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SHORT COMMUNICATIONS

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The Effect of Egg Yolk Sampling on Performance Parameters and Reproductive Indices of Northern Bobwhite Quail (*Colinus virginianus*) Eggs

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ABSTRACT: The sampling of 50 μl of the yolk (average 1.7% of the total volume) from northern bobwhite quail (*Colinus virginianus*) eggs on day 0, 6, 12, and 18 of incubation resulted in significantly ($P < 0.05$) increased embryo mortality in the day 12 and day 18 egg groups. Performance parameters and reproductive indices of the non-sampled eggs and the eggs sampled on day 0, and 6 of incubation did not differ from other reports on *C. virginianus*. No difference was found in postnatal responsiveness among hatchlings originating from controls and from all the groups of sampled eggs. As demonstrated in this representative galliform species, the technique can be applied for the screening of unembryonated eggs or early-embryogenesis-eggs of wild birds. If female birds are not accessible but their eggs are, information on the reproductive flock can be accessed without stressing adult birds.

Key words: Northern bobwhite quail, *Colinus virginianus*, egg yolk sampling, maternal antibodies.

In a variety of avian species, maternal antibodies (Ab) against a broad spectrum of pathogens are transferred prenatally to hatchlings via the egg yolk, as reviewed by Graczyk et al. (1994). Egg yolk and female parent serum Ab titers are strongly correlated (Brown et al., 1989). Because of this correlation, chicken egg yolk Ab titer was used to predict the Ab titer of the hens that laid these eggs (Brown et al., 1989). *Aspergillus* spp. Ab titer of Jackass penguin (*Spheniscus demersus*) females can be predicted based on the Ab titer of their egg yolk (Graczyk and Cranfield, 1995). Although the volume sufficient for serologic testing does not exceed 50 μl (Graczyk and Cranfield, 1995), crushing of an egg shell, and consequently egg loss, is commonly performed to obtain the yolk (Brown et al., 1989). If yolk sampling would not interfere with embryogenesis, the eggs of wild birds could be returned to the nest after sampling. This could provide serological information on bird Ab titer to the pathogens or on environmental toxicant residues in threatened or endangered birds without stressing the adults and without destroying the eggs. The yolk sample can be air-dried and stored on filter paper as it does not diminish Ab binding capacity (Graczyk et al., 1994; Graczyk and Cranfield, 1995). Our objective was to determine the effect of yolk sampling on performance parameters and reproductive indices of eggs from northern bobwhite quail (*Colinus virginianus*).

Fifty-five eggs of *C. virginianus* (flight-type) from Murray and McMurray Hatchery (Webster City, Iowa, USA) were randomly divided into five groups of 10 eggs each (group 1, 2, 3, 4, and 5) and one group of five eggs (group 6). The eggs of groups 1 to 5 were placed into an incubator with an automatic egg turner (one revolution/4 hr) (Turbofan Hova-Baton Incubator, GQF Co., Savannah, Georgia, USA) and incubated at 41 C and 95% to 97% relative humidity (Wilson et al., 1979). The eggs were weighed every 3 days and candled every day to determine fertility and embryo viability (Aboul-Ela et

TABLE 1. The effect of sampling of 50 µl of yolk from northern bobwhite quail (*Colinus virginianus*) eggs on egg (n = 10 per group) performance parameters and reproductive indices.

Egg performance parameters and reproductive indices	Control eggs (%)	Sampling incubation day for experimental eggs			
		0 (%)	6 (%)	12 (%)	18 (%)
Total mortality	30	30	30	70[a]	80[a]
Fertility	90	80	90	90	100
Total hatchability	60	50	60	0[a]	10[a]
Hatch of fertile eggs[b]	67	63	67	0[a]	10a
Viable 11-day embryos/number of eggs	80	80	70	70	90
Viable 21-day embryos/viable 14-day embryos[b]	100	100	100	100	75
Hatchlings/viable 21-day embryos[b]	75	63	86	0	33

[a] Chi-square test adjusted for small sample; $P < 0.05$.

[b] Sample sizes varied from eight to 10 eggs, since not all 10 eggs in each group were fertile.

al., 1992). Clear eggs or eggs with no viable embryos (opaque when candled) were broken out to determine if they were fertile or to determine a cause of embryo death, respectively. The embryos were examined for body or yolk-sac hemorrhages, and for body or yolk-sac deformations. On day 20 of incubation, the egg-turner was removed as to allow a non-assisted hatching. The chicks were transferred into a brooder, examined for responsiveness to maternal cues (McBride and Lickliter, 1994) and observed for social preferences (Banker and Lickliter, 1993). The eggs of group 1, 2, 3, and 4 were sampled on day 0, 6, 12, and 18 of incubation, respectively. Group 5 was not sampled and was used as a control. The width of an individual egg was measured (McGinnis et al., 1976), and 50 µl of the yolk was taken by inserting a 22-gauge needle up to a half egg width, attached to a 1.0 cm^3 syringe through the ventral midpoint egg shell aspect. The yolk was aspirated continuously after the needle reached a half of egg width. The insertion site was sealed with 20 µl of surgical glue (Nexaband, Veterinary Product Laboratories, Phoenix, Arizona, USA). The measure of reproductive indices followed the protocol of Piccirillo and Orlando (1985), and egg performance parameters were computed as described by Flunker et al. (1991). To determine the volume of sampled yolk compared to the total egg yolk volume, the eggs of group 6 which had been not incubated were frozen (−20 C) and opened longitudinally. The yolk was removed, cleaned of the chalaza, placed into a calibrated glass cylinder, thawed, and the volume was measured. A small sample-adjusted Chi-square test (Sokal and Rohlf, 1981) was used to asses the differences among the values of egg performance parameters.

