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EPA Small Pen Tests. II. Effects of Pen and Group Sizes, Sex Combinations, and Feeding Levels on Bobwhite Body Weight and Fat Content

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Short-term (small pen) field tests are required by the U.S. Environmental Protection Agency (EPA) in the registration process of pesticides when residue levels on or in avian foods are above certain levels (TUCKER 1975, MORROW et al. 1980). Protocol for small pen tests have been published by EPA (FEDERAL REGISTER 1978). An earlier study measured the effects of pen and group sizes, sex combinations, and feeding levels on bobwhite (Colinus virginianus) activity in EPA small pen tests (MORROW et al. 1980); this study evaluates effects of those preceding variables on body weights and ether-extractable fat content of bobwhites.

MATERIALS AND METHODS

Study site, source of experimental birds, pen construction, and general husbandry are detailed in MORROW et al. (1980). The study was conducted outdoors during the June-July breeding season of bobwhites in Kansas.

Ninety-six weight-stratified adult bobwhites were assigned randomly to 12 treatment combinations of group size, sex composition of the groups, and feed ration (Table 1). All 2-bird groups were placed into 4 X 5 X 1-ft (1.2 X 1.5 X 0.3-m) pens as recommended in EPA's small pen test protocol; the 6-bird groups into 8 X 8 X 1-ft (2.4 X 2.4 X 0.3-m) pens. The pens provided about 10 ft^2 (0.9 m^2) of space for each confined bird. Water was provided ad libitum during this 32-day study.

Birds were provided a pelleted ration consisting of 20.5% protein, 2.7% fat, and 3.6% crude fiber, plus vitamins and minerals. The feed was provided daily (except to those on restricted diets, see Table 1) by scattering it on the earthen floor of the pen. The full ration provided 140% of the bobwhite's existence energy requirement (see CASE and ROBEL 1974). Birds were captured and weighed every four days.

At the end of the 32-day study, all birds were killed by cervical dislocation, and their carcasses were quick-frozen and

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TABLE 1

Pen size, sex combinations, and bird groups used as treatments in this study.

Treatment Designation	Pen Size (feet)	Groupings	Ration[a] (%)	Sample Size
MF (Protocol Standard)	4 X 5	1 male + 1 female	100	3
M	4 X 5	2 males	100	3
F	4 X 5	2 females	100	3
MF-90	4 X 5	1 male + 1 female	90	1
MF-90 EOD	4 X 5	1 male + 1 female	90 EOD[b]	1
MF-80	4 X 5	1 male + 1 female	80	1
MF	8 X 8	3 males + 3 females	100	3
M	8 X 8	6 males	100	3
F	8 X 8	6 females	100	3
MF-90	8 X 8	3 males + 3 females	90	1
MF-90 EOD	8 X 8	3 males + 3 females	90 EOD	1
MF-80	8 X 8	3 males + 3 females	80	1

[a]Percent of maintenance ration (see text).

[b]180% of maintenance ration fed every other day (EOD).

stored at -20°C until analyzed for body fat. Each bird was analyzed individually. While frozen, birds were sawed lengthwise and processed through a Universal No. 3 food chopper (fine cutter). The chopped material was then dried 24 h at 60°C, again processed through the chopper and thoroughly blended. Moisture-free weight was determined after drying 2-g samples for 5 h at 110°C under vacuum (79 cm of Hg). Fat was extracted from duplicate 2-g samples of each bird in a Goldfisch extraction apparatus for 16 h with anhydrous diethyl ether as a solvent. Samples were then redried at 110°C for 5 h under vacuum and reweighed. Ether-extractable fat is expressed as a percentage of dry tissue weight.

Body weight and body fat data were treated as separate data sets and analyzed by the Statistical Analysis System (BARR and GOODNIGHT 1971). A one-way analysis of variance was used to test for significant differences (P = 0.05) between treatments for both data sets. Birds within treatments were used as the error term in the analysis for testing the hypothesis H_0: there are no effects due to treatments.

