

Presentation of the Avian Species Comparison Study - A Protocol Development Study for the Avian 2-Generation Tier II Assay

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From the DRP-Revised August, 2004

Table 6-1. Fitness Endpoints Specific to Endocrine Active Substances^(a)

| Fitness Endpoints | Critical Life Stages | | | | Type of Endocrine Activity | | |
|---|----------------------|-------------------|-------------------|------------|----------------------------|------------|---------------|
| | Embryo genesis | Early development | Sexual maturation | Egg-laying | Estrogenic | Androgenic | Thyroidogenic |
| <i>For F1 and F2 chicks</i> | | | | | | | |
| number eggs laid per pair | | | | yes | +(^b) | | |
| number fertile eggs per eggs laid PTOX ^(d) | | | | yes | +/- ^(c) | +/-- | |
| number cracked eggs at set and at 2 weeks ETOX ^(e) | | | | yes | | | |
| number eggs hatched per eggs set DTOX ^(f) | yes | | | yes | +/-- | +/-- | |
| number chicks surviving to 7 and 14 days per eggs set and per eggs hatched DTOX | | yes | | | | | |
| growth rate of chicks (weight at Days 1, 7, 14) | | yes | | | +/-- | +/-- | +/-- |
| eggshell strength or thickness ETOX | | | | yes | | | |
| early and late viability per eggs set DTOX | yes | | | | +/-- | +/-- | |
| sex ratio of chicks | yes | | yes | | +/-- | +/-- | |
| <i>For Parents</i> | | | | | | | |
| body weight at start and end of treatment PTOX | | | yes | yes | | +/-- | +/-- |
| food consumption weekly during treatment PTOX | | | yes | yes | | +/-- | +/-- |
| male copulatory behavior | | | yes | yes | | | |
| signs of toxicity | | | yes | yes | - | + | |
| survival | | | yes | yes | | | |

a) Adapted from Bennett et al. (2001).

b) + indicates positive activity (e.g., estrogenic, androgenic, thyroidogenic).

c) - indicates negative activity (e.g., antiestrogenic, antiandrogenic, antithyroidogenic).

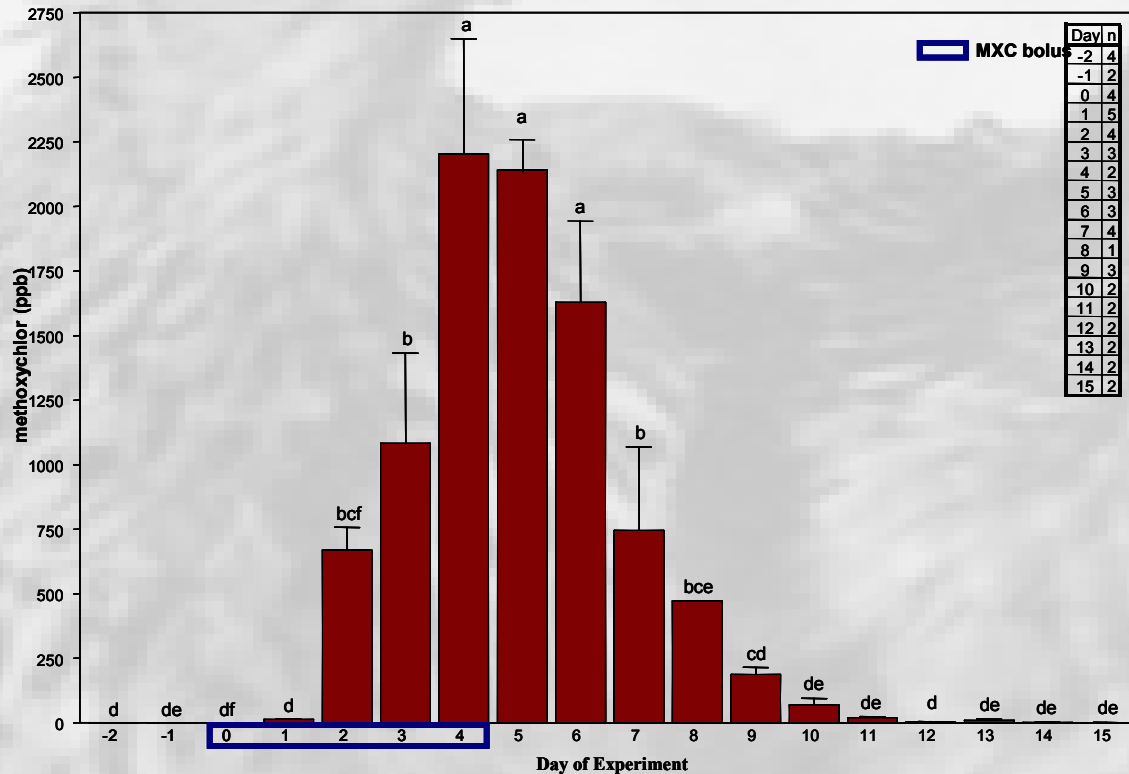
d) PTOX indicates endpoints that segregate into a group that reflect effects on *parental* health and reproduction.

e) ETOX indicates endpoints that segregate into a group that reflect effects on *egg shell quality*.

f) DTOX indicates endpoints that segregate into a group that reflect effects on *development*.

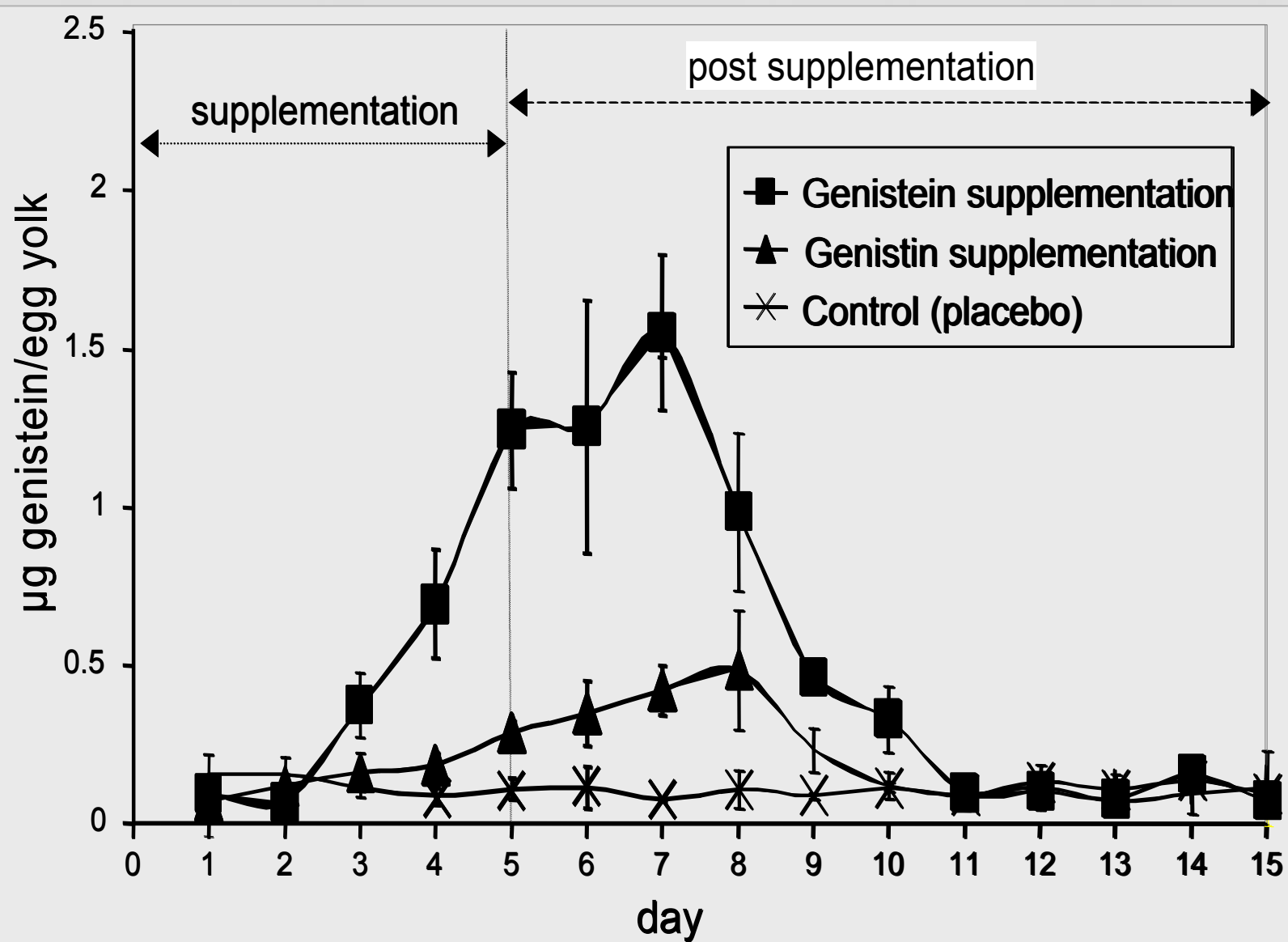
Maternal Transfer Study

MXC gel capsules (50 mg/day)



parental exposure → embryonic exposure

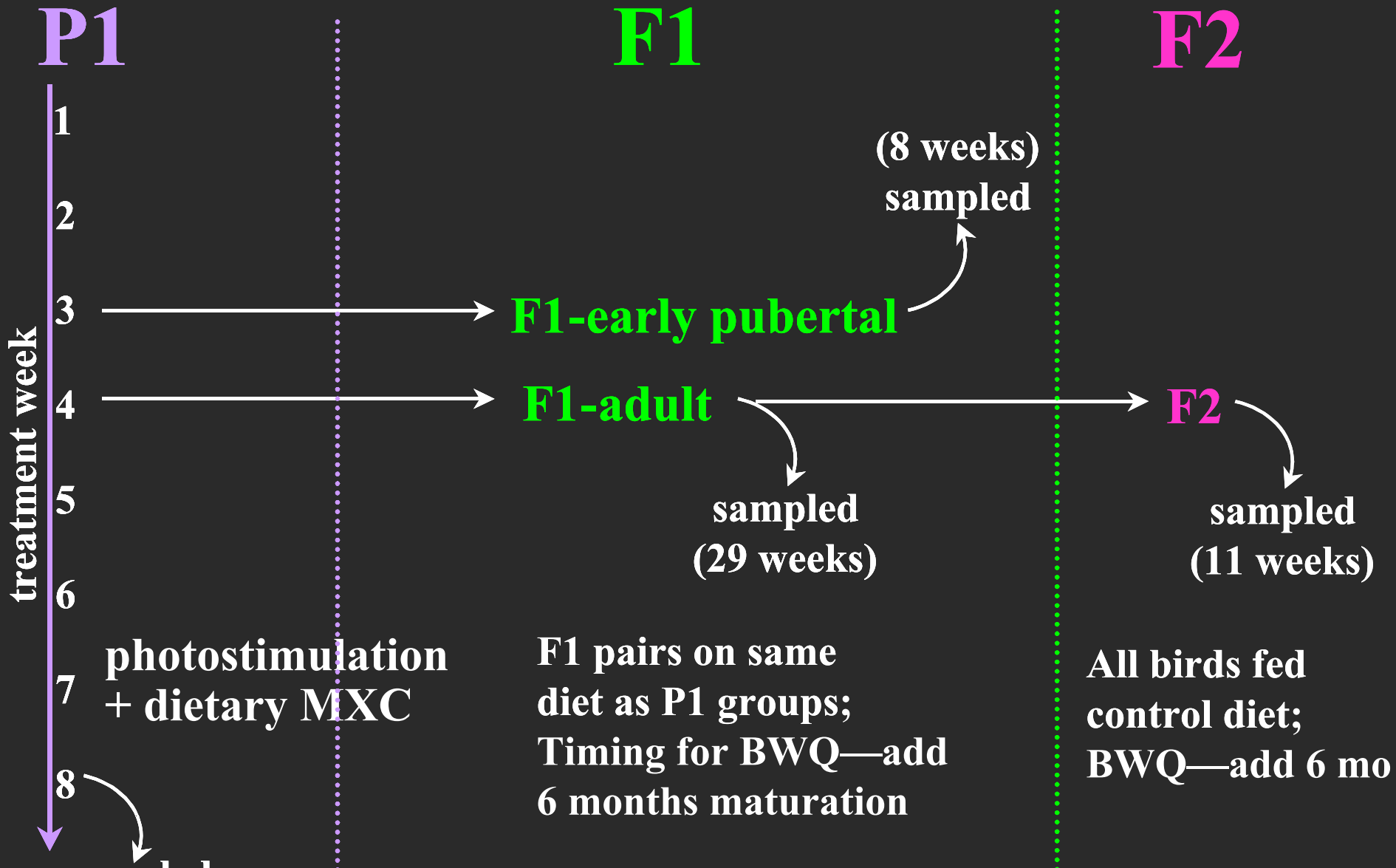
Wu, J., K. Henry, D. Donoghue, F. Schrenk, and M.A. Ottinger (2000).



Concentrations of Genistein in Japanese Quail egg yolks supplemented with genistein (aglycone), genistin (glucoside) or placebo capsules. Data points represent average of 4 replicates for treatment groups and 2 replicates for the control group. Modified from Lin et al, 2004.