The mean (±SE) egg weights varied from 8.8 g to 11.6 g; $\bar{x}$ = 10.4 ± 0.3 g. The values of all performance parameters of eggs sampled on day 0 and 6 of incubation were not significantly different (G = 1.16, $P > 0.05$) from those of controls (Table 1). Egg fertility varied insignificantly among the groups (G = 1.21, $P > 0.5$). The mean (±SE) values of reproductive indices for controls and the eggs sampled on day 0 and 6 of incubation were: 83 ± 3% for viable 11-day embryos per number of eggs; 96 ± 3% for viable 21-day embryos per viable 14-day embryos; and 77% ± 7% for hatchlings per viable 21-day embryos. The total embryo mortalities that occurred after sampling significantly increased for eggs sampled on day 12 and 18 of incubation, and consequently the total hatchability and the hatching of fertile eggs significantly decreased (Table 1). Mortality of embryos within 2 days after sampling was

0, 2, 5, and 2 for eggs probed on day 0, 6, 12 and 18 of incubation, respectively. Mortality of embryos that occurred at the end of incubation period was 2, 1, 2, and 6 for eggs probed on day 0, 6, 12 and 18 of incubation, respectively. One embryo died on day 16 of incubation (Group 1). Two embryos (Group 3) and one embryo (Group 4) died before sampling on day 9 and 10 of incubation, respectively. We could not determine the cause of death for the embryos which were viable for the entire incubation period but did not successfully hatch. Neither leakage of yolk from yolk-sac, body and yolk-sack deformation, nor hemorrhages were observed at necropsy. No differences in postnatal behavior to maternal cues (McBride and Lickliter, 1994) and social preferences (Banker and Lickliter, 1993) were observed between chicks hatched from the sampled eggs and the controls. The egg-yolk volume ranged from 2.41 ml to 3.16 ml, with a mean (±SE) of 2.90 ± 0.14. Thus, the 50 µl of sampled yolk constituted from 2.3% to 1.5%, with a mean (±SE) of 1.7% ± 0.1%.

The egg weight and size, and the incubation period were within the normal ranges reported for *C. virginianus* (McGinnis et al., 1976; Wilson et al., 1979). Performance parameters and reproductive indices of the controls and the eggs sampled on day 0, and 6 of incubation did not differ from the ranges reported by Piccirillo and Orlando (1985), Flunker et al. (1991), and Aboul-Ela et al. (1992). There was no difference in postnatal responsiveness to maternal cues and social preferences among hatchlings originating from controls and sampled eggs. The most apparent effect of yolk sampling was embryo mortality on the day after probing related to the difficulties of sampling the embryo yolk-sac and consequent embryo damage. At necropsy, these embryos had body and yolk-sack hemorrhages. We noted that sampling *C. virginianus* eggs after day 6 of incubation was associated with an increase of embryo mortality, and thus, should not be performed. However, this technique can be applied to freshly laid *C. virginianus* eggs or to eggs at the beginning of incubation. As shown recently (Graczyk and Cranfield, 1995), embryo sac Ab titer obtained by the sampling of embryonated eggs of Jackass penguins was not correlated with the Ab titer of the females that laid these eggs. Thus, the sampling of embryonated eggs has limited value for the assessment of the female Ab titer or immune status. We conclude that egg yolk sampling can be applied for the screening of unembryonated eggs of wild birds or to the eggs which are at the early stages of embryogenesis. This technique does not require egg loss and is rapid. The total procedure takes approximately 3 min for an experienced person. The eggs of *C. virginianus* are relatively small; thus the probing of larger eggs is expected to be easier, and the sampled yolk would represent a smaller fraction of the total egg-yolk volume. Although a single enzyme-linked immunosorbent assay (ELISA) can be performed with less than 50 µl, once collected, a sample can be screened for immunoresponses against a variety of pathogens. Yolk samples also can be used for the detection of environmental toxins. A 5 µl egg sample was sufficient to quantify by liquid gas chromatography the residues of organochlorine pesticides in chicken eggs from various geographical locations (Kahunyo et al., 1988). A 50 µl sample is sufficient to detect and quantify heavy metals and other inorganic compounds by inductively coupled plasma atomic emission spectrometry; a method applied to avocet (*Recurvirostra americana*) eggs (Fairbrother et al., 1994). The needle should be inserted up to a half egg width, thus it is not necessary to candle an egg before yolk collection. Sampling of eggs in the wild could generate a potential hazard by introducing a pathogen through the egg shell; thus, the use of sterile needle and surgical glue to seal the egg shell is recommended.

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