RELATIONSHIP AMONG PLASMA TRIGLYCERIDES, BODY MASS, AND REPRODUCTION OF NORTHERN BOBWHITES

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ABSTRACT

The earliest potential initiation of northern bobwhite (*Colinus virginianus*) reproduction is limited by photoperiod. Secondary factors such as lipid reserves, diet, and stress often limit the beginning of northern bobwhite reproduction, potentially reducing reproductive success and causing a shorter reproductive season. We measured late winter body masses and plasma triglycerides of wild northern bobwhites and subsequent reproductive timing and effort in 1997 and 1998 on the coastal prairie of Texas. Using body mass and plasma triglyceride levels as indices of body fat, we tested the hypothesis that the onset of reproduction and first clutch size was influenced by late winter lipid reserves. Northern bobwhite plasma triglycerides were higher ($P < 0.001$) and more variable ($P = 0.019$) in 1998, and nesting began 15 ± 1.6 days ($\chi \pm$ SE) earlier than in 1997. However, within each year, no combination of body mass and triglycerides was associated with timing of nesting or size of first clutch ($P > 0.1$). In addition, body masses were not correlated with plasma triglycerides ($P > 0.1$). Our findings suggest that individual plasma triglyceride levels and body mass are unsuitable variables for assessing within-population differences in reproductive timing. However, mean plasma triglycerides for a population may be useful for assessing differences in reproductive timing among years and locations. The relationship between triglycerides and hormones directly affecting gonadal recrudescence, such as luteinizing hormone (LH) and prolactin, is uncertain for wild northern bobwhites. Thus, future studies should assess causes and patterns of change in these hormones.

INTRODUCTION

The initiation of reproduction in birds is controlled by the endocrine system (Bahr and Johnson 1991). Photoperiod has an overriding influence on reproduction in many species, including northern bobwhite (Kirkpatrick and Leopold 1952), by regulating hypothalamic secretions of follicle stimulating hormone (FSH) and LH (Farner and Follett 1979, Carey 1996). After minimum photoperiod is available to birds, secondary factors may influence hormone levels and determine when individuals begin to reproduce. Physiological condition, diet, and stress are 3 interrelated secondary factors influencing northern bobwhite reproduction. For example, water deprivation in northern bobwhites decreases serum progesterone, delays or prevents egg production, decreases clutch size, and causes smaller eggs (Cain and Lien 1985, Koerth and Guthery 1991, Giuliano et al. 1995). Insufficient dietary energy increases serum corticosterone and decreases northern bobwhite egg production (Giuliano et al. 1996). Lower body mass in late winter may delay the onset of egg laying in pheasants (*Phasianus colchicus*), probably due to levels of stress and reproductive hormones (Gates and Woehler 1968). Physiological condition, diet, and stress are probably strongly correlated among wild birds at any particular time and place because these factors are largely dependent on environmental conditions. However, differences in condition, diet, and stress within a population may explain within-year variability in the timing of reproduction and reproductive effort.

Late winter lipid reserves probably influence future reproduction (Gates and Woehler 1968), although lipid reserves are less important to reproduction of grouses and presumably other galliforms as compared to waterfowl (Thomas 1988). Lipid reserves could increase size of the first clutch by allowing a female to maintain and recruit more follicles for ovulation, largely through the action of FSH (Carey 1996). Lipid reserves are correlated with northern bobwhite body masses during winter (Robel 1972, Frawley et al. 1999), and with both body masses and plasma triglycerides when measured in other avians (Bacon et al. 1989, Dabbert et al. 1997). Thus, late winter body masses and plasma triglycerides may explain within-year variability of reproductive timing and first clutch sizes. We tested the hypothesis that the onset of reproduction and first clutch size of northern bobwhites was influenced by late winter lipid reserves, using body masses and plasma triglyceride levels as indices of body fat.