Two Generation Test Design

(0, 5, and 10ppm Methoxychlor)



Overview of Experimental Schedule

JAPANESE QUAIL

BOBWHITE QUAIL

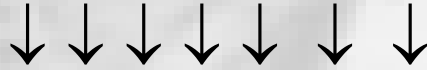
P1

P1



WEEK OF EXPERIMENT

WEEK OF EXPERIMENT



WK1 fert/ viab
WK2 fert/ viab
WK3 used for F1's
WK4 fert/ viab
WK5 fert/ viab
WK6 Day 1 pre pub.
WK7 ESS Post pub Behav Test Fecal

WK1 fert/ viab
WK2 fert/ viab
WK3 pre pub sample
WK4 used for F1's
WK5 fert/ viab
WK6 ESS
WK7 Day 1
WK8

6 months maturation!

F1

F1



WEEK OF EXPERIMENT

WEEK OF EXPERIMENT



WK1 fert/ viab
WK2 fert/ viab
WK3 used for F2's
WK4 fert/ viab
WK5 fert/ viab
WK6 Day 1 Behav Test
WK7 ESS

WK1 fert/ viab
WK2 fert/ viab
WK3 used for F2's
WK4 Day 1 fert/ viab
WK5 fert/ viab
WK6 fert/ viab
WK7 ESS

6 months maturation!

F2

F2



WEEK OF EXPERIMENT

WEEK OF EXPERIMENT

- F2's observed for onset of sex. Maturation
- Eggs used for fert/viab checks plus ESS
- Body and feed intake weights recorded
- Males Behaviorally Tested

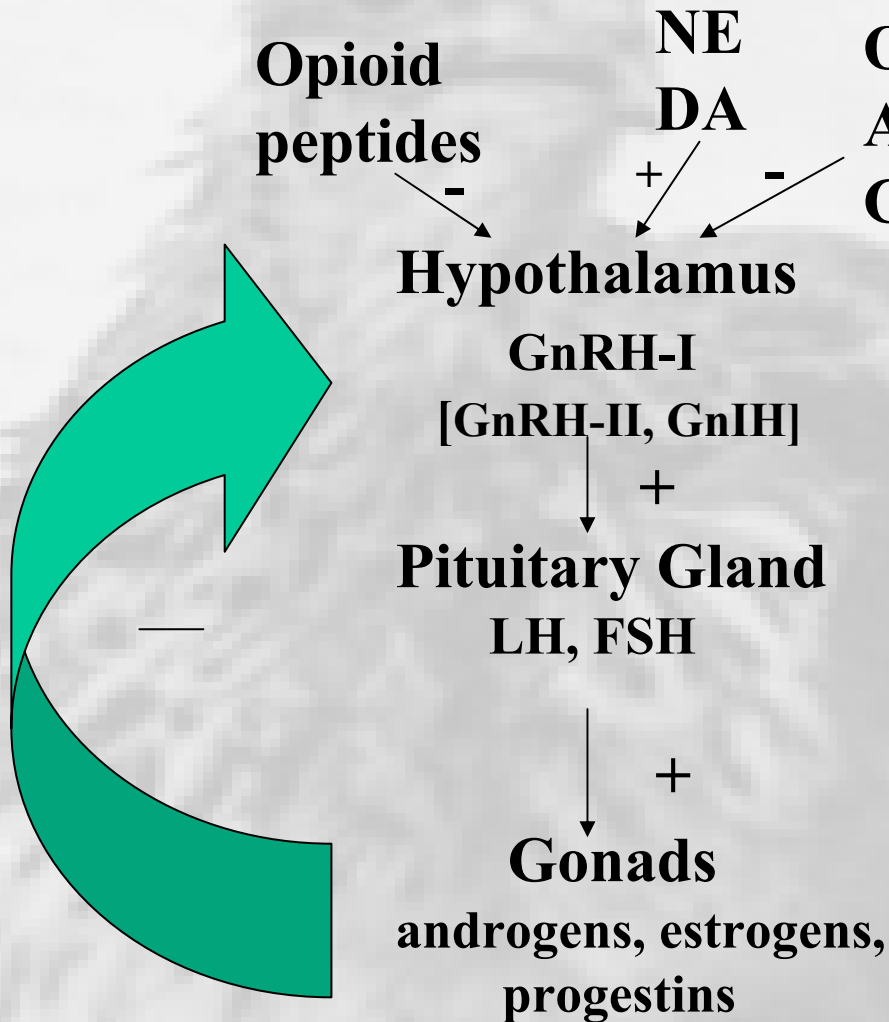
ESS: Egg Shell Strength
Fecal: Fecals Collected

fert/viab: Fertility/Viability Measured
Behav Test: Behaviorally Tested Males

pre pub: prepubertal sample
Day 1: Sampled on Day 1

post pub: post pubertal sample

Hypothalamic-Pituitary-Gonadal Axis in Japanese Quail



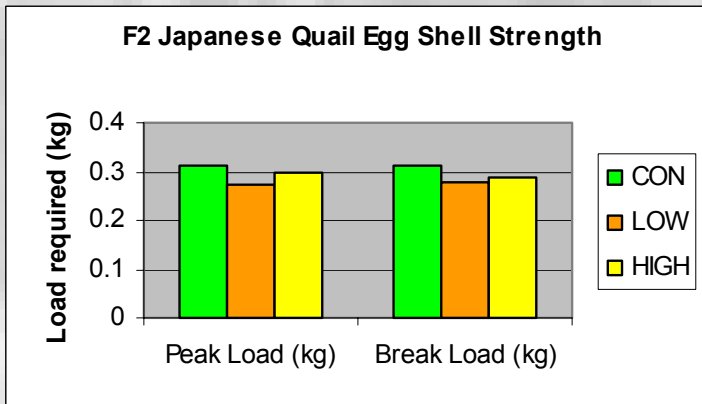
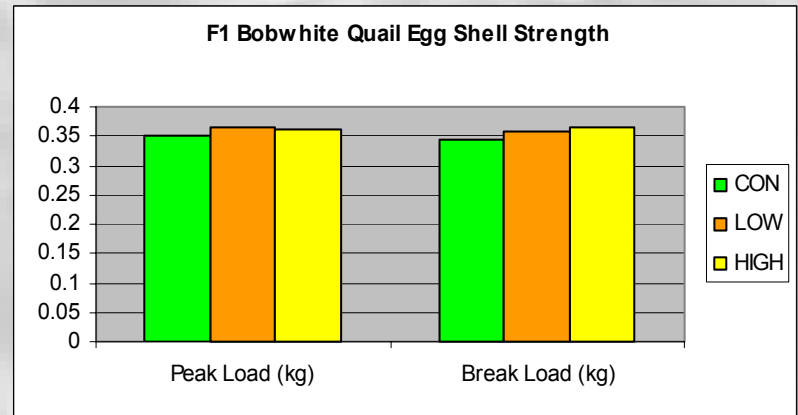
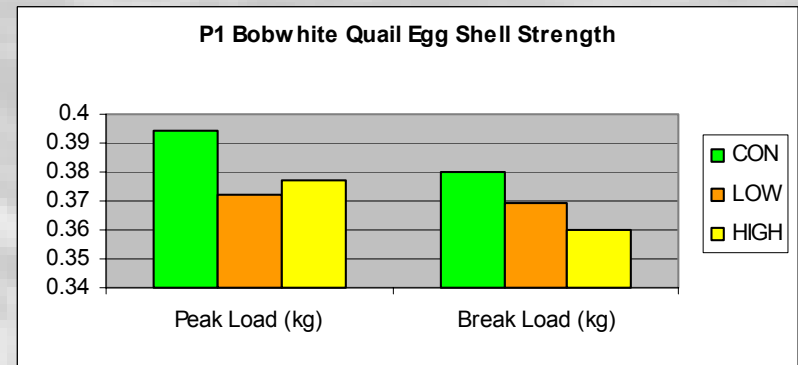
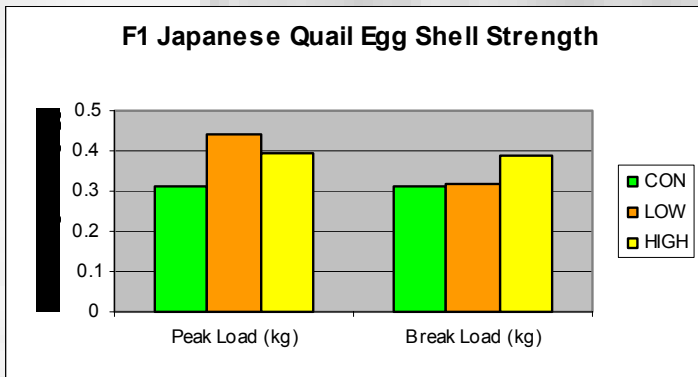
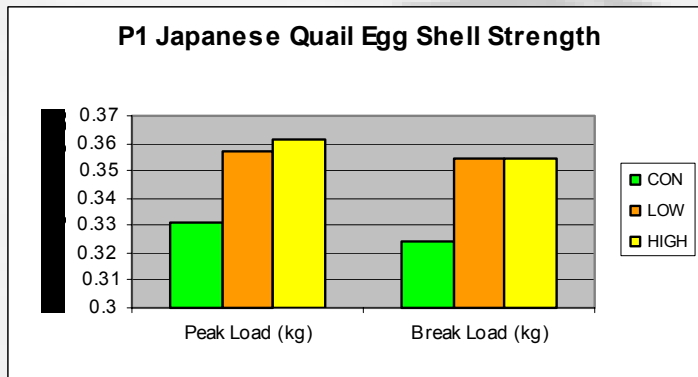
Neuroendocrine systems regulate endocrine and behavioral components of reproduction.

Potential Measurement Endpoints: Reliability and Sensitivity

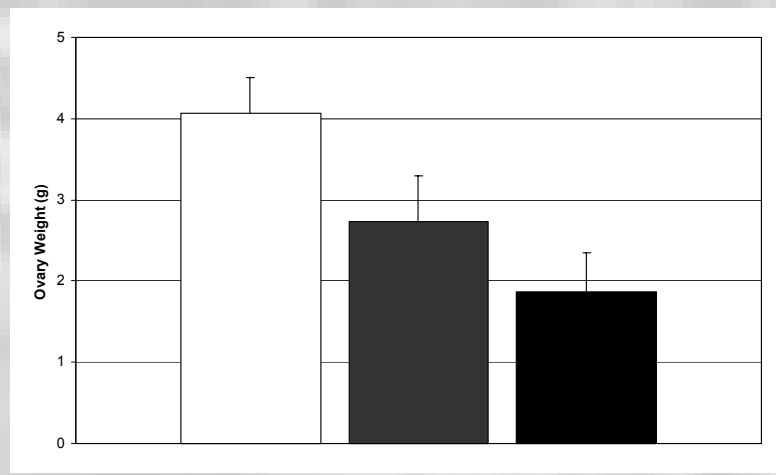
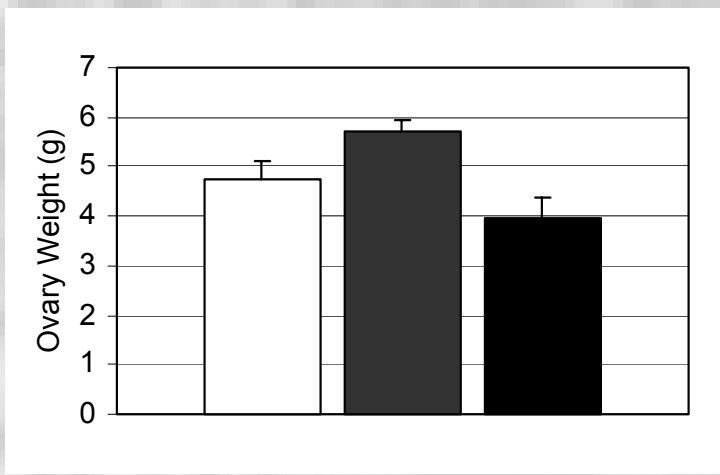
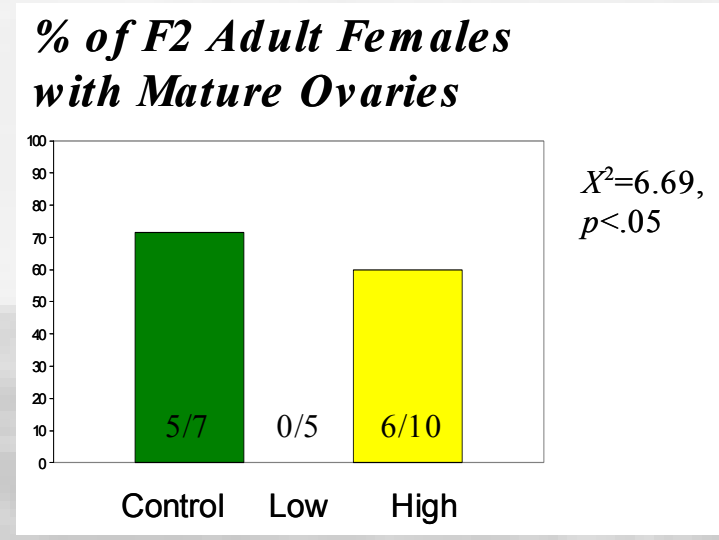
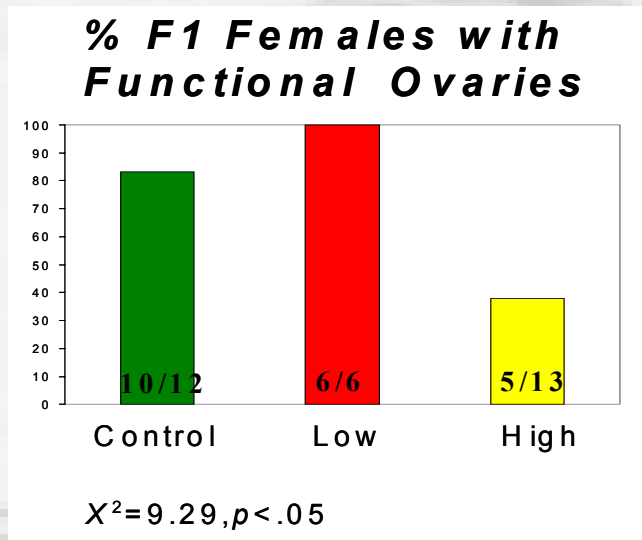
| Type of endpoint | Duration | Sensitivity |
|--|---------------------|----------------|
| Fitness (fertility, hatch, growth, sexual maturation) | short and long term | less sensitive |
| Behavioral (motor test, sexual behavior) | long term | very sensitive |
| Endocrine (plasma/fecal hormones, gonad morphology) | short and long term | variable |
| Neuroendocrine (NE, DA, AROM, GnRH-I) | short and long term | very sensitive |

from OECD Expert Group on Assessment
of EDC Effects in Birds Report, 2001.

