Dietary Ferulic Acid, Biochanin A, and the Inhibition of Reproductive Behavior in Japanese Quail (*Coturnix coturnix*)

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DE MAN, E. AND H. V. S. PEEKE. *Dietary ferulic acid, biochanin A, and the inhibition of reproductive behavior in Japanese quail* (Coturnix coturnix). PHARMAC. BIOCHEM. BEHAV. 17(3) 405–411, 1982.—A study was conducted to determine the effects of two dietary compounds, biochanin A and ferulic acid, on the reproductive performance of Japanese quail, *Coturnix coturnix*. These compounds may be present in the diet of wild birds during dry years or at the end of the growing season during normal years. Four treatment groups, each fed a diet supplemented with biochanin A or ferulic acid, and a control group were tested for morphological and behavioral effects before, during and after treatment. Fertility and hatchability data were collected during treatment only. Ferulic acid was shown to inhibit male copulatory behavior during treatment. Biochanin A inhibited male copulatory behavior during and after treatment. Neither drug affected female copulatory behavior though egg-laying was inhibited by the low dose of biochanin A. Ferulic acid significantly reduced the hatchability of eggs. Biochanin A increased the fertility of males and the hatchability of eggs fertilized by them. A correlation between results of this study and natural conditions of reproduction is discussed. It is concluded that both biochanin A and ferulic acid exert inhibiting influences on the reproductive behavior of *Coturnix*.

Ferulic acid   Biochanin A   Dietary supplements   Sexual behavior   Quail

A POSITIVE correlation exists between the reproductive success of California quail, *Lophortyx californicus*, and high seasonal rainfall [26,34]. Influencing this relationship is an early breeding season of long duration that enhances the chances of producing double broods [38]. The amount of rainfall is known to affect the diet of quail; more grasses are ingested during dry years than during wet years when the diet contains more forbs and their seeds [25, 26, 28, 33]. It is possible, therefore, that the key to reproductive performance of California quail may be found in their food source.

Extracts from clovers and forage plants have estrogenic effects on rabbits [16,27], sheep [10], mice [12–15, 22, 30], rats [36], and guinea pigs [3]. Other investigations identified these plant estrogens, or phytoestrogens, as isoflavones: genestein, foromonetin and biochanin A [8,17], and daidzein [29].

Other substances found in food sources also have adverse effects on reproduction in mammals. These include phenolic compounds 4-vinylguaiacol and 4-vinylphenol, their parent cinnamic acids, ferulic acid and p-coumaric acid [11], and unidentified estrogenic inhibitors [4,5].

Isoflavones are more prevalent in forbs during years of sparse rainfall than during wet years. Forbs grown during dry years inhibit the reproductive capabilities of California quail [33]. Phenols, 4-vinylphenol (4-hydroxystyrene) and 4-vinylguaiacol (4-hydroxy-3-methoxystyrene) are most prevalent in various food sources at the end of the vegetative growing cycle [11, 23, 31]. Effects of phenols on reproduction in wild quail are unknown.

From the above, we hypothesized that both types of compounds, isoflavones and phenols, might inhibit reproduction in quail at times when the food crop is not adequate to support a large population. On an annual basis phenols might inhibit reproduction at a time which coincides with the termination of a plentiful food supply. Over a longer period of time, phytoestrogens may also inhibit reproduction in years of low rainfall.

Biochanin A, 5,7-dihydroxy-4' methoxyisoflavone, was

the isoflavone chosen for investigation in this study. It has been found in a variety of plants eaten by quail [8, 9, 29] and in crop samples taken from California quail [33]. Ferulic acid, 4-hydroxy-3-methoxycinnamic acid, the precursor of 4-vinylguaiacol, was chosen to represent phenolic plant compounds in this study, as it was readily available in pure form. Ferulic acid has been found in many plant species but predominantly in the Gramineae (84% frequency of occurrence) [6].

While the original hypothesis is based on observations of California quail, Japanese quail, *Coturnix coturnix*, were chosen for this study because of their unique suitability to the study of reproductive behavior. Reproductive behavior of male Japanese quail can be broken down into several, hormonally dependent, components [7]. Receptivity in the female *Coturnix* is also easily measured and hormonally dependent [35]. Male *Coturnix* have an external cloacal gland, the size of which is androgen-dependent and reflects the state of the internal gonads [39], and indicates their readiness to mate [44]. Measurements of the cloacal diameter of females [2,35] and number of eggs laid are measures of ovarian activity.

