

## **EPA Small Pen Tests I. Effects of Pen and Group Sizes, Sex Combinations, and Feeding Levels on Bobwhite Activity**

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Short-term (small pen) field tests are required by the Environmental Protection Agency (EPA) in the registration process of a pesticide if residue levels on or in avian food are greater than 20% of the 5-day dietary LC<sub>50</sub>, or if 10 or fewer granules of the pesticide contain a quantity of pesticide in excess of the oral LD<sub>50</sub> (TUCKER 1975). Protocol for small pen tests have been published by EPA (FEDERAL REGISTER 1978). The unknown effects of several variables involved with these small pen tests could lead to uncertainty in interpreting results from the tests. This study was conducted to measure effects of pen and group size, sex composition of the groups, and feeding levels on the activity of bobwhites (*Colinus virginianus*), an accepted species for small pen tests.

### **MATERIALS AND METHODS**

This study was conducted on the Konza Prairie Research Natural Area, an 8,616-acre (3,490 ha) Kansas State University research area located 10 miles (16 km) south of Manhattan, Kansas. The study site on Konza Prairie consisted of approximately one acre (2.5 ha) of high quality bottom land that had been in cultivation for at least 30 years.

Ninety-six weight-stratified adult bobwhites, obtained from a commercial hatchery, were selected at random from a pool of 225 and assigned to 12 treatment combinations of group size, sex composition of the groups, and feed ration (Table 1). All 2-bird groups were placed in 4 X 5 X 1-ft (1.22 X 1.52 X 0.30 m) pens as recommended in EPA's small pen test protocol (FEDERAL REGISTER 1978). The wood-framed pens were covered on tops and sides with 1/2-inch (1-cm) hardware cloth and equipped with a 1-quart watering font and a 12 X 12 X 8-inch (30 X 30 X 20-cm) wooden shelter. The 6-bird groups were confined in wood-framed 8 X 8 X 1-ft (2.4 X 2.4 X 0.30-m) pens covered on tops and sides with 1/2-inch hardware cloth and equipped with a 1-quart watering font and a 21 X 21 X 18-inch (53 X 53 X 20-cm) wooden shelter. These pens provided about 10 ft<sup>2</sup> (0.90 m<sup>2</sup>) of pen space for each confined bird. Square foot (0.09 m<sup>2</sup>) grids constructed of 5/8-inch

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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 <sup>a</sup> (%)	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 <sup>b</sup>	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

<sup>a</sup>Percent of maintenance ration (see text).

<sup>b</sup>180% of the maintenance ration fed every other day (EOD).

(1.6-cm) vinyl plastic flagging were placed on the bottom of each pen.

After the birds had been randomly assigned to pens, they were allowed 16 days to acclimate to study procedures and environmental conditions of the study site. Birds were provided a pelletized ration daily (except those groups on restricted diets, see Table 1). This ration provided 140% of the quail's existence energy requirement (see CASE and ROBEL 1974); water was provided ad libitum. Every fourth day, all birds were captured and weighed.

A 16-day testing period followed the 16-day acclimation period, during which quantitative measurements of bird activity and movement were collected. Activity and movement data were collected randomly during daylight hours except 0900-1115 h, when birds were fed and watered and pens were maintained.

Activity and movement data were collected from each pen during 16 20-min observation periods during the 16-day testing period. Observations were made from blinds constructed of burlap and situated adjacent to the pens. Both birds in the 4 X 5-ft pens were observed simultaneously for a 20 min, whereas only two of the six birds (picked at random prior to each observation period) in an 8 X 8-ft pen were observed during a particular observation period. An adjustment time of 5 to 15 min preceded data collection during each observation period. Observations (made every 2 min during a 20-min period) consisted of recording the location and activity of the two birds with reference to the grid on the pen floor. Bird movement was determined by totaling for a 20-min period the straight-line distance a bird moved between each of 11, 2-min location recordings; bird activity, by totaling the number of times a bird was active (walking, running, feeding, or drinking) out of the 11, 2-min recordings. Birds dusting, preening, lying down, or not moving were considered inactive. In addition, any abnormal behavior exhibited by the birds was recorded. Normal behavior was considered to be that as described by STOKES (1967).

Activity and movement data were treated as separate data sets and analyzed by using 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 the treatments for both data sets. Pen within treatments was used as the error term in the analysis for testing the hypothesis  $H_0$ : there are no effects due to treatments.

## RESULTS AND DISCUSSION

Activity and movement data were provided by 24 bobwhites in 4 X 5-ft pens during 192 individual 20-min periods and by 72 birds in 8 X 8-ft pens during 189 individual 20-min periods. A total of 4224 bits of activity and movement data provided the

basis for analyzing activity and movements of paired birds, whereas 4158 bits of data each for activity and movement provided the basis for analyzing activity and movements of birds in the 6-bird groups.

Male-female pairs of birds in 4 X 5-ft pens (EPA protocol recommendation) on a full maintenance ration had a mean activity level of 5.2 per 11 observations (Table 2), not significantly different from that of male-male pairs or female-female pairs. Although pairs of bobwhites given 80% of the daily maintenance ration or 90% of the maintenance ration every other day (EOD) exhibited lower activity than did pairs of birds on a 90% maintenance ration fed daily, the differences were not significant. The activity of the pairs of bobwhites on restricted rations did not differ significantly from that of those on full maintenance rations (Table 2).

TABLE 2

Mean number of times a bird was active per 11 observations during 20-min observation periods.