No differences were observed in body weight, feed intake, egg production, or egg shell quality.



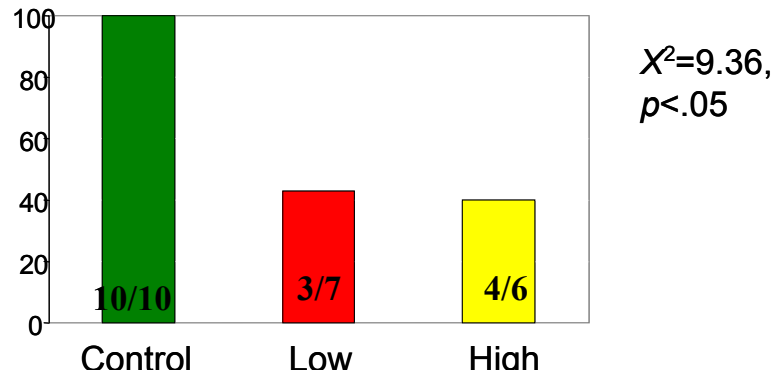
Japanese Quail: Ovarian Development and Maturation



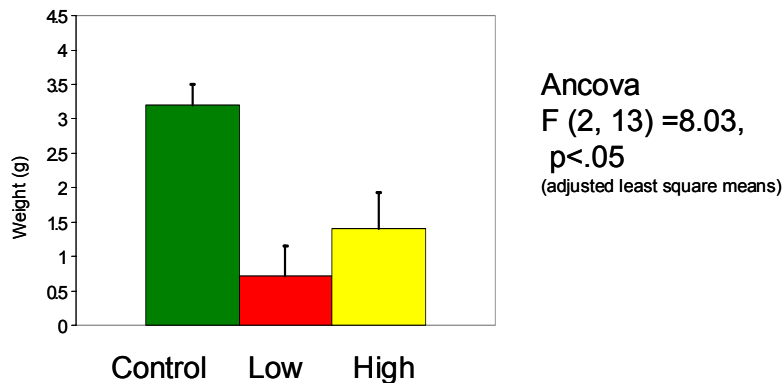
F1 hens in the high MXC group and both MXC groups in the F2 hens appeared to mature later; adults did not differ.

Sexual Maturation and Male Reproduction

% of F2 Males with Developed Testes at 7 Weeks of Age



F2 Testes Weight in Males at 7 weeks



Testes weight did not differ in adult males. No effects of dietary MXC were observed on testes weight in F1 males potentially due to sampling after sexual maturation was completed in all treatments. F2 males were sampled during maturation; MXC exposed F2 males appeared to be delayed relative to sexual maturation.

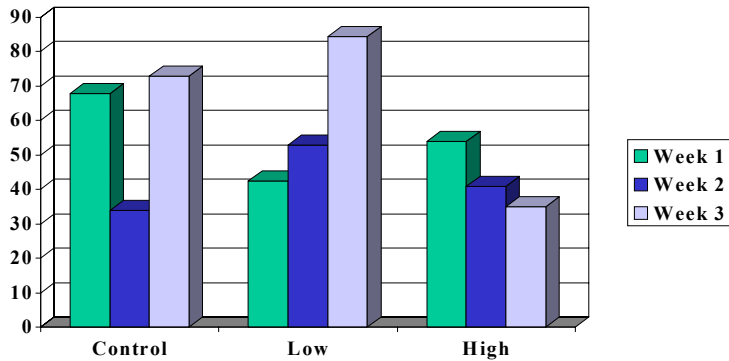
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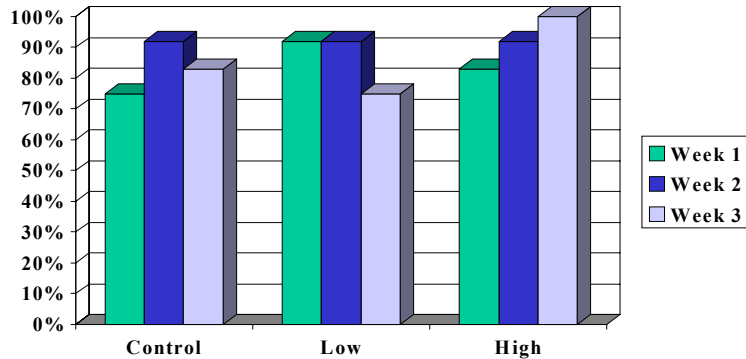
from OECD Expert Group on Assessment
of EDC Effects in Birds Report, 2001.

Open Field Runway Test

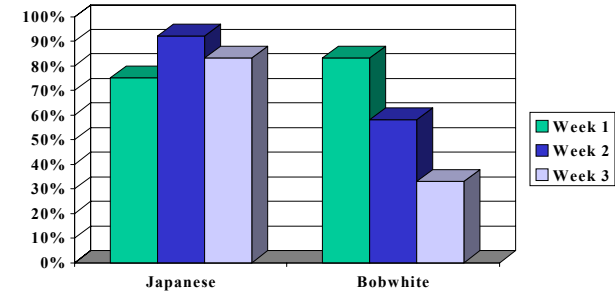
Average Time for the Japanese Quail to Cross the Finish Line (in sec.)



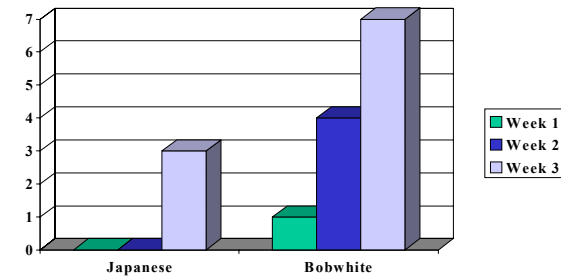
Percent of Japanese Quail to Cross the Finish Line



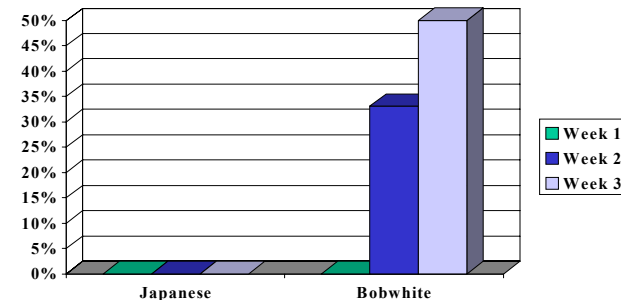
Percent of Japanese Quail Compared to Bobwhite Quail to cross the finish line



Lag Time for Bobwhite Quail Compared to Japanese Quail (number of birds with a lag time greater than 10 seconds)

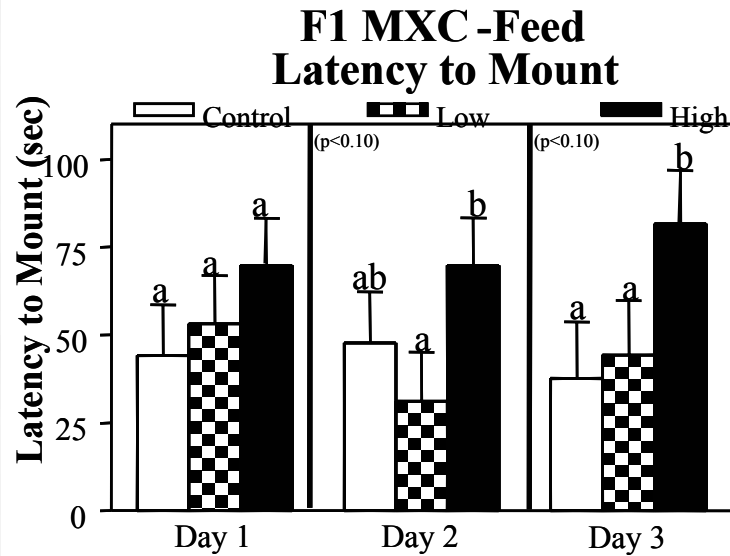


Percent of Japanese Quail Compared to Bobwhite Quail Immobile

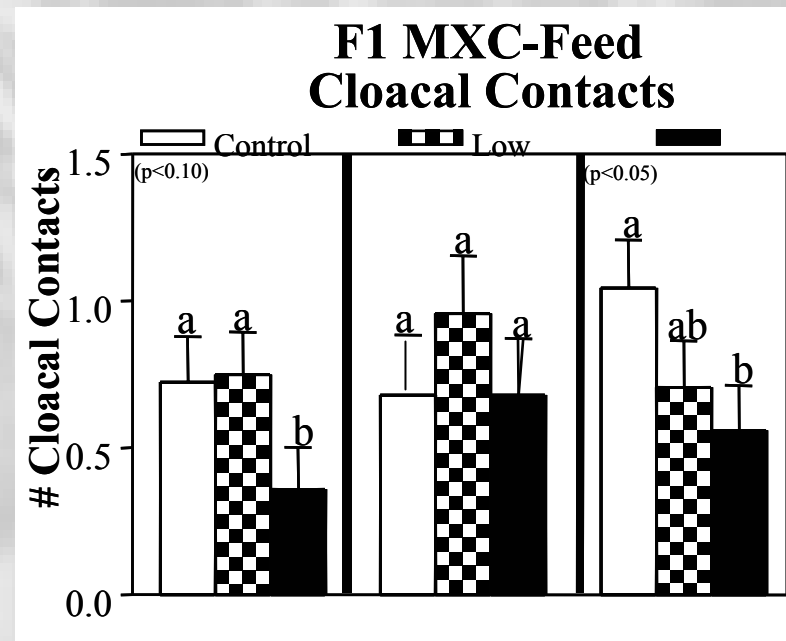
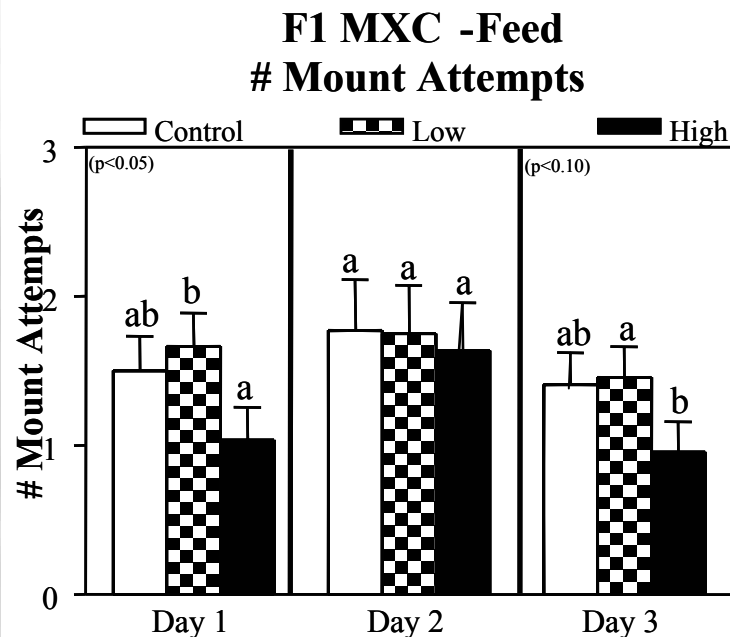


Young Japanese quail performed well in a test that required them to rejoin conspecifics (from Ottinger, Wu, Humphries, and Ottinger, unpublished data).