METHOD

Animals

Two hundred 3-week-old Japanese quail were obtained from the Department of Avian Sciences, University of California, Davis. Birds were housed in a communal brooder until they reached sexual maturity. Light was provided continuously to ensure maturity at six weeks of age [46]. While kept in the brooder, chicks were fed Purina Game Bird Startena, a 30% protein diet formulated by the Ralston Purina Company, St. Louis, MI. All birds, except stimulus females, were then transferred to battery-type cages for the duration of the experiment, and fed Purina Game Bird Layena, a 20% protein chow. They were maintained at about 21°C on a 16L 8D photoperiod as recommended by Ernst [24] in order to maintain egg production and mating activity.

Initial Screening

A total of 60 males (10 in each of five experimental groups and 10 stimulus males) and 75 females (10 in each of five experimental groups and 5 in each of five stimulus female groups) were used. Each male was pretested by placing him in the observation box with a female. If the male did not initiate and complete the entire copulatory sequence within one minute he was not used in the experiment. Those that did were arbitrarily assigned to one of the experimental groups or to the stimulus group. Each female used in the study was given this experience to acclimate her to the process.

A total of six battery cages were used, five housing the twenty experimental birds in each group (10 males and 10 females), the sixth housing the 10 stimulus males. Stimulus females were housed separately in five groups, each group corresponding to an experimental group of males. Males and females were housed back to back and were visually isolated from each other.

Dosages and Food Preparation

Dosages of biochanin A and ferulic acid used in this experiment were chosen to bracket concentrations which might be found in the diet of wild California quail: 1 mg biochanin

TABLE 1
DOSAGES AND DURATION OF ADMINISTRATION OF DRUGS USED IN EXPERIMENTAL FEEDING

Group	Drug	Dosage mg/g feed	Number of days administered
A	none	—	—
B	ferulic acid	2.00	19
C	ferulic acid	5.25	19
D	biochanin A	0.50	9
E	biochanin A	1.50	9

A/gram food and 4 mg ferulic acid/gram food [21]. It should be born in mind that the dosages based on one species and administered to another not closely related species can only be a rough approximation. Dosages and administration times are listed in Table 1.

Food was prepared as follows. The appropriate quantity of either biochanin A or ferulic acid was dissolved in 150 ml of 100% ethanol and poured into the feed. The ethanol was evaporated at 40°C for 2 hours. The treated food was fed to the birds for the time periods specified in Table 1. Water was provided ad lib.

Data Collection

Surface area of the cloacal gland of experimental males was determined by multiplying the length of the gland by its width [39]. Cloacal width only was measured on experimental females [2].

Behavioral observations were made between 1000 and 1300 hours. A male was placed in the introduction chamber and a female in the observation box. The observation box was 60.9 cm wide, 45.7 cm deep, and 25.4 cm high. Three walls and the ceiling were plywood; the remaining wall and the floor were 1.3 cm hardware mesh cloth. A 75-watt light bulb placed 91.4 cm in front of the observation box provided illumination.

The 17.8 cm long introduction chamber, made of 10.2 cm diameter PVC pipe, was attached to a side wall of the observation box. The male was placed into the introduction chamber through a rear door of opaque acrylic. A clear acrylic door blocked his access to the observation box but allowed him to see the female. The birds were given 30 seconds to acclimate before behavioral observations were initiated.

The observer sat 3 feet in front of the observation box and recorded data on an electrically-controlled events recorder. The clear acrylic door was operated remotely by the observer. Once opened, the doorway allowed the male access to the female. Behavioral observations began as soon as the male's head crossed into the observation box.

Experimental males were tested for three minutes. Latency, duration, and frequency of the four specific behavior patterns (neck grab, mount, wing spread, and cloacal contact movement), described by Wilson and Bermant [44], were recorded. Males were allowed to complete any behaviors initiated before the three minutes were up. Latency was measured as the time that elapsed between the male's entering the observation box and initiating the neck grab. If a male repeated a single behavior in less than one second, it was counted as a single event.
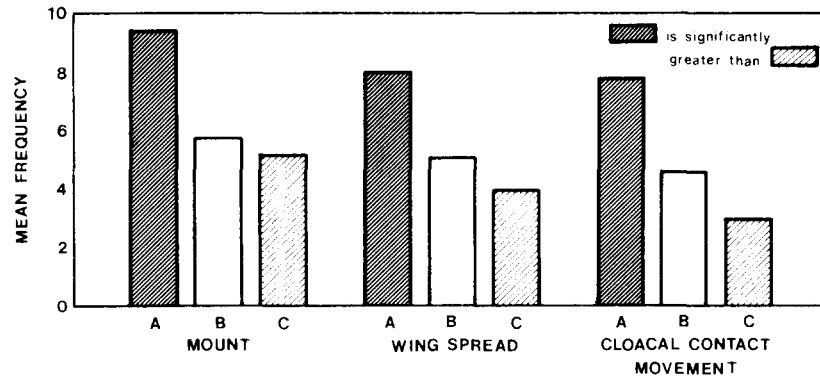
FIG. 1. Mean frequencies of behaviors of males in groups A, B, and C during treatment.

