H., "Practical Physiological Chemistry," 13 ed., p. 619. McGraw-Hill (Blakiston), New York (1954).
6. Evelyn, K. A., and Malloy, H. T., *J. Biol. Chem.* 126, 655 (1938).
7. Diven, R. H., Pistor, W. J., Reed, R. E., Trautman, R. J., and Watts, R. E., *Amer. J. Vet. Res.* 23, 497 (1962).
8. Von Issekutz, B., *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmacol.* 193, 567 (1939).
9. Tanaka, T., and Rosenberg, M. M., *Poultry Sci.* 34, 1429 (1955).
10. Kilgore, L., Stasch, A. R., and Barrentine, B. F., *Amer. J. Clin. Nutr.* 14, 52 (1964).

Received Oct. 9, 1969. P.S.E.B.M., 1970, Vol. 133.