RESULTS AND DISCUSSION

This 32-day study simulated EPA small pen tests by being conducted outdoors under natural field conditions during the bobwhite reproductive season in Kansas, a period that coincides with applications of pesticides for which registration is required. Had our study been an actual EPA small pen test, the 32-day period would have been divided into a 16-day pretreatment control period and a 16-day treatment period with the pesticide being tested applied.

Three males and 4 females of the 96 original experimental bobwhites died during the study, all in 8 X 8-ft pens; 4 birds died of injuries inflicted by other birds in the pen, 1 from crop impaction, and 2 from unknown causes. Each of the 4 birds killed by injuries (2 males and 2 females) was in a different pen containing only males or only females, and the group was being provided a full ration.

The mean weight of the 48 male bobwhites was 187 g, approximately 11% less than the 210 g mean weight of females. Because of the weight differential, male and female weight changes were analyzed separately.

Male bobwhites gained weight during all treatments (Table 2), and the amount gained was not significantly different among treatments within pen size or between pen sizes. Weight gain reflected excess energy intake, indicating that the 140% of existence energy requirement was too much food for bobwhites confined in our small and large pens. The 140% level may be appropriate for free-living birds as suggested by WEST (1967) and ROBEL et al. (1974), but is too much for bobwhites in EPA small pen tests. Even 80% of the

TABLE 2

Mean body weights of bobwhites confined 32 days outdoors under different treatment conditions.

Treatment[a]		Mean Body Weights (g)					
		4 X 5-ft Pens			8 X 8-ft Pens		
		Begin	End	Change	Begin	End	Change
MF	Males	199	213	14	187	205	18
	Females	216	255	39	215	226	11[b]
M		194	203	9	184	197	13
F		217	229	12	194	221	22[b]
MF-90	Males	180	197	17	184	203	19
	Females	204	223	19	233	231	-2[b]
MF-90 EOD	Males	170	172	2	194	198	4
	Females	215	226	11	205	195	-10[b]
MF-80	Males	176	186	10	183	204	21
	Females	206	238	32	197	208	11[b]

[a]See Table 1 for treatment designations.

[b]Significantly different (P = 0.05) from corresponding weight change by birds in 4 X 5-ft pens.

baseline diet (112% of the existence energy requirement) resulted in body weight gain. It might be advisable to use a diet providing no more than the existence energy requirement during EPA small pen tests with male bobwhites.

Monitoring weight changes of female bobwhites was complicated by egg laying during the 32-day trial. Weight gain data were adjusted to compensate for weight changes associated with eggs laid. No treatment-related weight changes were associated with sex combinations of diet levels within pen sizes, however, significant differences in weight changes were detected between pen sizes (Table 2). When males were with females in the 8 X 8-ft pens, females lost significant amounts of weight, but when only females were in the 8 X 8-ft pens, they gained weight. As with males, a diet providing 140% of the existence energy needs of egg-laying females was too much for female bobwhites in EPA small pen tests. A diet providing only existence energy needs might be more appropriate, especially if females are confined with males in 4 X 5-ft pens.

Carcasses of male bobwhites contained less fat than female carcasses (Table 3), and because of this, male and female body fat data were analyzed separately. Differences in body size have been shown to be correlated with body fat content in bobwhites (ROBEL 1972), but the 23-g difference in mean male and female body weights in our study would account for only a 39 to 48% difference in body fat, much less than the over 200% difference in fat content observed between the males and females. High fat levels in females likely reflected the reproductive condition of those females during our study.

In the 4 x 5-ft pens, the least body fat was detected in male-only pair combinations (Table 3) and probably stemmed from stress-associated with aggressive interactions. Male groups provided a full ration and males in male-female groups on 80% of the full ration had significantly less fat than did males in other treatments in the 8 X 8-ft pens, the former probably reflecting behavioral stress; the latter, possibly dietary stress (however, weight data do not support the dietary-stress hypothesis).