Experimental females were tested for two minutes. Upon initiation of the neck grab and subsequent behaviors of the stimulus male, the female would respond either by squatting, standing, or avoiding the male. Criteria for these behaviors have been previously described by Noble [35].

Experimentation began two weeks after the birds were introduced into the battery cages. Control data were collected for each individual on the day prior to beginning experimental feeding. Morphological characteristics and behavioral responses were recorded. These data were collected every fifth day until the tenth day following the termination of experimental feeding.

Eggs were collected from both experimental (x) and stimulus (s) females three times a day between 0900 hours on the fifth day after the termination of experimental feeding and 0900 hours on the eighth day. Eggs were maintained at 60°F for no more than 6 days before incubation as recommended by Woodard et al. [48]. All eggs were set on the same day. The Marsh Farms Roll-X Incubator was used and maintained at 37.5°C and 60% relative humidity. The incubator was fitted with an automatic turning device, designed to turn the eggs once each hour. Eggs hatched from the 16th to the 20th day of incubation.

Those eggs which did not hatch after 20 days were opened and examined to determine if they had been fertilized. Development beyond the sixth day was easily recognizable [37]. Therefore, eggs fertilized but not developed beyond day six were counted as unfertilized.

Statistical Analysis

Behavioral data, along with measures of cloacal gland surface area were tested using a one-way analysis of variance, completely randomized design [18]. Cloacal diameter of the females and the number of eggs laid were also treated in this fashion. Data showing a significant difference ($p < 0.05$) were investigated further using the Newman Keuls' test to determine which groups differed.

Since the duration of the experimental female's response was dependent upon the behavior of the stimulus male, it was important to determine whether the interaction time varied between groups or over the course of the study. Total interaction time is the sum of the duration of the stimulus males' neck grabs and was tested using the two-factor mixed design: repeated measures on one factor [18].

During the neck grab a female might respond by squat-

ting, standing, or avoiding. The duration of each response was analyzed using a three-factor mixed design: repeated measures on two factors (treatment and response). This design permits a comparison of the three groups' (ABC and ADE) performance and evaluates the responses throughout the study period.

The proportions of eggs which hatched, were fertilized but did not hatch, and which were not fertilized were determined for each group of females. The values obtained for each experimental and stimulus group were then compared with the corresponding values obtained for the appropriate control group, A-experimental or A-stimulus, using the test for significance of difference between two proportions [18].

RESULTS

Morphology and Behavior

Males. Unless discussed below, the results obtained from experimental animals were not significantly different (that is, $p > 0.05$) from those obtained from control animals. During treatment, the frequencies of mounts, wing spreads and cloacal contact movements were significantly less for group C than for group A (Fig. 1). After treatment ended, frequencies of these behaviors returned to values not significantly different from the control group except that the mean duration of mount was significantly less ($p = 0.037$) for group C (2.0 seconds) than for group A (2.5 seconds) during these post-treatment tests.

During treatment, males in group E had significantly fewer neck grabs but neck grabs were of a longer duration than those of groups D and A (Fig. 2). Mean durations for total interaction time were also significantly greater for group D than for group E both during and after treatment (Fig. 3), though as the figure shows, any changes were slight. After treatment, frequencies of neck grabs, mounts, wing spreads, cloacal contact movements, (Fig. 4) and mean durations for total interaction (Fig. 3) were significantly less for group E than for group A. Mean duration of neck grabs was greater for group D than for group A (Fig. 5), but the frequency of neck grabs was less (Fig. 4).

Females. The total interaction time between stimulus males and experimental females did not differ between the experimental groups or over the duration of the experiment. All females squatted more than they stood or more than they avoided during the interaction. There were no changes in female behavioral response as a result of drug treatment.

There were, however, differences in morphology and egg-laying during treatment (Fig. 6). Mean cloacal diameter (c.d.) was significantly greater for group B (1.34 cm) than for group C (1.19 cm). Females of group D laid fewer eggs (.75 eggs/day) than females in the control group A.

Fertility and Hatchability

There was no significant difference between experimental groups and the control group in the proportion of eggs that hatched (Table 2). However, groups Bx and Cx had a greater proportion of fertilized eggs which did not hatch and group Cx also had significantly fewer unfertilized eggs than control group Ax.