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METHODS

Female northern bobwhites were captured from 1 February through 23 April in 1997 and 1998 primarily with walk-in funnel traps (Smith et al. 1981) baited with milo. All hens were weighed, marked with an aluminum legband (National Band and Tag Co., Newport, Kentucky), radiomarked (American Wildlife Enterprises, Montacello, Florida), bled, and released. Each hen was bled by pricking the ulnar or brachial vein with a 25-gauge needle and collecting blood into heparinized capillary tubes. Capillary tubes were sealed with clay, transported to a field lab, and centrifuged. Tubes were then scored with a diamond-tipped pen and broken immediately above the white blood cell layer. Plasma was pipetted into cryovials, frozen initially at $-20°C$ and then $-84°C$, and shipped on dry ice to a commercial laboratory (Veterinary Associates Laboratory, Edmond, Oklahoma) for measurement of triglycerides using a Technicon RA-1000® clinical chemistry analyzer (Bayer Corporation, Pittsburgh, Pennsylvania). Hemolytic and lipemic samples were discarded due to potential measurement errors (Alleman 1990).

From late April until mid-July, most radiomarked hens were located at least once every other day to find nests. Each bird was approached on foot until it flushed or moved away, was observed, or had been circled by the researcher, indicating that it might be on a nest. Two sets of stake flags were placed at a distance of 5 and 10 m from the nest, oriented so that each set was aligned with the nest. The position of the nest could be determined on subsequent visits by sighting down both sets of stake flags and determining the point where the 2 lines intersected. This marking method was used so that predators investigating the stake flags were not drawn directly to the nest. Hens were never intentionally flushed from nests and rarely flushed inadvertently. To confirm the nest location and clutch size, the nest site was checked at times when the hen might be away from the nest feeding. When the nest was unoccupied, the number of eggs was recorded. The date that eggs in a nest began to be incubated was estimated by averaging the last date that a hen was not found on a nest and the first date that a hen was found incubating eggs on a nest. The date that nesting began was estimated by subtracting the number of eggs in a nest from the estimated date that incubation of eggs began.

Data Analysis

The value of late-winter body mass and plasma triglycerides as predictors of first clutch size and date of first nest initiation was assessed using multiple linear regression. Body masses and plasma triglycerides were recorded from birds captured 6–16 February 1997 and 4 February–7 March 1998. Records for 5 nests initiated >30 days after the earliest nests each year were censored because it was suspected that the initial nests for these birds were not detected. First nests would probably not have been detected if they

Table 1. First clutch sizes, dates of nest initiation, late winter body masses (g), and plasma triglyceride levels (mg/dL) of northern bobwhites in 1997 ($n = 17$) and 1998 ($n = 23$), Refugio County, Texas.

Variable	1997		1998	
	$\bar{x}$	SE	$\bar{x}$	SE
First clutch size	15.7	0.3	15.2	0.4
Date of nest initiation	3 May	1.9	18 April	1.4
Body mass	167.7	2.6	161.1	1.9
Plasma triglyceride	122.2	9.6	250.1	21.0

were depredated before incubation began or if the male incubated the eggs.

Residuals were tested for normality using the Shapiro–Wilk test (Shapiro and Wilk 1965). Homoscedasticity and linearity were assessed by examining plots of residual and predicted values. Analyses were conducted using SPSS for Windows, release 6.0 (Norušis 1993). Statistical significance was determined with $\propto$ = 0.05. Means are reported ± 1 SE.