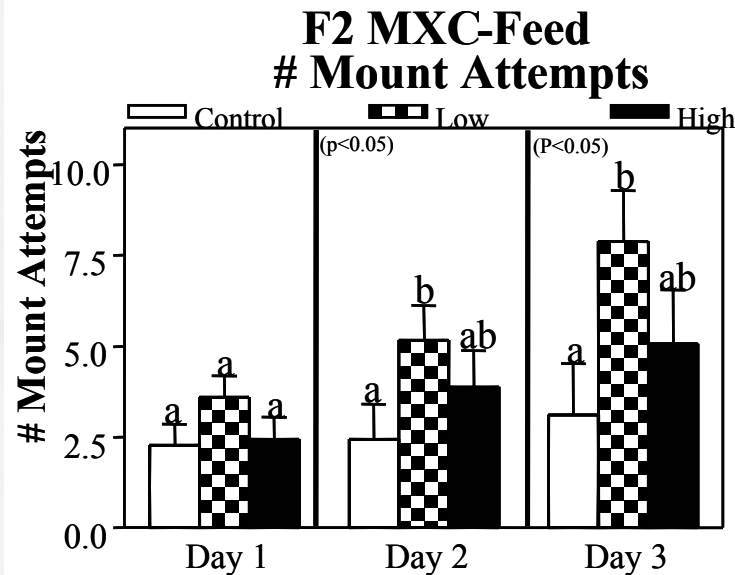
Male Reproductive Behavior: F1



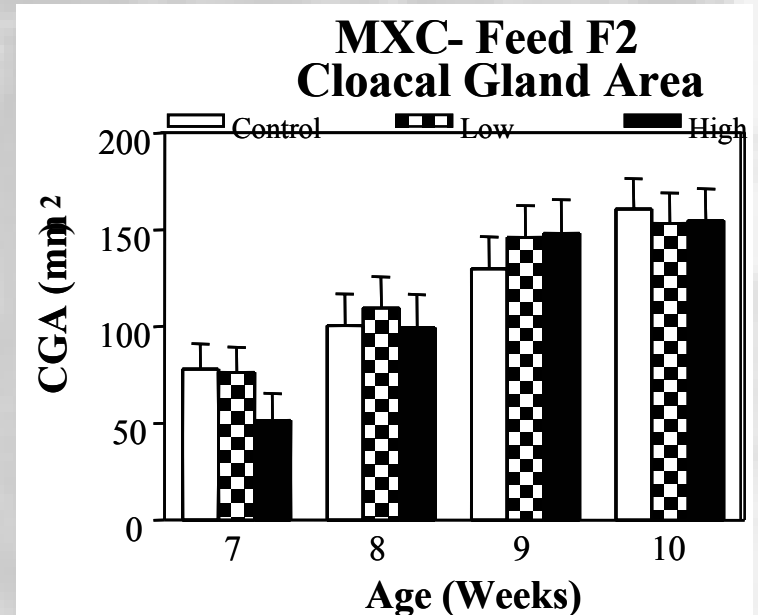
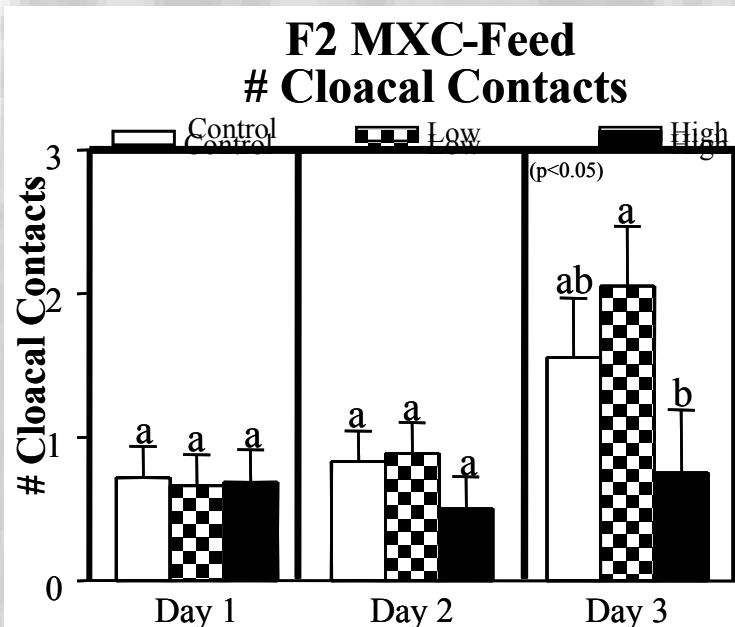
Males were separately housed; reproductive behavior was tested on 3 consecutive days. Dietary MXC was associated with impaired mating (longer latency to mate and reduced mating) in high MXC treatment males.



Male Reproductive Behavior: F2



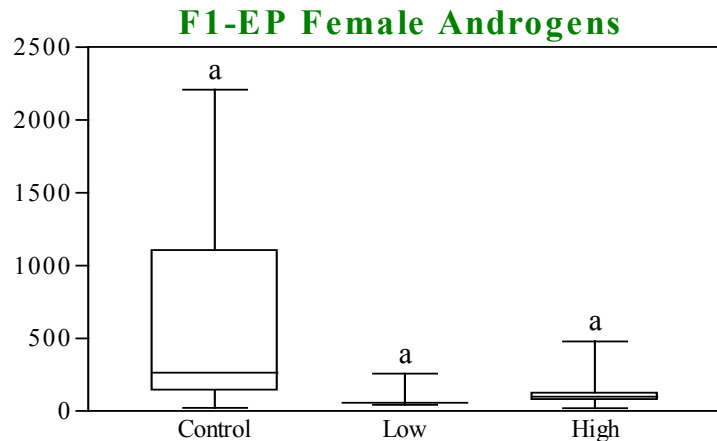
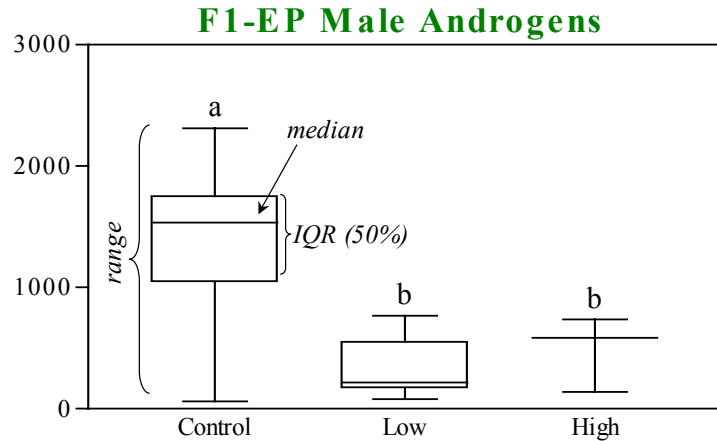
F2 Males continued to show behavioral effects in spite of rearing on control feed.



Potential Measurement Endpoints: Reliability and Sensitivity

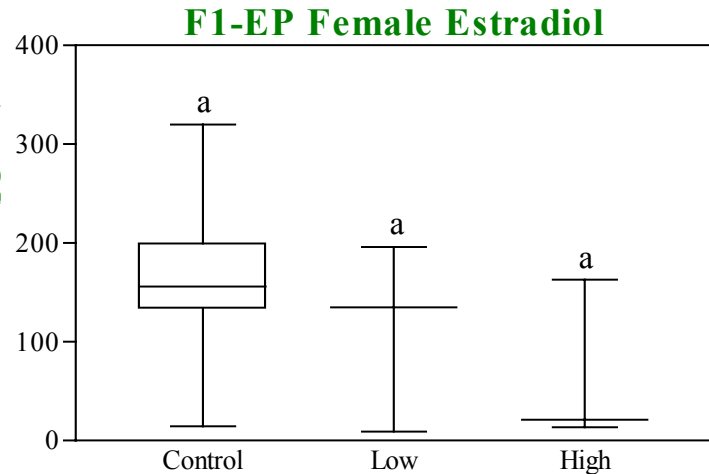
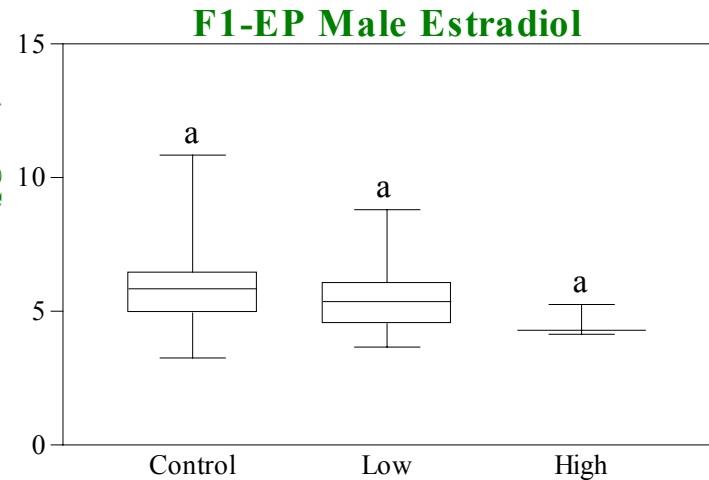
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from OECD Expert Group on Assessment
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Androgens:

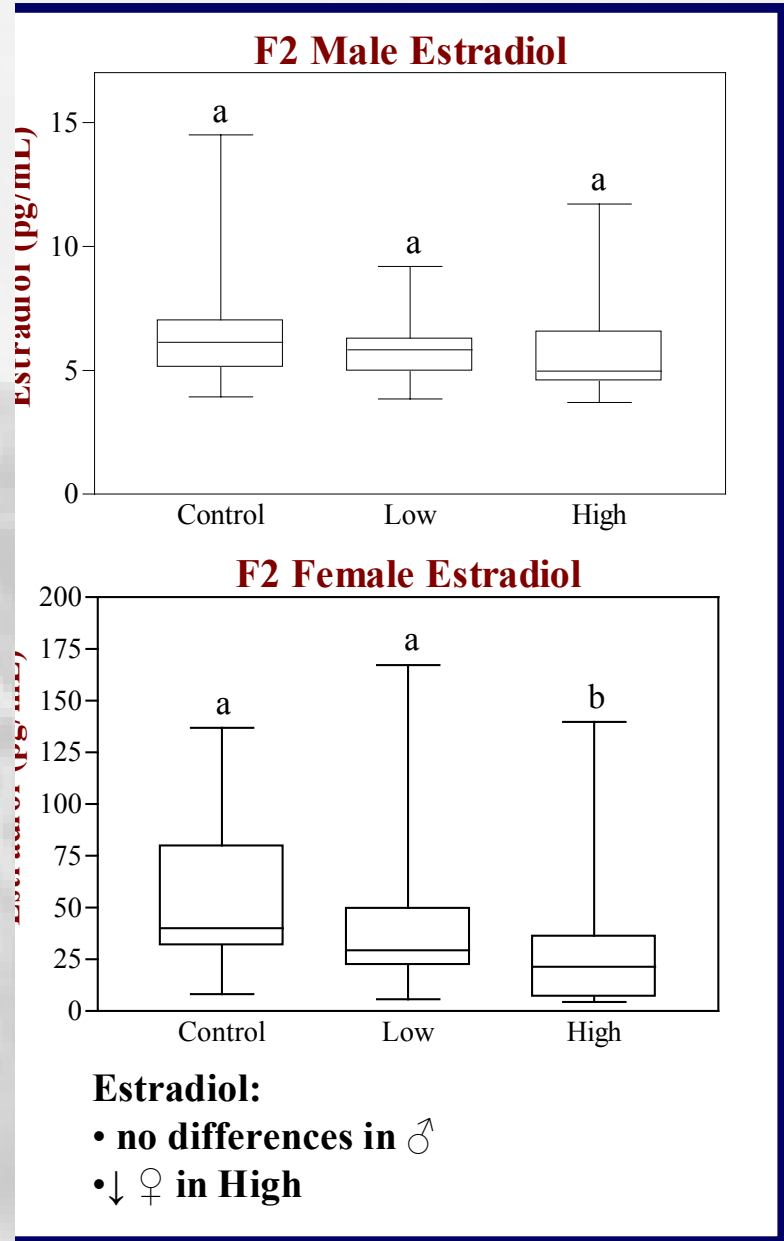
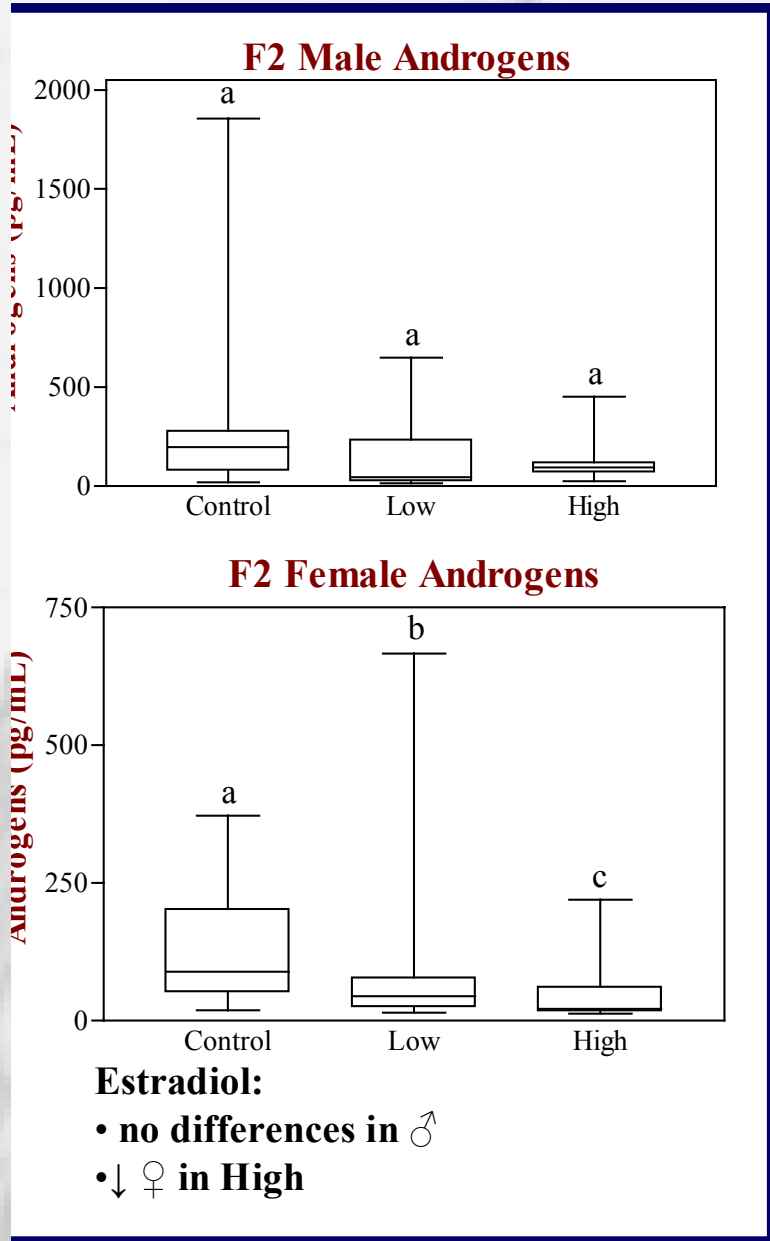
- ♂ ↓ with treatment
- No differences in ♀
- ↑ variability in Control birds



Estradiol:

- No differences in ♀ or ♂

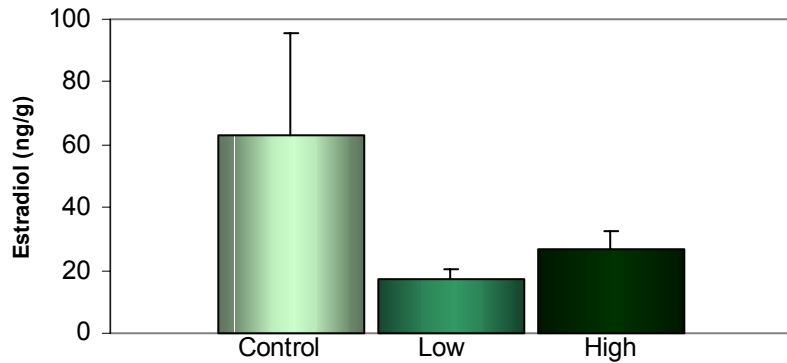
Plasma androgen was reduced in young males; variability was high during maturation in controls due to individual differences in onset of maturation.