Proportions of fertilized eggs that did not hatch did not differ between stimulus and control groups (Table 3). But groups Cs and Es had a greater proportion of hatched eggs and group Es had a greater proportion of fertilized eggs.

DISCUSSION

Behavior and Morphology

Ferulic Acid. In males, treatment with the higher dose of ferulic acid caused a reduction in the frequency of mounts, wing spreads, and cloacal contact movements: these copulatory behaviors are mediated by estrogen and are only indirectly affected by testosterone [1]. Despite this reproductive inhibition, there were no changes in cloacal gland surface area, the only testosterone-dependent character measured in this study. This suggests that ferulic acid has some anti-estrogenic activity.

In females, the only significant result of treatment with ferulic acid was a greater mean cloacal diameter for those receiving the lower dose, B, than for those receiving the higher dose, C. Since cloacal diameter is an effective measure of both behavioral receptivity and estrogen treatment in Japanese quail [35] it appears that estrogenic activity was greater in group B than in group C. While the exact nature of the action of ferulic acid is not understood, its anti-estrogenic properties may help explain the small cloacal diameters of group C, though it does not explain why they are smaller than group B.
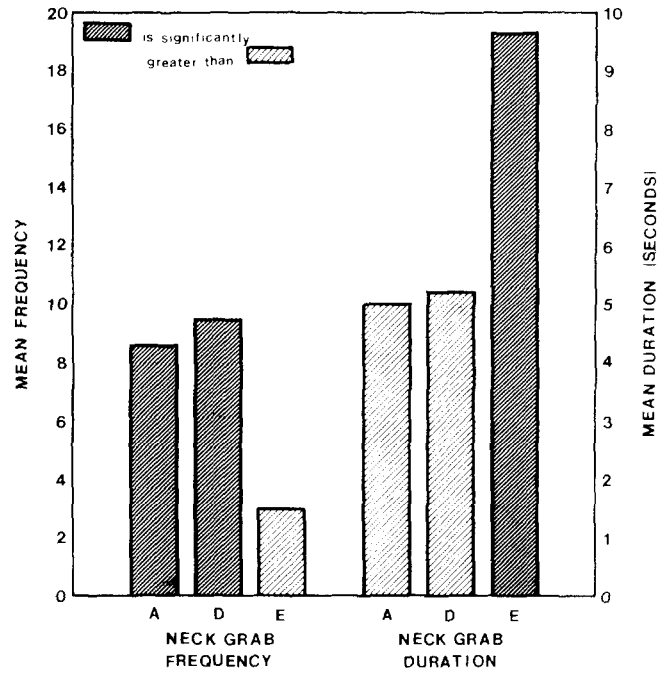

FIG. 2. Mean frequencies and durations of neck grabs in groups A, D, and E during treatment.

Noble's study revealed that females do not respond by squatting if their cloacal diameter is less than 1.4 cm. The results of Adkins and Adler [2] suggest that a cloacal diameter of 1.2–1.6 cm is normal, and that functional castration is not apparent until the cloacal diameter has shrunk to 0.6 to 0.8 cm. Despite the relatively low values obtained for cloacal diameter in this study, squatting was the prevalent behavioral response. This led us to believe either that cloacal diameter is a more sensitive measure of estrogenic activity, or that ferulic acid has little effect on the estrogenic factors affecting receptivity.
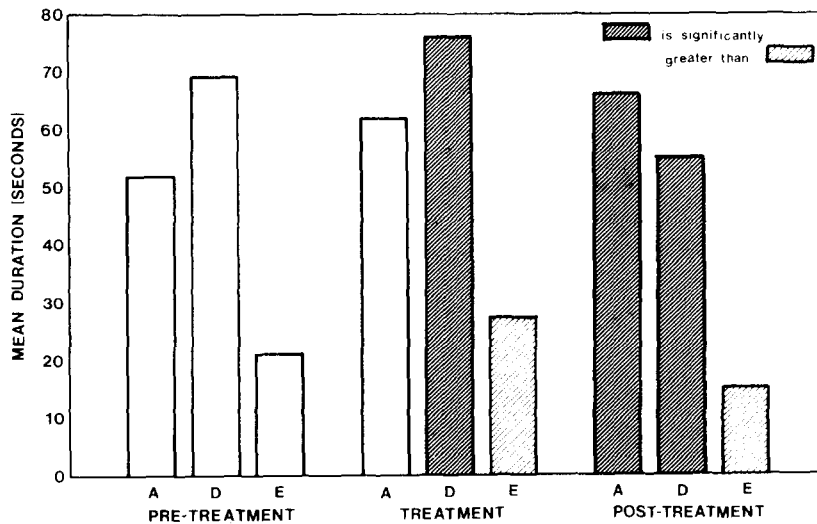