The relatively high body fat content of female bobwhites if to be expected during nesting season. However, egg laying made female body-fat data difficult to interpret. We did not attempt to adjust any of the fat data for eggs laid during the 32-day study or for the presence of partially formed eggs in the bird when analyzed. A female laying an egg on day 31 of the study would have less total body fat than a female killed on day 32 with a fully formed egg still in her uterus. Those types of situations probably accounted for much of the variability in fat content of female bobwhites, e.g., ranging from 4.5 to 11.6% of body weight. In general the weight data do not support the body fat data, further substantiating complications due to egg formation and laying. Even though statistical analyses disclosed several treatment effects on body fat

TABLE 3

Mean ether-extractable fat in carcasses of *bobwhites* confined 32 days outdoors under different treatment conditions.

Treatment[a]	Mean Ether-extractable Body Fat (% dry weight)[b]			
	Males		Females	
	4 X 5-ft Pens	8 X 8-ft Pens	4 X 5-ft Pens	8 X 8-ft pens
MF	3.4[a]	3.0[a]	10.3[a]	9.1[a]
M	1.8[b]	2.0[b]		
F			7.4[b]	9.0[a]
MF-90	2.9[ab]	2.4[ab]	10.4[a]	9.4[a]
MF-90 EOD	3.5[a]	2.5[ab]	7.3[b]	8.6[a]
MF-80	3.1[ab]	1.4[b]	7.3[b]	6.3[b]

[a]See Table 1 for treatment designations.

[b]Means joined by underlining do not differ significantly; nor do values with common superscripts within a column (P = 0.05).

content of females, we believe the only one which might be biologically significant is the low body fat content of females on an 80% diet in 8 X 8-ft pens (Table 3).

Because of more fat in female bobwhites than males, and females periodically eliminating fat via egg production, female bobwhites may not be the most appropriate experimental animals for EPA small pen tests when fat-soluble pesticides are being evaluated. Data from female bobwhites certainly cannot be compared directly with data from males. Data from this study also showed more variability in body weights of females than males. Because of the preceding and because EPA small pen tests are not designed to evaluate effects of pesticides on avian reproduction, it may be more appropriate to use only male bobwhites in EPA small pen tests to reduce weight-fat complications associated with egg laying.

CONCLUSIONS

Weight gains by bobwhites during simulated EPA small pen tests showed that a diet providing 140% of the existence energy requirement of bobwhites was too much and that a diet providing only existence energy needs might be adequate for males or females in 4 X 5-ft pens. Carcasses of males had less ether-extractable fat than carcasses of females. Egg laying by female bobwhites added variability to the body weight and body fat data, and made it difficult to interpret. Because of high fat levels in female bobwhites and the periodic elimination of fat via egg production, female bobwhites may not be appropriate experimental animals for EPA small pen tests involving fat-soluble pesticides. Data from this study and our earlier one (MORROW et al. 1980) support the need to develop standardized experimental conditions for EPA small pen tests before they are used to evaluate the hazards to wildlife of various pesticidal compounds.

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REFERENCES

BARR, A. J., and J. H. GOODNIGHT: Statistical Analysis System. Dept. Statistics, North Carolina State Univ., Raleigh, N.C. (1971).

CASE, R. M., and R. J. ROBEL: J. Wildlife Management 38, 638 (1974).

FEDERAL REGISTER: 43, 29732 (1978).

MORROW, M. E., R. J. ROBEL, A. D. DAYTON, D. S. HARAKAL, S. M. MIDDENDORF, and T. J. SNODGRASS: Bull. Environm. Contam. Toxicol. 24, 840 (1980).

ROBEL, R. J.: In: Proc. 1st National Bobwhite Quail Sympos. J. A. Morrison and J. C. Lewis (Eds). Stillwater, Oklahoma, Oklahoma State Univ., Research Foundation (1972), p. 139.

ROBEL, R. J., R. M. CASE, A. R. BISSET, and T. M. CLEMENT, JR.: J. Wildlife Management 38, 653 (1974).

TUCKER, R. K.: Criteria Upon Which to Trigger Exercise of Conditional and Special Tests for Avian Wildlife. Ecological Effects Branch, Criteria and Evaluation Division, Office of Pesticides Programs. U.S. Environmental Protection Agency, Washington, D.C. Unpubl. Rept. 20 pp. (1975).

WEST, G. C.: Ecology 48, 58 (1967).

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