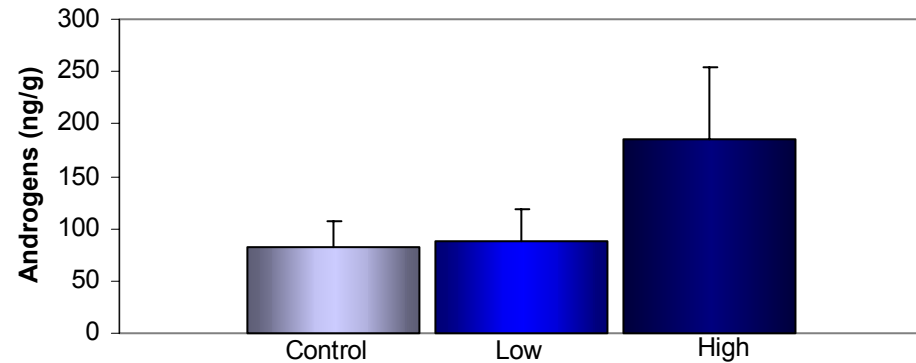
Adult females had reduced plasma estradiol as well as individual variability in plasma steroids.

F1 Post-Pubertal

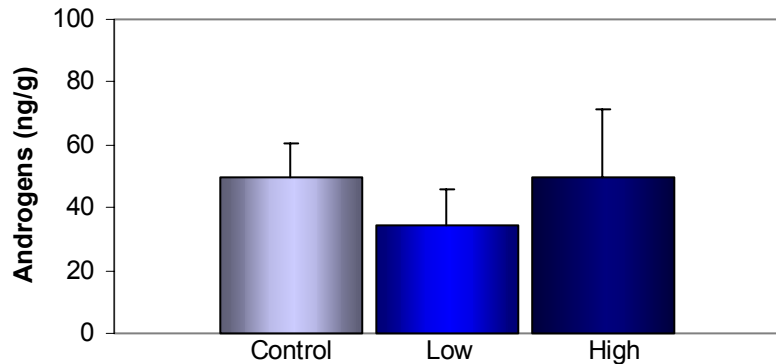
**JQ Post-Pubertal F1 Female
Fecal Estradiol**



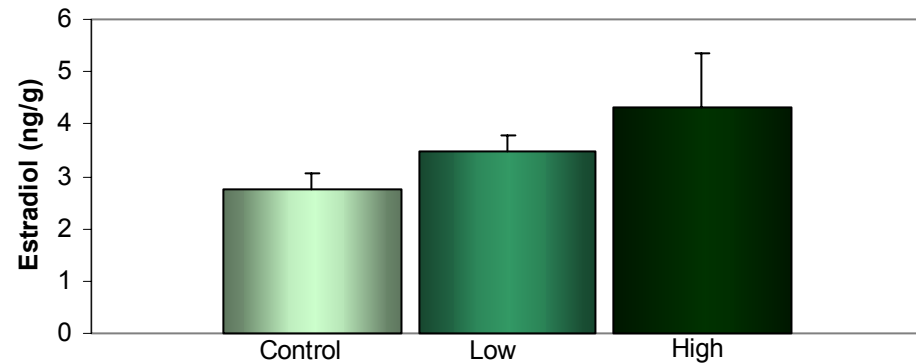
**JQ Post-Pubertal F1 Male
Fecal Androgens**



**JQ Post-Pubertal F1 Female
Fecal Androgens**

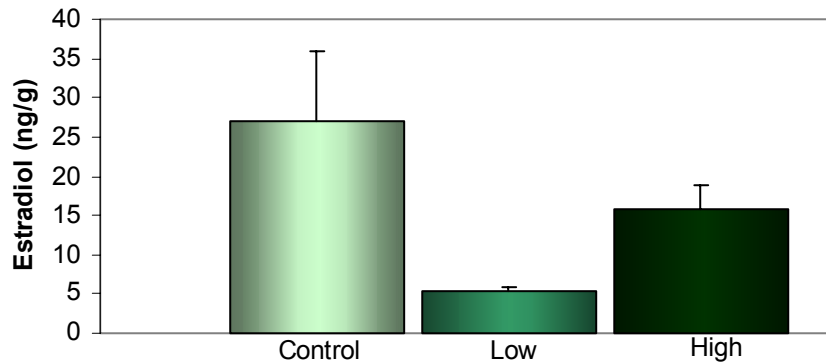


**JQ Post-Pubertal F1 Male
Fecal Estradiol**

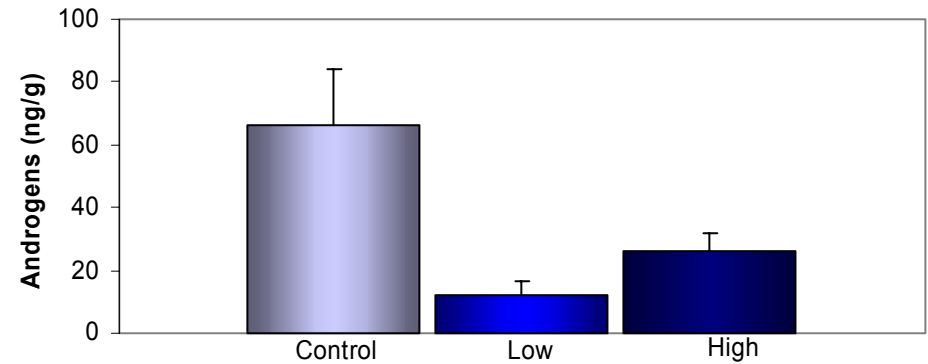


F2 Pre-Pubertal Fecal Steroids

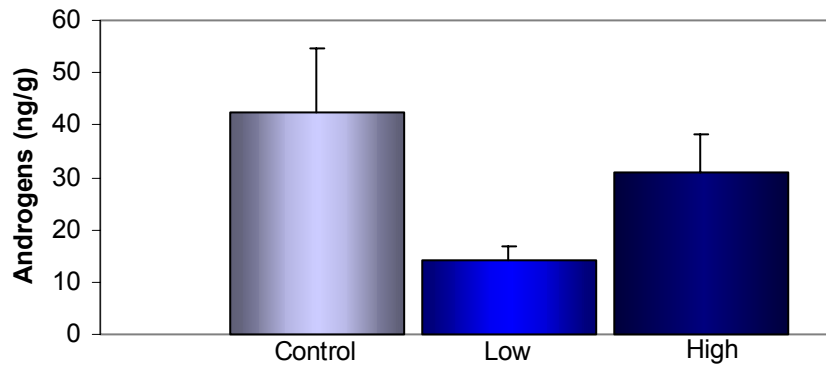
JQ Pre-Pubertal F2 Female
Fecal Estradiol



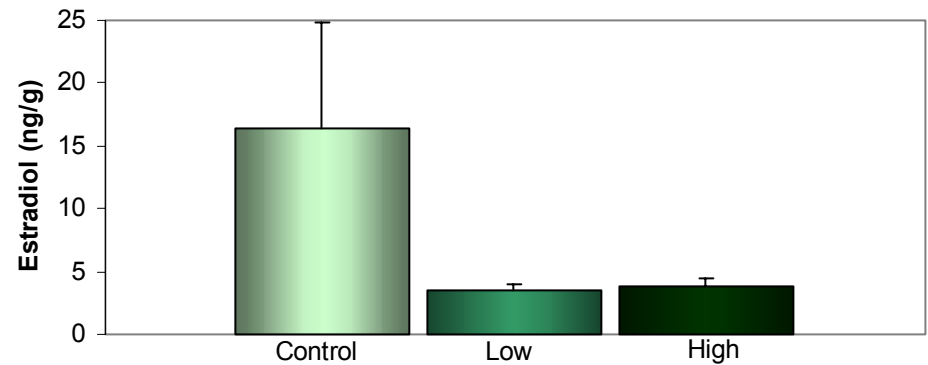
JQ Pre-Pubertal F2 Male
Fecal Androgens



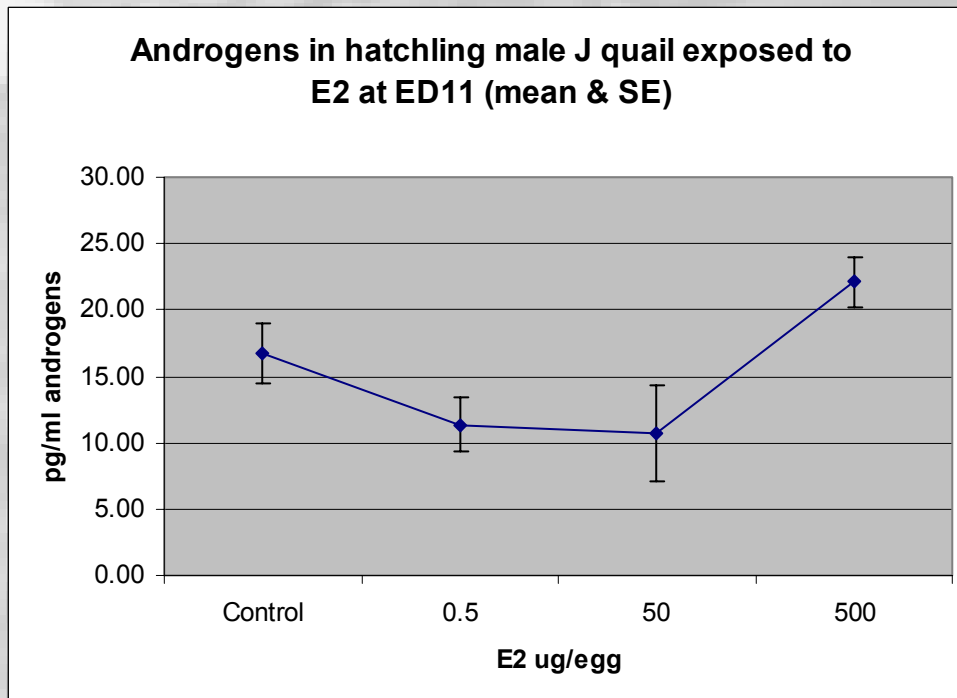
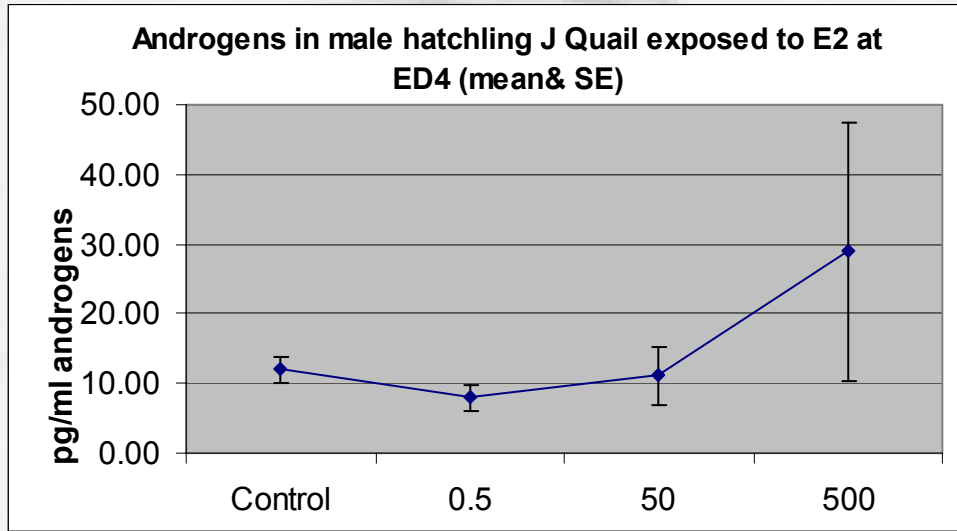
JQ Pre-Pubertal F2 Female
Fecal Androgens



JQ Pre-Pubertal F2 Male
Fecal Estradiol



Plasma Androgen in Hatchling Quail with Embryonic E2



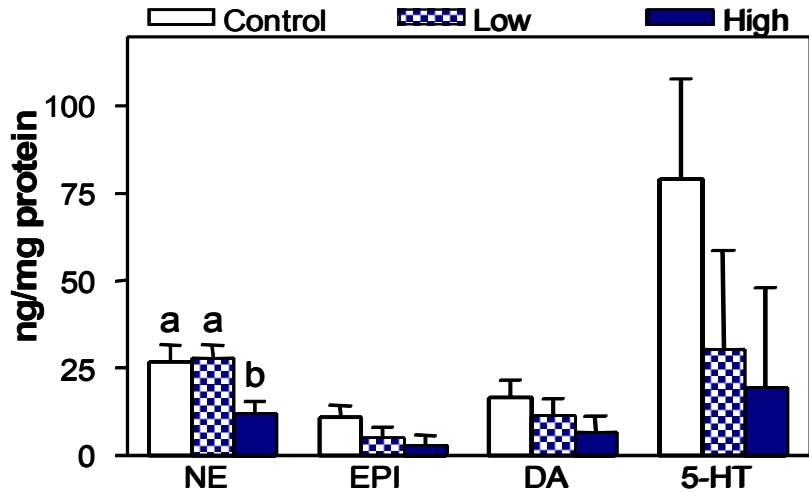
Male quail have elevated plasma androgen at hatch. In ongoing experiments, dose and timing of E2 exposure are under investigation.