FIG. 3. Mean durations of total interaction times for groups A, D, and E.
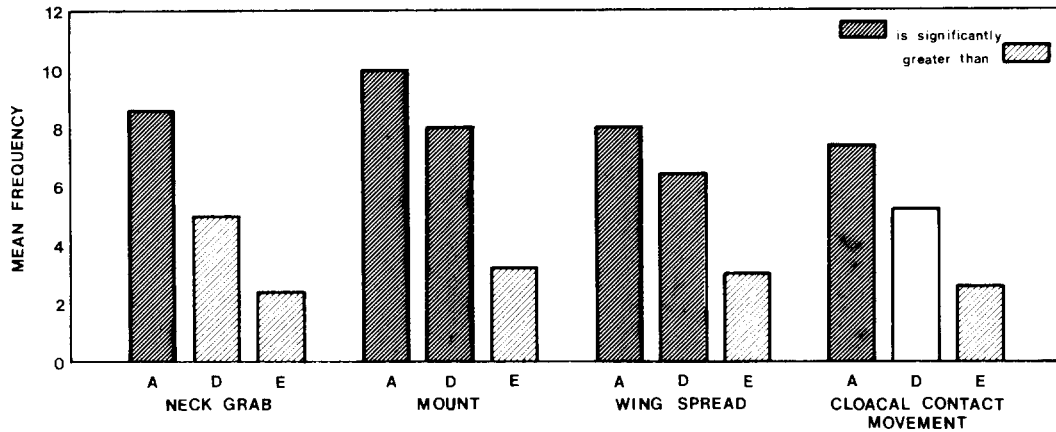
FIG. 4. Mean frequencies of neck grabs, mounts, wing spreads, and cloacal contact movements for groups A, D, and E after treatment.
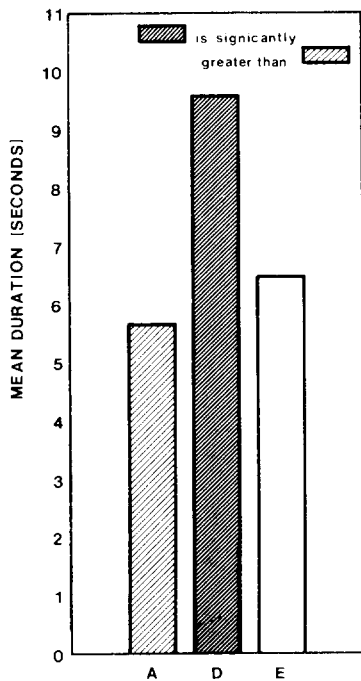

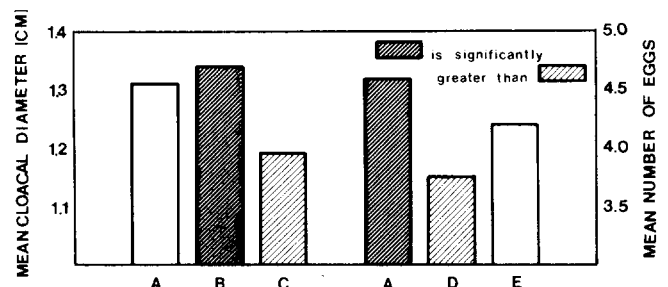FIG. 5. Mean durations of neck grabs in groups A, D, and E after treatment.

FIG. 6. Mean cloacal diameters of females in groups A, B, and C and mean numbers of eggs laid by females in groups A, D, and E during treatment.

Females receiving the low dose of biochanin A laid fewer eggs. However, behavioral responses did not differ from the control or high dose group. The high proportion of squats suggests normally receptive females with a normal hormonal milieu. However, the reduced number of eggs laid indicates a possible inhibition or impairment of LH function since this hormone is most directly responsible for ovulation [41, 42, 45].

Hatchability and Fertility

Experimental females. Hatchability of fertilized eggs depends on egg composition, eggshell thickness and toxic substances entering the egg either before or after it is laid. Many of these were influenced when certain chemical substances were administered to the hen [19, 32, 40]. At this point it is impossible to determine how ferulic acid inhibited the hatching success of fertilized eggs and how it may have enhanced fertility at the same time. Biochanin A had no effect on either fertility or hatchability of eggs.