RESULTS

Body masses, plasma triglyceride levels, dates of nest initiation, and sizes of first clutches were obtained for 17 northern bobwhite hens in 1997 and 23 in 1998 (Table 1). Northern bobwhites began nesting 15 ± 1.6 days earlier in 1998 ($t_{38} = 6.70$, $P < 0.001$). Clutch sizes did not differ between years ($t_{38} = 0.86$, $P = 0.394$). The mean and variance of plasma triglycerides were higher in 1998 (mean: t-test for unequal variances, $t_{30} = -5.53$, $P < 0.001$; variance: Levene's [1960] test, $F_{1,38} = 5.99$, $P = 0.019$). Body masses were lower in 1998 (t-test for equal variances, $t_{38} = 2.09$, $P = 0.043$). Plasma triglycerides were not correlated with body mass either year (1997: $R^2 = 0.01$, $F_{1,15} = 0.20$, $P = 0.660$; 1998: $R^2 = 0.12$, $F_{1,21} = 2.87$, $P = 0.105$; Fig. 1).

Tests for relationships among variables were conducted separately for each year because birds began reproducing earlier in 1998. No combination of body mass and triglycerides successfully predicted date of nest initiation or first clutch size (Table 2). Assumptions of normality and homoscedasticity were violated for analyses of clutch size in 1998 due to a clutch of 22 eggs. The analysis was repeated with this record removed with identical results.

DISCUSSION

Late winter body mass and plasma triglycerides were not related to date of nest initiation or first clutch size either year. This contradicts patterns found in turkeys (*Meleagris gallopavo*) (Badyaev et al. 1996), where body mass in February–March and nest initiation date were the best predictors of clutch size of first nests. However, in the turkey study (Badyaev et al. 1996), the partial r for female body mass was 0.10. Thus, turkey body mass accounted for only an additional 1% of the variation in clutch sizes given the
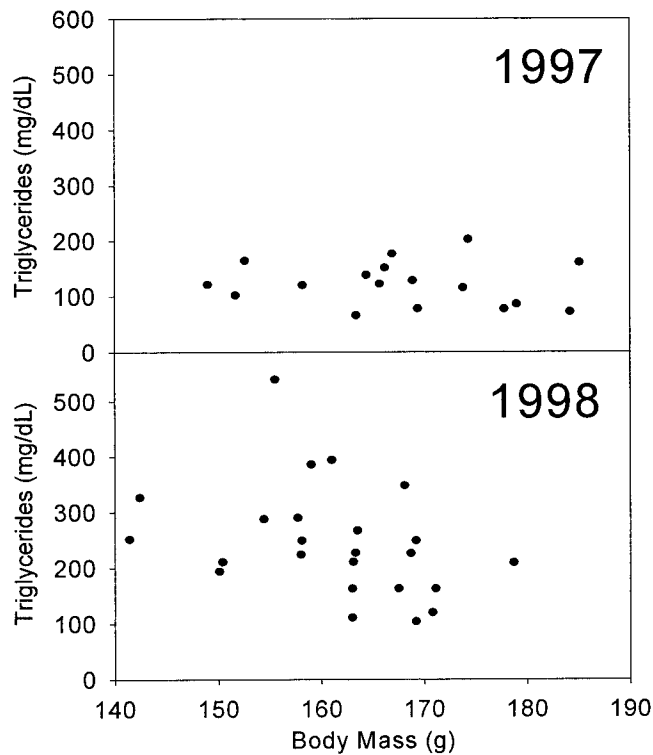
Fig. 1. Lack of linear relation between plasma triglycerides and body mass of northern bobwhites in 1997 and 1998, Refugio County, Texas (1997: $R^2 = 0 = 0.01$, $F_{1,15} = 0.20$, $P = 0.660$; 1998: $R^2 = 0.12$, $F_{1,21} = 2.87$, $P = 0.105$).

Table 2. R^2 and significance of late winter body mass (BM) and plasma triglycerides (TRIG) as predictors of first clutch size and date of nest initiation for northern bobwhites in Refugio County, Texas, 1997.