Potential Measurement Endpoints: Reliability and Sensitivity

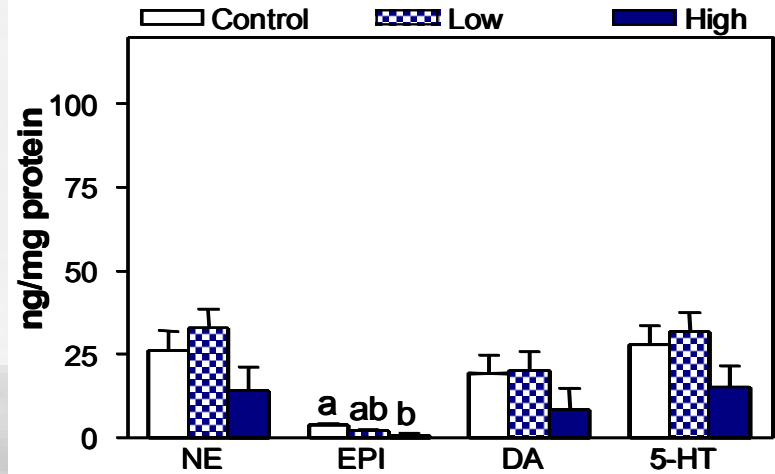
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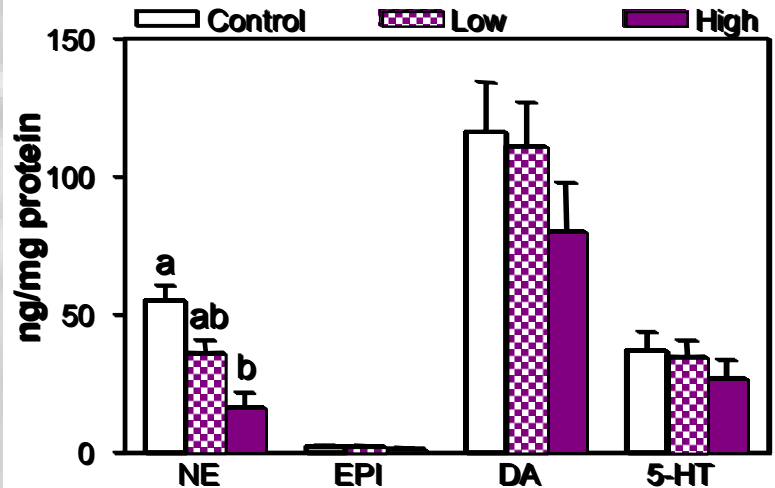
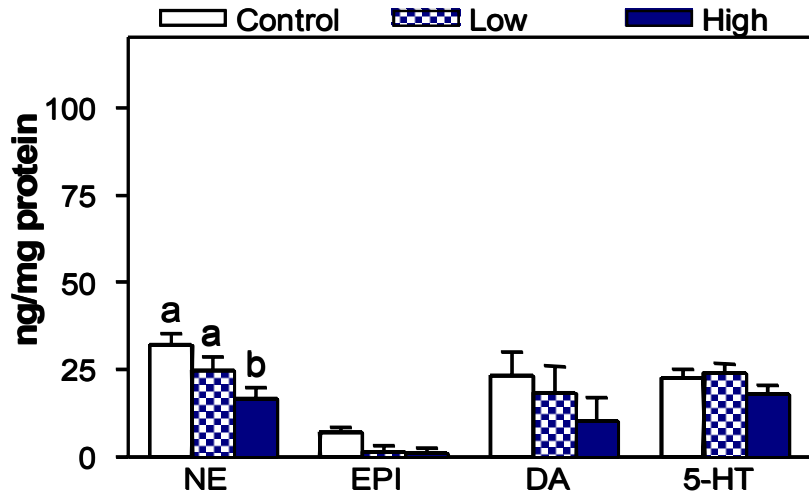
F1-Wk3 (female)



F1-Wk6 (female)



F2 d1 (female)



Altered hypothalamic catecholamines are found in post hatch MXC exposed birds.

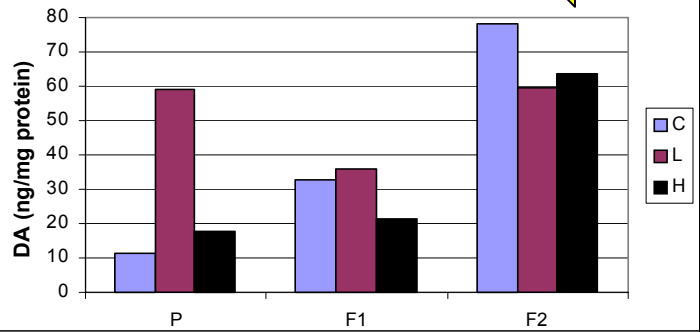
Anterior region

FEMALES

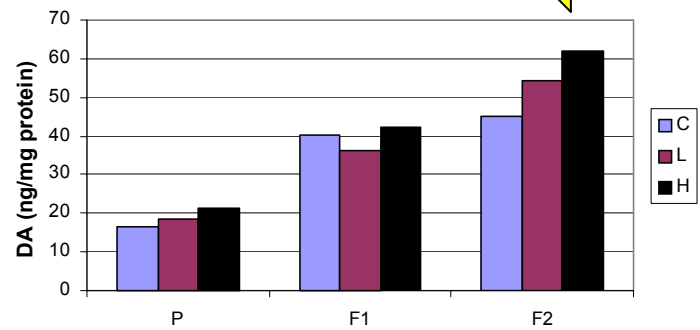
Posterior region



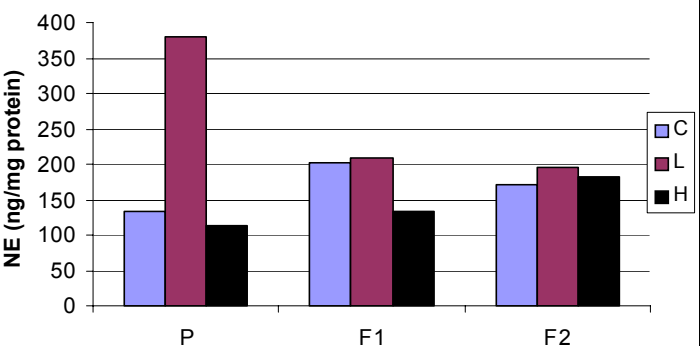
DA JQ F A



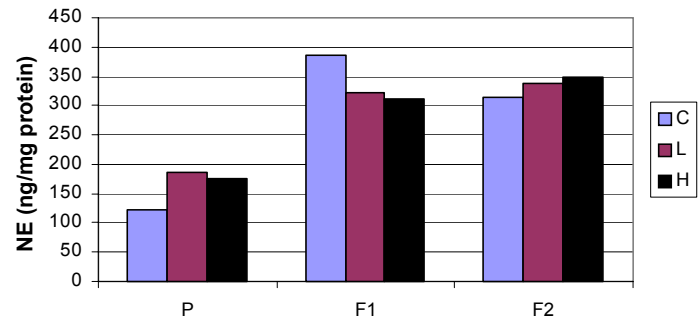
DA JQ F B



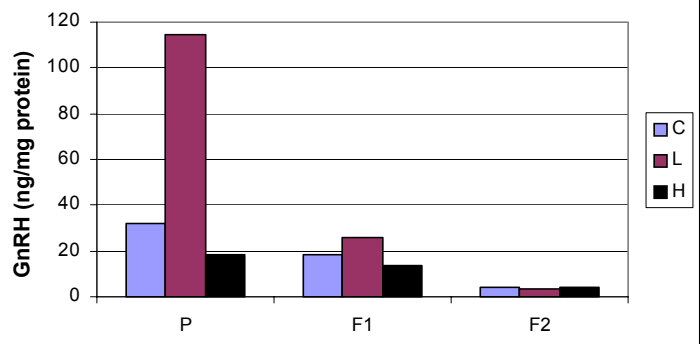
NE JQ F A



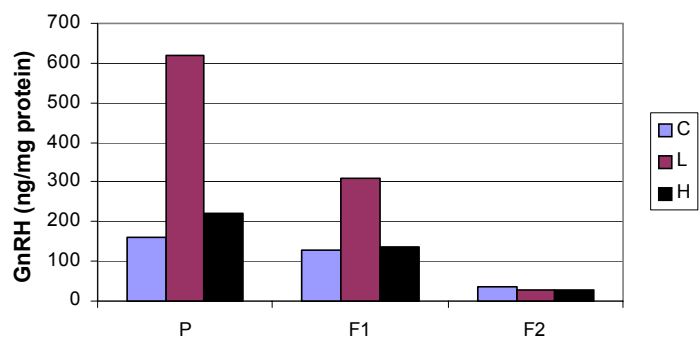
NE JQ F B



GnRH JQ F A



GnRH JQ F B



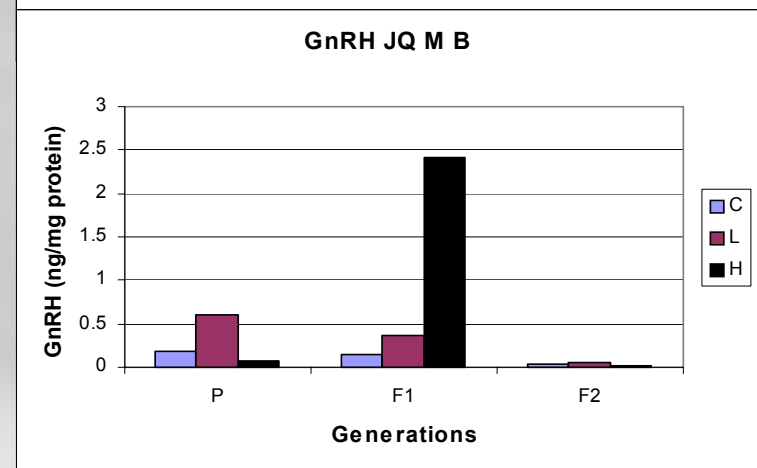
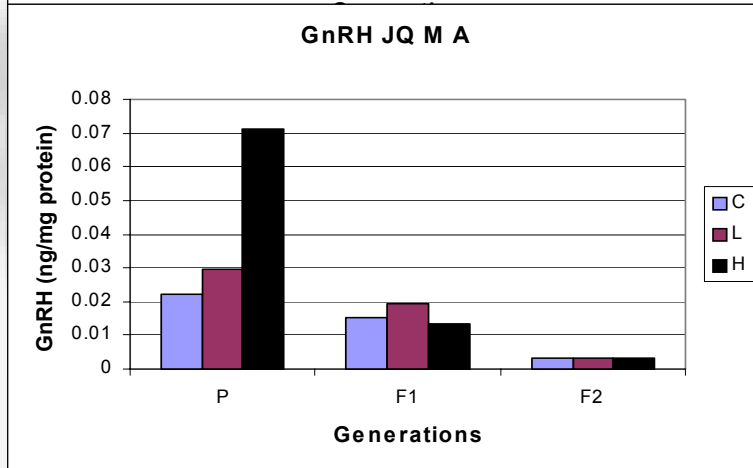
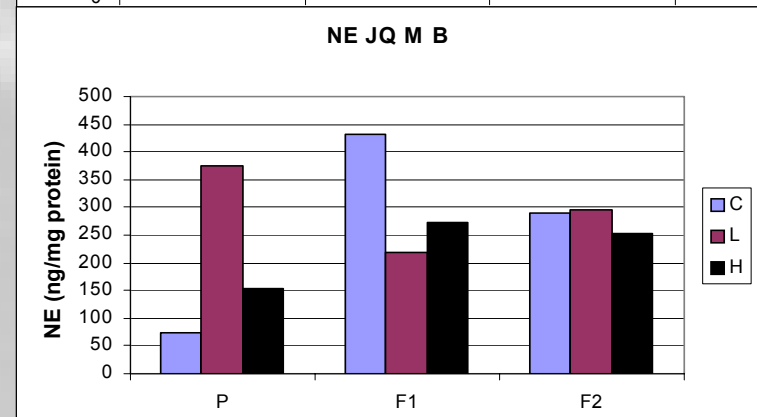
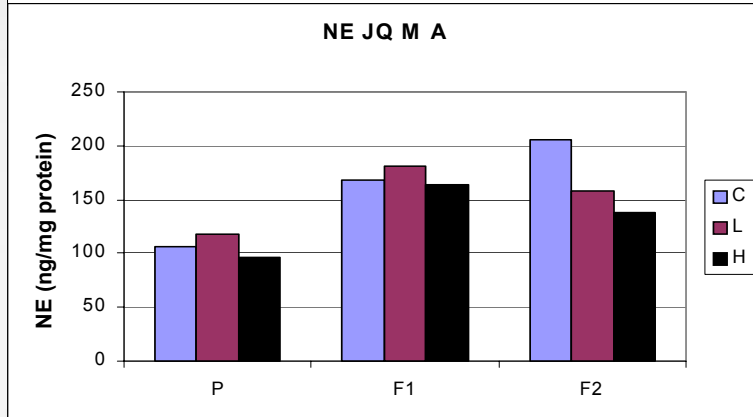
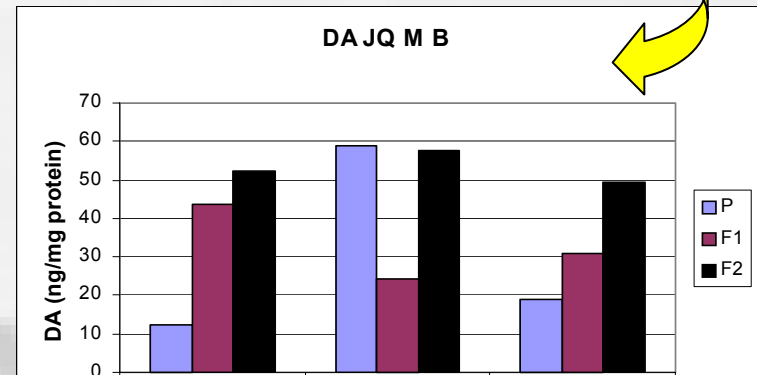
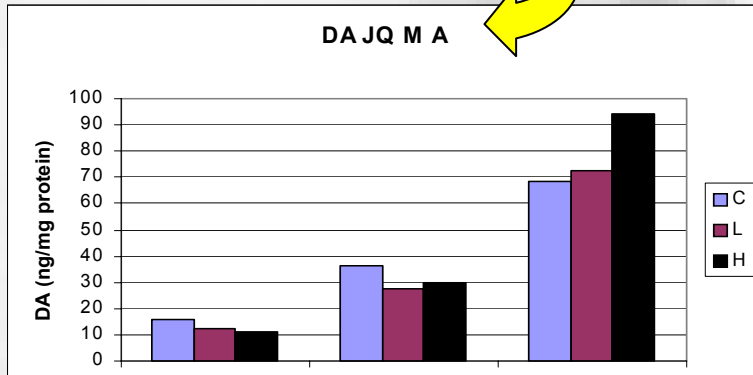
Generations