Stimulus females. Hatchability and fertility of eggs laid by stimulus females are measures of the reproductive success of experimental males. Both biochanin A and ferulic acid in high doses caused an increase in hatchability. Biochanin A also caused an increase in fertility. If reproductive success is measured by that proportion of hatched eggs, then both ferulic acid and biochanin A, when ingested by males, enhance reproductive performance.

Biochanin A. During treatment with biochanin A two behaviors were affected: neck grabs were of less frequency and longer duration, and total interaction time was shorter.

Estrogen is known to stimulate copulatory behavior [1,2]. In this study, the higher dose of biochanin A, which usually mimics the effects of estrogen [8,10], induced the opposite of the expected result. During the post-treatment period, males in group E had fewer neck grabs, mounts, wing spreads, and cloacal contact movements, and shorter interaction times than the control males. A possible explanation is that biochanin A inhibits endogenous estrogen. The low dose stimulated copulation in comparison to the high dose, but not in comparison to the control. Again, there was no effect on the testosterone-dependent cloacal gland.

TABLE 2

SUMMARY OF RESULTS OF TESTS FOR SIGNIFICANCE OF DIFFERENCE BETWEEN PROPORTION OF EGGS HATCHED. FERTILIZED BUT NOT HATCHED, AND UNFERTILIZED: COLLECTED FROM EXPERIMENTAL FEMALES

Group	Eggs hatched	Eggs fertilized; not hatched	Eggs not fertilized
Bx	n.s.	*	n.s.
Cx	n.s.	*	*
Dx	n.s.	n.s.	n.s.
Ex	n.s.	n.s.	n.s.

n.s. = No significant difference $(p > 0.05)$.
* = Significant difference $(p \leq 0.05)$.

TABLE 3

SUMMARY OF RESULTS OF TESTS FOR SIGNIFICANCE OF DIFFERENCE BETWEEN PROPORTIONS OF EGGS HATCHED. FERTILIZED BUT NOT HATCHED, AND UNFERTILIZED: COLLECTED FROM STIMULUS FEMALES

Group	Eggs hatched	Eggs fertilized: not hatched	Eggs not fertilized
Bs	n.s.	n.s.	n.s.
Cs	*	n.s.	n.s.
Ds	n.s.	n.s.	n.s.
Es	*	n.s.	*

n.s. = No significant difference $(p > 0.05)$.
* = Significant difference $(p < 0.05)$.

Groups Cs and Es had a greater proportion of eggs hatch, presumably because of the increased fertility of the males in these two experimental groups. Androgens and estrogen are known to depress pituitary activity and the secretion of pituitary gonadotropins, thereby inhibiting spermatogenesis [43]. In this study, spermatogenesis may have been enhanced by anti-estrogenic effects of ferulic acid and biochanin A. Motility and maturation of sperm also influence fertility. These are known to be inhibited by treatment with certain chemicals [20]. How this effect interacts with the seemingly inhibitory effects on reproductive behavior needs to be studied in more detail before one can determine the exact roles biochanin A and ferulic acid play in the annual and seasonal regulation of reproduction.

Conclusion

Both ferulic acid and biochanin A, when administered orally inhibit reproductive behavior in male Japanese quail. Although it is true that neither drug administered in quantities approximating those found in the wild caused a cessation of reproduction, it is important to remember that each of these chemicals is but a single component of a complex chemical network in naturally occurring food sources. Effects of these chemicals in combination with the other components may have synergistic effects on reproductive inhibition.

This study suggests that the behavior of the male *Coturnix* is more malleable than that of the female, since female behavior was not affected by either treatment. Surface area of the cloacal gland, a male morphological characteristic known to be androgen-dependent, was not affected. The one female morphological character known to be estrogen-dependent, cloacal diameter, responded to treatment with ferulic acid.

The purpose of this study was to determine whether biochanin A, a phytoestrogen found in the stunted plants growing during a drought, and ferulic acid, a parent compound to a phenol found in plants at the end of the growing season, could be responsible for inhibiting reproduction during dry years and at the end of the growing season. This expeirment shows that both may play a role in reducing the reproductive capacity, primarily by altering male behavior.