Dependent variable	Year	Independent variables	R^2	P
First clutch size	1997	BM + TRIG	0.19	0.235
		BM	0.12	0.172
		TRIG	0.09	0.249
	1998	BM + TRIG	0.03	0.752
		BM	0.00	0.970
		TRIG	0.03	0.466
Date of nest initiation	1997	BM + TRIG	0.03	0.794
		BM	0.02	0.582
		TRIG	0.02	0.634
	1998	BM + TRIG	0.08	0.414
		BM	0.03	0.463
		TRIG	0.03	0.435

other variable in their model. If body mass influenced clutch size this weakly in our study, we could not have detected it. Both studies support earlier conclusions that galliform reproduction relies very little on lipid reserves (Thomas 1988).

In 1997, plasma triglyceride levels from our study were similar to winter plasma triglyceride levels reported for northern bobwhites housed in outdoor pens and fed a commercial gamebird feed *ad libitum* (Hill and Murray 1987). However, triglyceride levels in 1998 were twice as high and twice as variable. We examined the relationship between sampling date and plasma triglycerides in 1998 to determine if our sampling interval extended into the beginning of the breeding season when triglycerides increase 3- to 6-fold (Hill and Murray 1987). If this occurred, we predicted that plasma triglycerides would increase during the 31-day sampling interval in 1998. However, we found no linear relationship between plasma triglycerides and sampling date ($F_{1,21} = 0.5$, $P = 0.507$). We suspected that differences in sampling locations also may have caused the observed annual differences. In 1997, 13 of 17 samples were obtained from birds captured within a 1-km radius. In 1998, samples were obtained from 8 locations separated by >2 km, and the largest number of samples from any single location was 7. To test whether the differences in the means and variability of plasma triglycerides were due to location, we compared the samples collected in 1997 from a single location with 4 samples collected from that location in 1998. Surprisingly, the means and

trends for 1997 and 1998 of plasma triglyceride levels and body masses from this single location were nearly identical to that of all locations. Thus, we do not know why triglyceride levels were so much greater and variable in 1998.

Higher plasma triglycerides in 1998 were associated with earlier reproduction. However, we found no relationship between individual plasma triglyceride levels and date of reproduction. We suspect that northern bobwhites in our study were differentially affected by numerous extrinsic factors, such as differences in diet caused by habitat quality and disturbance by predators. In addition, some extrinsic factors probably affected all of the birds to nearly the same extent within a given year; these effects would include winter severity and precipitation. Our results suggest that plasma triglycerides may be useful as an indicator of population trends in timing of reproduction, but not for predicting reproductive timing of individuals.

Body mass and plasma triglycerides were not positively related (Fig. 1). In fact, the pattern in 1998, though not statistically significant, was a negative relationship. These results indicate a need for a better understanding of how body mass, plasma triglycerides, and body fat vary among wild birds subjected to stressful late winter conditions. In addition, better information is needed on the timing of changes in these variables as photoperiod increases to levels that can stimulate gonadal recrudescence.

CONCLUSION

Our ability to predict northern bobwhite production is hampered by an incomplete understanding of the factors controlling reproductive timing and effort. Earlier nesting of galliforms is advantageous due to lower nest predation, higher hatchability, and greater potential for renesting (Lehmann 1946, Klimstra and Roseberry 1975, Guthery et al. 1988, Badyaev et al. 1996). Photoperiod limits the potential reproductive period in northern bobwhites (Kirkpatrick and Leopold 1952), but secondary factors such as lipid reserves, diet, and stress can constrain the realized reproductive

period (Gates and Woehler 1968; Cain and Lien 1985; Koerth and Guthery 1991; Giuliano et al. 1995, 1996). Our findings indicate that plasma triglycerides and body masses may be useful for comparing populations among years and locations, but these variables do not explain within-population variation in reproductive timing and effort. Future research on reproduction of northern bobwhites should measure hormones more closely linked to gonadal recrudescence and ovulation and inhibitory effects of stress hormones such as corticosterone. Research on reproduction of wild northern bobwhite can build on the excellent foundation of recent laboratory studies that measured reproductive and stress hormones (Cain and Lien 1985; Giuliano et al. 1995, 1996).

ACKNOWLEDGMENTS

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