Treatment <sup>a</sup>	Mean Activity Levels <sup>b</sup>	
	4 X 5-ft Pens	8 X 8-ft Pens
MF	5.2 <sup>c</sup>	3.6 <sup>c</sup>
M	4.2 <sup>c</sup>	3.7 <sup>c</sup>
F	5.4 <sup>c</sup>	4.8 <sup>c</sup>
MF-90	5.6 <sup>c</sup>	3.3 <sup>c</sup>
MF-90 EOD	4.4 <sup>c</sup>	4.0 <sup>c</sup>
MF-80	4.1 <sup>c</sup>	3.3 <sup>c</sup>

<sup>a</sup>See Table 1 for treatment designations.

<sup>b</sup>Value of 0 = not active during 20-min observation period; value of 11 = constant activity. Means joined by underlining are not significantly different; values with common superscripts within a column are not significantly different (P = 0.05).

The equally balanced male-female 6-bird groups in 8 X 8-ft pens had a mean activity of 3.6 per 11 observations, not significantly different from that of the all-male or all-female 6-bird

groups (Table 2). Male-female 6-bird groups on daily 90% or daily 80% maintenance rations had the lowest activity exhibited by 6-bird groups, but not significantly lower than that of other 6-bird groups in the study. Although activity levels of bobwhites in 6-bird groups contained in 8 X 8-ft pens were consistently lower than those of pairs of birds contained in 4 X 5-ft pens, the differences were not significant (Table 2).

During 11 observations, male-female pairs of bobwhites in 4 X 5-ft pens traveled a mean distance of 14.7 ft out of a possible 50 ft (Table 3). That value was not significantly different from that for male-male pairs or female-female pairs. Although male-female pairs given 80% of the daily maintenance ration or 90% of the maintenance ration EOD moved on the average less distance than did the pairs of birds on a 90% daily maintenance ration, the differences were not significant. Those pairs on a restricted ration in 4 X 5-ft pens did not travel a distance significantly different from that of the male-female groups on full maintenance ration (Table 3).

TABLE 3

Mean distance traveled by a bird during 11 observations per 20-min period. Maximum possible distance traveled per bird was 50 ft for birds in 4 X 5-ft pens; 98 ft for birds in 8 X 8-ft pens.

Treatment <sup>a</sup>	Mean Distance (feet) Traveled <sup>b</sup>	
	4 X 5-ft Pens	8 X 8-ft Pens
MF	14.7 <sup>c</sup>	22.6 <sup>c</sup>
M	13.2 <sup>c</sup>	17.0 <sup>d</sup>
F	13.9 <sup>c</sup>	20.0 <sup>cd</sup>
MF-90	<u>15.8<sup>c</sup></u>	<u>19.2<sup>cd</sup></u>
MF-90 EOD	<u>13.1<sup>c</sup></u>	<u>18.5<sup>cd</sup></u>
MF-80	12.2 <sup>c</sup>	20.9 <sup>cd</sup>

<sup>a</sup>See Table 1 for treatment designations.

<sup>b</sup>Means joined by underlining are not significantly different; values with common superscripts within a column are not significantly different (P = 0.05).

During 11 observations, the male-female 6-bird groups in the 8 X 8-ft pens traveled a mean distance of 22.6 ft out of a possible 98 ft, a significantly greater ( $P < 0.01$ ) distance than the 17.0 ft traveled by the all-male 6-bird groups (Table 3). The male-female 6-bird groups fed a 90% ration EOD moved on a average less distance than did the 6-bird groups given either a 90% daily maintenance ration or an 80% daily maintenance ration. However, those differences were not significant. Bobwhites in the male-female, male-male and female-female 6-bird groups as well as the 6-bird group provided an 80% daily maintenance ration, traveled a significantly greater distance ( $P < 0.05$ ) during the 11 observations than did the corresponding pair groups in 4 X 5-ft pens. The remaining 6-bird groups consistently traveled greater distances than did the corresponding pair groups; however, the differences were not significant (Table 3).

In addition to the data discussed above, we noted numerous occurrences of agonistic encounters among the birds in 6-bird groups in 8 X 8-ft pens. Few such encounters were noted among the pair groups in 4 X 5-ft pens. These observations agree with those of AL-RAWI and CRAIG (1975) on domestic chickens, i.e., frequency of aggressive encounters increase as group size increases.

#### CONCLUSIONS

Six-bird groups of bobwhites in large pens exhibited significantly more movement than did corresponding pairs of bobwhites in small pens, indicating a need to standardize pen and bird-group sizes in EPA small pen tests if results are to be comparable. Although in this study we did not detect any statistically significant differences in activity levels of bobwhites attributable to pen or bird-group size, the fact that the activity of pairs of bobwhites in small pens were consistently higher than those of 6-bird groups in larger pens reinforces the need to standardize pen and bird-group sizes in EPA small-pen tests. Higher levels of aggression noted in the 6-bird groups compared with the 2-bird groups further substantiate this need. Additionally, the all-male 6-bird groups traveled significantly less distance than did male-female 6-bird groups, suggesting the need to also standardize sex combinations of birds used in EPA small pen tests. Dietary levels did not appear to affect either activity levels or movements of birds. Therefore this study produced no data to support the need to standardize dietary levels provided birds used in EPA small pen tests.

Additional research is needed to separate the effect of group size and pen size on bird activity and movements before these parameters are standardized in EPA small pen test protocol.

#### ACKNOWLEDGMENTS

This is contribution 80-268-j, Division of Biology and Department of Statistics, Kansas Agricultural Experiment Station, Kansas State University Manhattan, KS 66506. Financial support was provided by the Kansas Agricultural Experiment Station and the National Science Foundation (Grant SPI-786934).

#### REFERENCES

- AL-RAWI, B., and J. V. CRAIG: Applied Animal Ethology 2, 69 (1975)
- 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).
- STOKES, A. W.: Auk 84, 1 (1967).
- 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 Pesticide Programs. U.S. Environmental Protection Agency, Washington, D.C. Unpubl. Rept. 20 pp. (1975).