REFERENCES

1. Adkins, E. K. Effects of diverse endrogens on the sexual behavior and morphology of castrated male quail. *Hormones Behav.* 8: 201–207, 1977.
2. Adkins, E. K. and N. T. Adler. Hormonal control of behavior in the male Japanese quail. *J. comp. physiol. Psychol.* 81: 27–36, 1972.
3. Alexander, G. and R. H. Watson. The assay of estrogenic activity of *Tri. Sub. L.* by increase in uterine weight in spayed guinea pigs. II. *Aust. J. agric. Res.* 2: 480–493, 1951.
4. Allen, M. R. and W. D. Kitts. The effect of yellow pine (*Pinus ponderosa* Laws) needles on the reproductivity of the laboratory female mouse. *Can. J. anim. Sci.* 41: 1–8, 1961.
5. Allison, C. A. and W. D. Kitts. Further studies on the anti-estrogenic activity of yellow pine needles. *J. Anim. Sci.* 23: 1155–1159, 1954.
6. Bate-Smith, E. C. Ferulic, sinapic and related acids in leaves. *Chem. Ind.* 73: 1457–1458, 1954.
7. Beach, F. A. and N. G. Inman. Effects of castration and androgen replacement on mating in male quail. *Proc. natn. Acad. Sci. U.S.A.* 54: 1426–1431, 1965.
8. Beck, A. B. The oestrogenic isoflavones of subterranean clover. *Aust. J. agric. Res.* 15: 223–230, 1964.
9. Beck, A. B. and J. R. Knox. The acylated isoflavone glycosides from subterranean clover and red clover. *Aust. J. Chem.* 24: 1509–1518, 1971.
10. Bennetts, H. W., E. J. Underwood and F. L. Shier. A specific problem of sheep on subterranean clover pastures in Western Australia. *Aust. vet. J.* 22: 2–12, 1946.
11. Berger, P. J., E. H. Sanders, P. D. Gardner and N. C. Negus. Phenolic plant compounds functioning as reproductive inhibitors in *Microtus montanus. Science* 195: 575–577, 1977.
12. Bickoff, E. M., A. N. Booth, A. L. Livingston, A. P. Hendrickson and R. L. Lyman. Determination of estrogenic activity in fresh and dried forage. *J. Anim. Sci.* 18: 1000–1009, 1959.