Generations

Anterior region

MALES

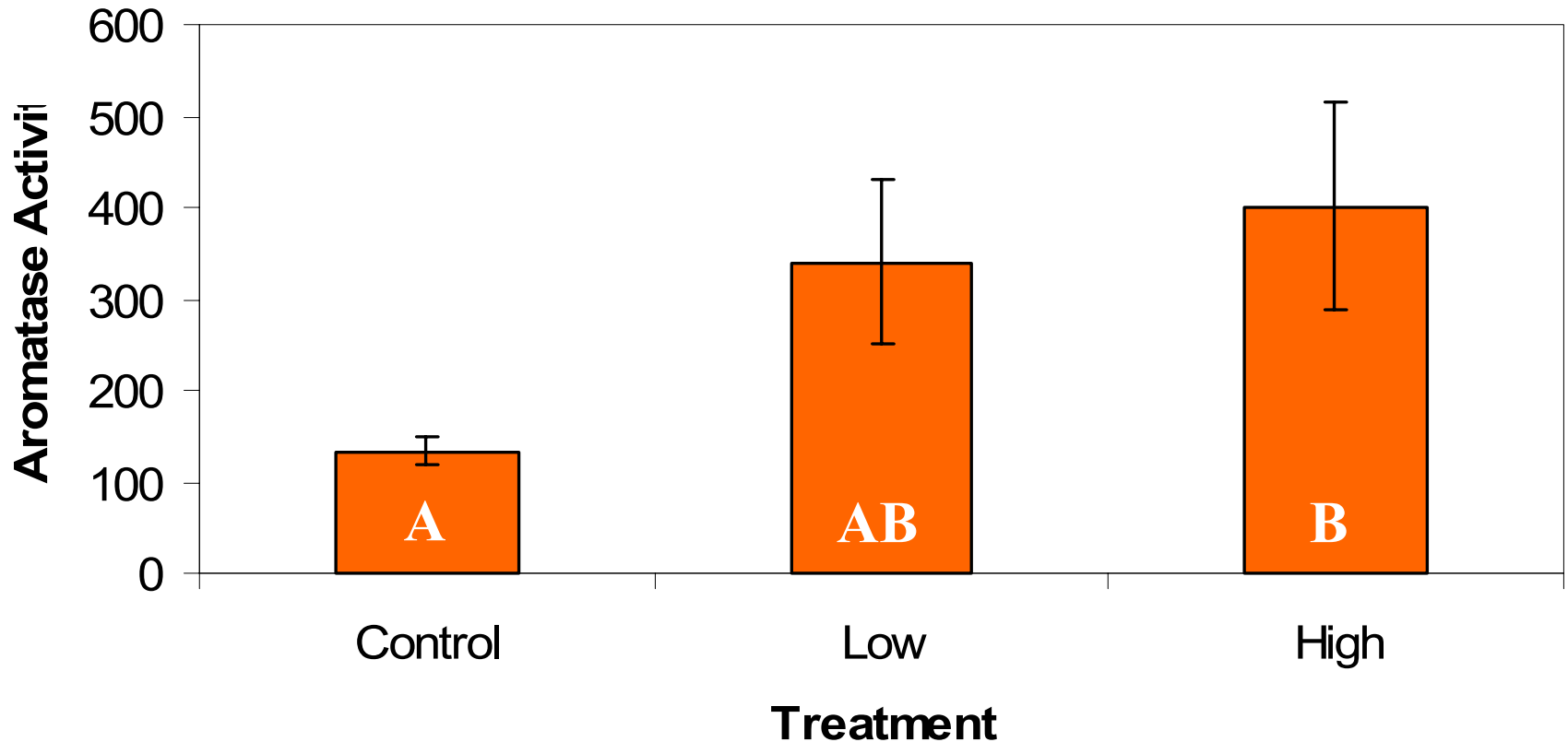
Posterior regions



Hypothalamic Aromatase Activity in Japanese Quail (F1) Exposed to Dietary Methoxychlor (5 and 10ppm)

Aromatase Activity
(fmol/hr*organ)/mg protein

P = 0.0534



| Group | N | Gender | Aromatase Activity \pm SE |
|--------------|----------|---------------|---|
|--------------|----------|---------------|---|

| | | | |
|--|-----------|----------|------------------------------------|
| | 10 | F | 124.3 \pm 19.1 |
|--|-----------|----------|------------------------------------|

| | | | |
|--|-----------|----------|-------------------------------------|
| | 13 | F | 414.0 \pm 158.7 |
|--|-----------|----------|-------------------------------------|

| | | | |
|--|-----------|----------|-------------------------------------|
| | 15 | F | 396.0 \pm 155.0 |
|--|-----------|----------|-------------------------------------|

| | | | |
|--|-----------|----------|------------------------------------|
| | 10 | M | 142.7 \pm 27.4 |
|--|-----------|----------|------------------------------------|

| | | | |
|--|-----------|----------|------------------------------------|
| | 12 | M | 260.5 \pm 81.6 |
|--|-----------|----------|------------------------------------|

| | | | |
|--|-----------|----------|-------------------------------------|
| | 11 | M | 406.7 \pm 173.2 |
|--|-----------|----------|-------------------------------------|

Potential Measurement Endpoints: Reliability and Sensitivity

| Type of endpoint | Duration | Sensitivity |
|--|---------------------|----------------|
| Fitness (fertility, hatch, growth, sexual maturation) | short and long term | less sensitive |
| Behavioral (motor test, sexual behavior) | long term | very sensitive |
| Endocrine (plasma/fecal hormones, gonad morphology) | short and long term | variable |
| Neuroendocrine (NE, DA, AROM, GnRH-I) | short and long term | very sensitive |

Anything Else???

from OECD Expert Group on Assessment
of EDC Effects in Birds Report, 2001.

Measurements

Reproductive maturation:

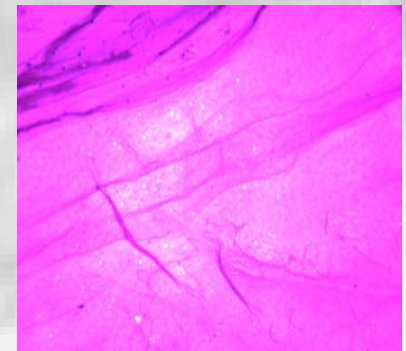
1. Female onset of puberty
2. Ovarian follicle counts
3. Male proctodeal gland size
4. Adult ovarian and testicular weights

Reproductive behavior:

1. Latency to mount
2. Latency to successful copulation
3. # of successful cloacal contacts

Sperm quality:

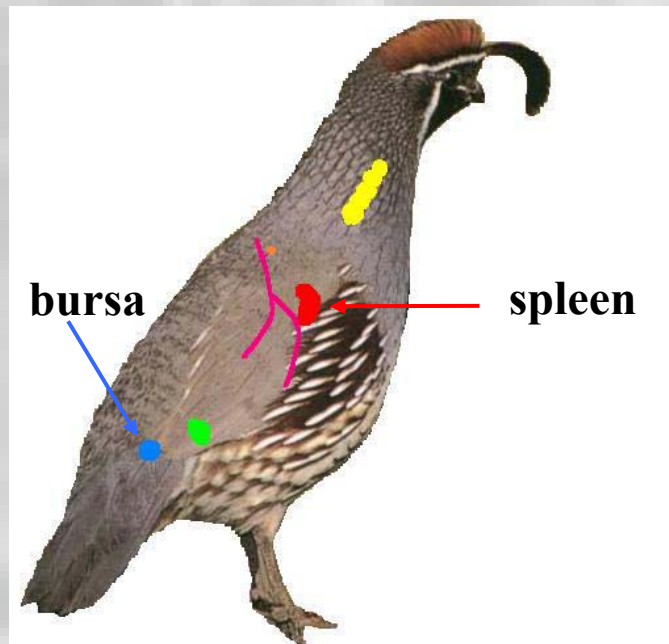
1. Motility
2. Penetration of the germinal disc



Measurements

Immune system development:

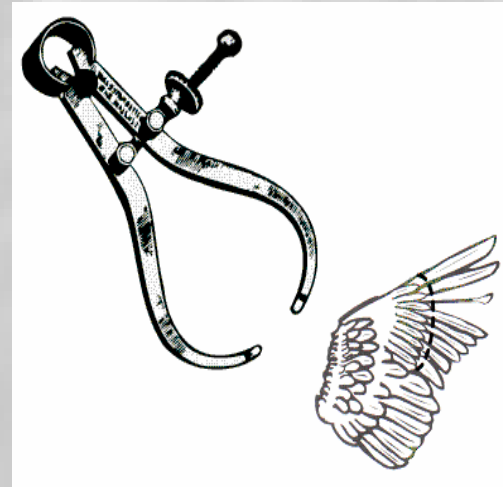
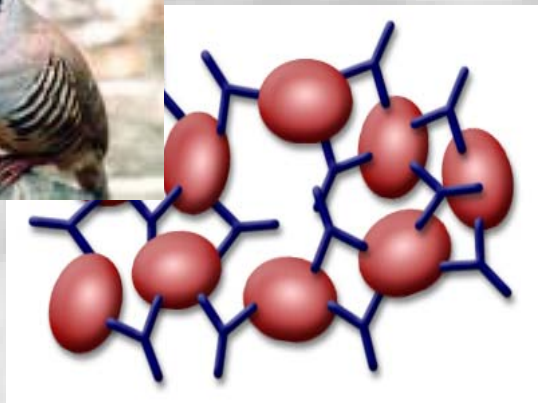
- 1. Organ weights – bursa and spleen**
- 2. Bursal morphology**
- 3. Lymphocyte counts**



Measurements

Immune system function:

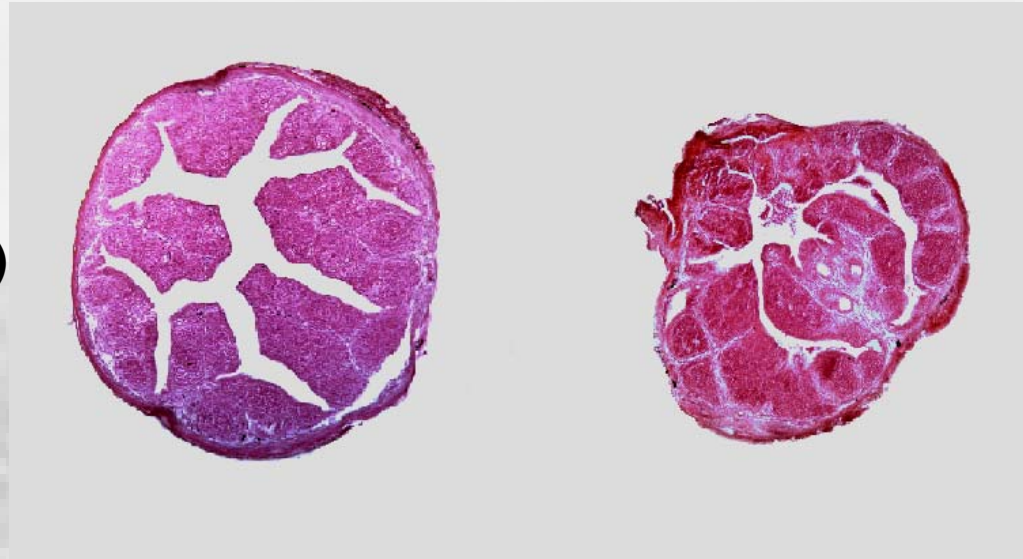
- 1. Humoral – response to chukar red blood cells**
- 2. Cell-mediated – PHA-P wing web test**



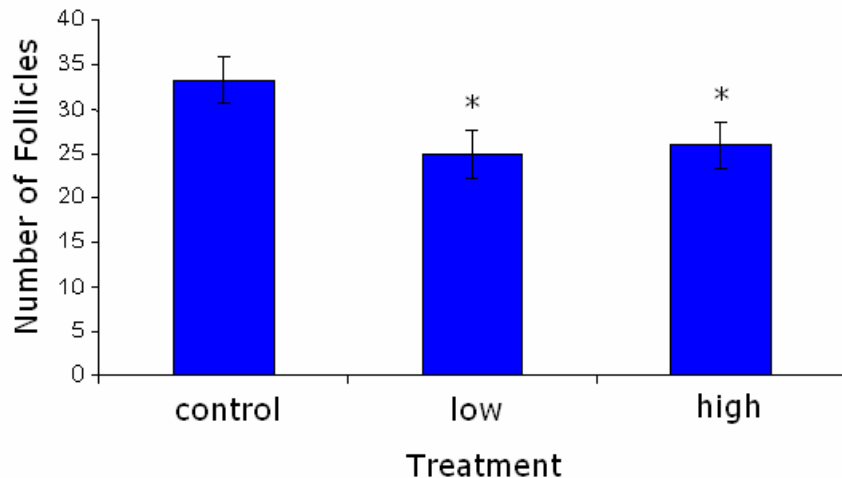
DDE Treatment Effects

(p,p'-dichlorodiphenyldichloroethane)
egg injection (E1 into yolk)

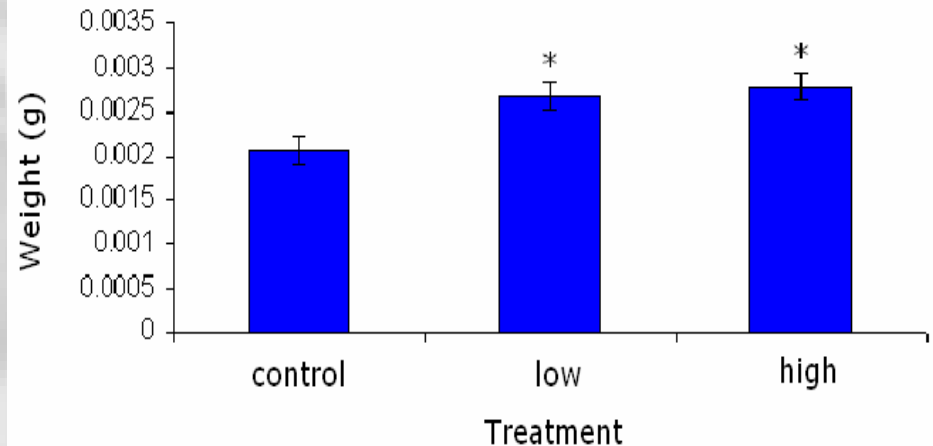
- sesame oil (vehicle control)
- 20 μg DDE (low)
- 40 μg DDE (high)



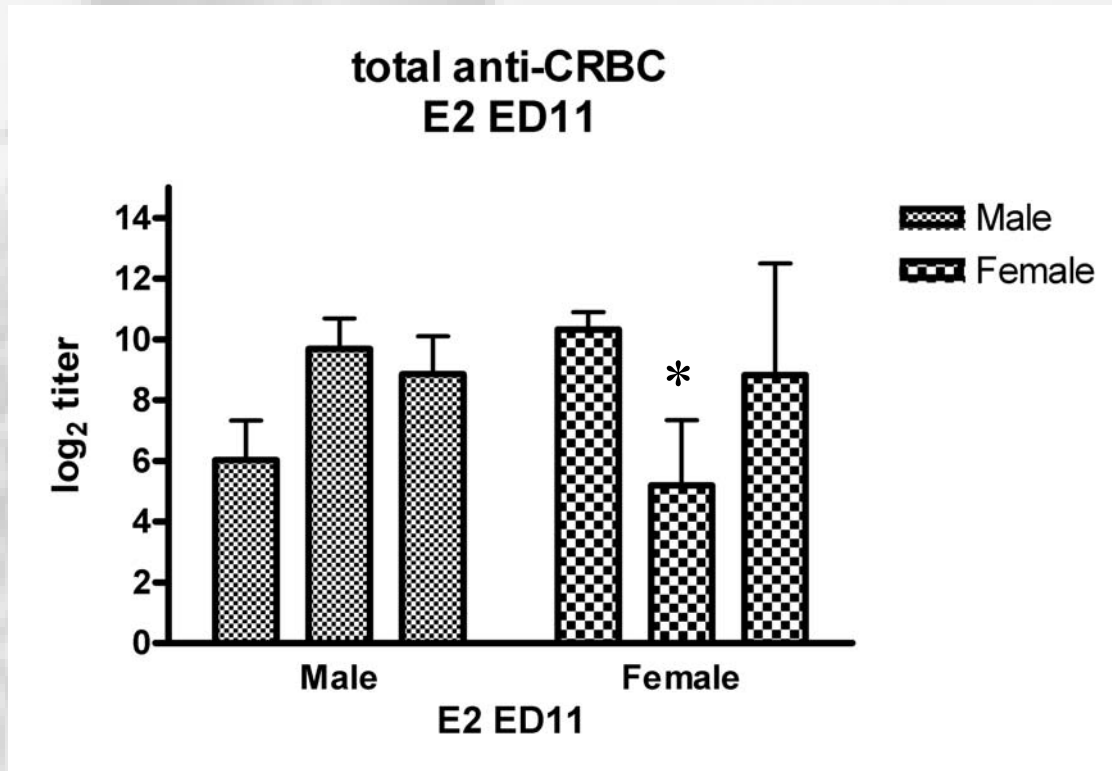
Follicle Numbers



Bursa Weights



E2 Affected Some Measures of Immune Response



Antibody titers to chuckar red blood cells, bars are control, 50 and 500 ug E2/egg at ED 11, female response decreased ($p < 0.05$) from control.

Some other considerations....

- Strain of Quail
 - Use white egg strain (Wada? And UMCP)
 - Size (Japan and Hawaii—wildtype strains)
 - Marks, Delaney, Siegel genetically characterized strains
- Separate Toxicological from Endocrine End Points
- Management (diet, aggression, lighting, environment)
- Baseline Fertility and Productivity of Strain
- Apply to Risk Assessment in the Field
 - Interpret birds being there and reproducing!!!

Effects of an Estrogenic PCB

Exposure at E4 by egg injection resulted in decreased reproductive performance (fertility and egg production), due in part to reduced male reproductive behavior.

| Generation | Treatment | n | Latency (sec) | Attempts | Cloacal Contacts |
|------------|-----------|----|---------------|------------|------------------|
| P1 | Control | 13 | 35 ± 10.05 | 5.5 ± 1.98 | 2.16 ± 0.45 |
| | PCB | 8 | 77.8 ± 19.45 | 5.9 ± 0.97 | 2.0 ± 0.44 |
| F1 | Control | 11 | 42.6 ± 14.44 | 8.4 ± 1.95 | 2.3 ± 0.47 |
| | PCB | 6 | 107.0 ± 14.97 | 2.8 ± 0.82 | 0.3 ± 0.20 |
| F2 | Control | 16 | 49.8 ± 9.96 | 6.0 ± 0.86 | 2.2 ± .40 |
| | PCB | 12 | 77.0 ± 13.25 | 5.3 ± 2.6 | 0.9 ± 0.33 |

PCB= 2',4',6'-trichloro-4-biphenylol

P1= parent generation

F1= first generation

F2= second generation

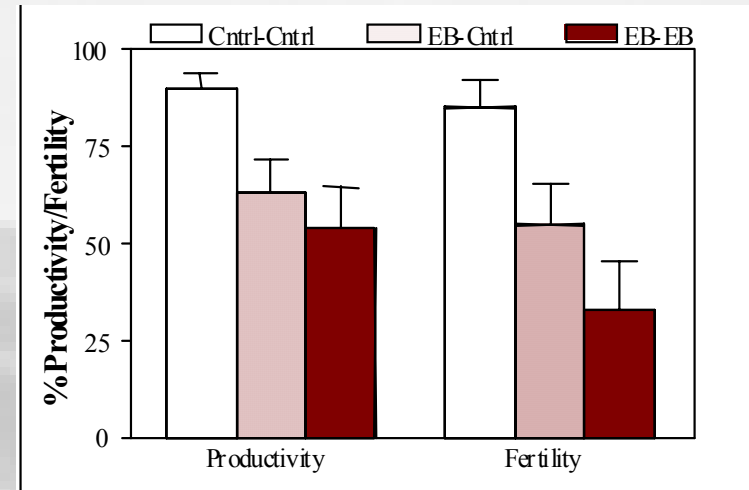
n= number of tested males/day

Henry et al., unpublished data

Effects of Injected EDCs: Estradiol

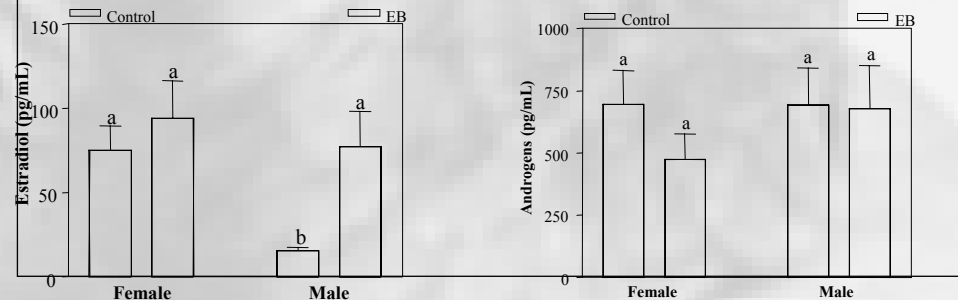
Effects of Embryonic Estradiol Benzoate on Reproductive Parameters

| SEX | TRAIT | TREATMENT | | |
|---------|--|------------------------|-----------------------|------------------------|
| | | Control injected | Oil injected | EB |
| Females | Age at first egg (n=15-30/trt) | 53±0.72 ^a | 51±0.95 ^a | 61±1.7 ^b |
| | Age at which 20% lay (n=15-34) | 56 | 52 | 67 |
| | Age at which 50% lay (n=15-34) | 60 | 56 | 74 |
| | Lay rate (%) (n=14-34) | 81 ± 2.5 ^a | 81 ± 4.1 ^a | 52 ± 8.9 ^b |
| | Ovary weight (%) (n=14-16) | 3.9 ± 0.2 ^a | Not determined | 4.0 ± 0.5 ^a |
| | Body weight (g) (n=14-16) | 152 ± 2.6 ^a | Not determined | 143 ± 2.6 ^b |
| Males | Age at cloacal foam production (n=23-33) | 49±0.7 ^a | 48 ± 1.0 ^a | 49 ± 2.0 ^a |
| | Cloacal gland area (mm ² ; n=6-9) | 177±16 ^a | 178 ± 13 ^a | 172±8 ^a |
| | Body weight (g) | 116 ± 2 | 115±6 | 114±2 |



EB Injection: Plasma Steroids

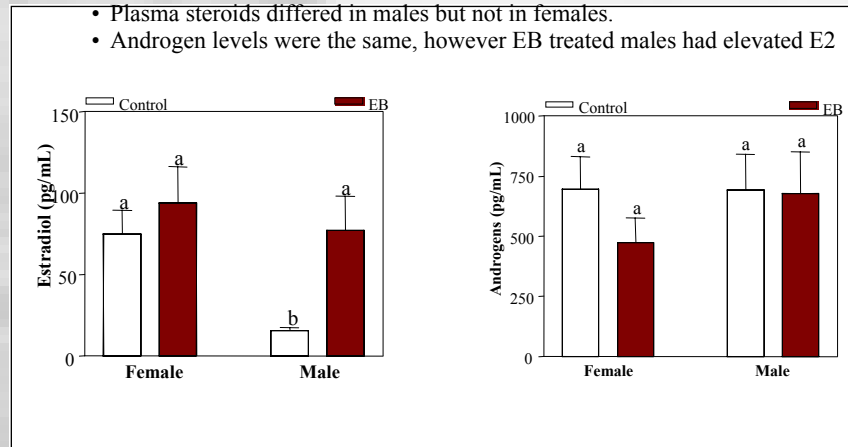
- Plasma steroids differed in males but not in females.
- Androgen levels were the same, however EB treated males had elevated E2



Embryonic estradiol treatment resulted in reduced male reproductive behavior. Plasma androgen was unaffected; however plasma estradiol was higher in treated males.

EB Injection: Plasma Steroids

- Plasma steroids differed in males but not in females.
- Androgen levels were the same, however EB treated males had elevated E2



EB injection: Sexual Behavior

- Sexual behavior was measured in photoregressed males given T-implants.
- EB treated males showed increased latency to mount and decreased mating behavior.