13. Bickoff, E. M., A. N. Booth, R. L. Lyman, A. L. Livingston, C. R. Thompson and G. O. Kohler. Isolation of a new estrogen from ladino clover. *J. agric. Fd Chem.* **6:** 536–539, 1958.
14. Bickoff, E. M., A. L. Livingston, A. N. Booth, A. P. Hendrickson and G. O. Kohler. Estrogenic activity in dehydrated and suncured forages. *J. Anim. Sci.* **19:** 189–197, 1960.
15. Biggers, J. D. and D. H. Curnow. Oestrogenic activity of subterranean clover 1. The oestrogenic activity of genestein. *Biochem. J.* **58:** 278–282, 1954.
16. Bradbury, J. T. The rabbit ovulating factor in plant juice. *Am. J. Physiol.* **142:** 487–493, 1944.
17. Bradbury, R. B. and D. E. White. The chemistry of subterranean clover. Part 1. Isolation of foromonetin and genestein. *J. chem. Soc.* **182:** 3447–3449, 1951.
18. Bruning, J. L. and B. L. Kintz. *Computational Handbook of Statistics.* Palo Alto, CA: Scott, Foresman and Company, 1977.
19. Consuesra, P. F. Recent findings in the inhibition of avian ova sustension. In: *Some Recent Findings in the Inhibition of Avian Reproductivity,* edited by D. K. Wetherbee. U.S. Fish and Wildl. Serv. Spec. Sci. Rep. Wildl. No. 67. Washington, DC: U.S. Government Printing Office, 1962, pp. 23–24.
20. Crawford, R. D. Recent findings in the inhibition of avian sperm sustension. In: *Some Recent Findings in the Inhibition of Avian Reproductivity,* edited by D. K. Wetherbee. U.S. Fish and Wildl. Serv. Spec. Sci. Rep. Wildl. No. 67. Washington, DC: U.S. Government Printing Office, 1962, pp. 3–22.
21. deMan, E. Dietary influence on the reproductive performance of Japanese quail. Master's thesis. San Francisco State University, San Francisco, CA.
22. Dohan, F. C., E. H. Richardson, R. C. Stribley and P. Gyorgy. The estrogenic effects of extracts of spring rye grass and clover. *J. Am. vet. med. Ass.* **118:** 323–324, 1951.
23. El-Basyouni, S. and G. H. N. Towers. The phenolic acids in wheat. 1. Changes during growth and development. *Can. J. Biochem.* **42:** 203–210, 1964.
24. Ernst, R. A. *Raising and Propagating Japanese Quail.* University of California Division of Agricultural Sciences Leaflet 2738, 1965.
25. Erwin, M. J. Comparison of the reproductive physiology, molt and behavior of the California quail in two years of differing rainfall. Master's thesis. University of California, Berkeley, CA 1975.
26. Francis, W. J. The influence of weather on population fluctuations in California quail. *J. Wildl. Mgmt* **34:** 249–266, 1970.
27. Friedman, M. J. and G. S. Friedman. Gonadotropic extracts from green leaves. *Am. J. Physiol.* **124:** 486–490, 1939.
28. Glading, B., H. H. Biswell and C. F. Smith. Studies on the food of the California quail in 1937. *J. Wildl. Mgmt* **4:** 128–144, 1940.
29. Guggolz, J., A. L. Livingston and E. M. Bickoff. Detection of daidzein, formononetic, genestein and biochanin A in forages. *J. agric. Fd Chem.* **9:** 330–332, 1961.
30. Kitts, W. D., E. Swierstra, V. C. Brink and A. J. Wood. The estrogen-like substances in certain legumes and grasses. I. *Can. J. anim. Sci.* **39:** 6–13, 1959.
31. Kuwatsuka, S. and H. Shindo. Behavior of phenolic substances in the decaying process of plants. I. Identification and quantitative determination of phenolic acids in rice straw and its decayed product by gas chromatography. *Soil Sci. Pl. Nutr.* **19:** 219–227, 1973.
32. Landy, M. J. Recent findings in the inhibition of avian embryogenesis. In: *Some Recent Findings in the Inhibition of Avian Reproductivity,* edited by D. K. Wetherbee. U.S. Fish and Wildl. Serv. Spec. Sci. Rep. Wildl. No. 67. Washington, DC: U.S. Government Printing Office, 1962, pp. 35–52.
33. Leopold, A. S., M. Erwin, J. Oh and B. Browning. Phytoestrogens: adverse effects on reproduction in California quail. *Science* **191:** 98–99, 1976.
34. McMillan, I. I. Annual population changes in California quail. *J. Wildl. Mgmt* **28:** 701–711, 1964.
35. Noble, R. Hormonal control of receptivity in female quail (*Coturnix coturnix japonica*). *Hormones Behav.* **4:** 61–72, 1963.
36. Ostrovsky, D. and W. D. Kitts. Effect of estrogenic plant extracts on the uterus of the laboratory rat. *Can. J. anim. Sci.* **43:** 106–112, 1963.
37. Padgett, C. S. and W. D. Ivey. The normal embryology of the *Coturnix* quail. *Anat. Rec.* **137:** 1–11, 1960.
38. Raitt, R. J. and R. E. Genelly. Dynamics of a populatoin of California quail. *J. Wildl. Mgmt* **28:** 127–141, 1964.
39. Sachs, B. D. Photoperiodic control of the cloacal gland of the Japanese quail. *Science* **157:** 201–203, 1967.
40. Somes, R. G., Jr. Recent findings in the inhibition of avian oogenesis. In: *Some Recent Findings in the Inhibition of Avian Reproductivity,* edited by D. K. Wetherbee. U.S. Fish and Wildl. Serv. Spec. Sci. Rep. Wildl. No. 67. Washington, DC: U.S. Government Printing Office, 1962, pp. 53–72.
41. Sturkie, P. D. Hypophysis. In: *Avian Physiology,* edited by P. D. Sturkie. New York: Springer-Verlag, 1976, pp. 287–300.
42. Sturkie, P. D. and W. J. Mueller. Reproduction in the female and egg production. In: *Avian Physiology,* edited by P. D. Sturkie. New York: Springer-Verlag, 1976.
43. Wentworth, B. C. Recent findings in inhibition of spermatogenesis. In: *Some Recent Findings in the Inhibition of Avian Reproductivity,* edited by D. K. Wetherbee. U.S. Fish and Wildl. Serv. Spec. Sci. Rep. Wildl. No. 67, 1962, pp. 73–86.
44. Wilson, M. I. and G. Bermant. An analysis of social interactions in Japanese quail. *Coturnix coturnix japonica. Anim. Behav.* **20:** 252–258, 1972.
45. Wilson, W. O. A review of the physiology of *Coturnix* (Japanese quail). *Wld's Poult. Sci. J.* **28:** 413–429, 1972.
46. Wilson, W. O., U. K. Abplanalp and L. Arrington. Sexual development of *Coturnix* is affected by changes in photoperiod. *Poult. Sci.* **41:** 17–22, 1962.
47. Woodard, A. E. and H. Abplanalp. The effects of mating ratio and age on fertility and hatchability in Japanese quail. *Poult. Sci.* **46:** 383–388, 1967.
48. Woodard, A. E., H. Abplanalp, W. O. Wilson and P. Vohra. *Japanese Quail Husbandry in the Laboratory.* Davis, CA: Department of Avian Sciences, 1963.