# ORGAN WEIGHTS OF SINGLE COMB WHITE LEGHORN COCKERELS FED PBB

Thesis for the Degree of M. S.
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ABSTRACT

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By

Edward H. Kowaleski Jr.

The recent accidental feeding of livestock and poultry with polybrominated biphenyl (PBB) has caused widespread concern as to the exact effects of this chemical on exposed animals. This study was undertaken to determine the effects of feeding PBB to Single Comb White Leghorn cockerels. The parameters studied were feed intake, body weight and various organ weights; heart, liver, thyroid, testes, comb and spleen.

Symptoms observed after chronic administration (0-250 ppm) of PBB to 180 chicks and then to 210 chicks in a pair-fed study (0-200ppm) included 1) depressed body weight as a result of decreased feed intake, 2) decreased comb, testes and spleen weights, 3) decreased heart weight as a result of decreased feed intake, 4) increased liver and thyroid weight.

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A THESIS

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# INTRODUCTION

The dispersion of chemicals into the environment has become an issue of primary concern. The problem can be especially acute as when an accidental contamination occurs as it did in Michigan in 1973. The fire retardant, polybrominated biphenyl (PBB), was inadvertantly mixed into livestock and poultry feeds, resulting in considerable economic loss and concern for human contamination.

PBB is used in the thermoplastics industry mainly as a component of the housings of business machines and in industrial and electric equipment. It is a relatively new compound and thus has received very little toxicological research.

The present study is a report of the effects of PBB on body weight, various organ weights and on feed consumption in the Single Comb White Leghorn cockerel, when administered continuously in the feed.

## Objectives

This research was conducted to measure the effects of feeding polybrominated biphenyl to Single Comb White Leghorn cockerels.

Specific objectives were;

1. To determine the effect on body weight gains;
2. To determine the effect on feed consumption;
3. To determine the effect on weights of various organs of the body including the heart, liver, spleen, testes, comb and thyroid.

## REVIEW OF LITERATURE

Toxicological effects of polybrominated biphenyls (PBB)[1] have not been studied in animals as extensively as the chemically related polychlorinated biphenyls (PCB) but with the recent accidental exposure of livestock and poultry to PBB in the state of Michigan it is evident that research is greatly needed.

PBB is an industrial compound used mainly in the thermoplastics industry to reduce flammability. It is a mixture of brominated biphenyls with the average number of bromine atoms being six per biphenyl molecule. The mixture is as follows;

| | |
|---|---|
| Tetrabromobiphenyl | 2.0% |
| Pentabromobiphenyl | 10.6% |
| Hexabromobiphenyl | 62.8% |
| Heptabromobiphenyl | 13.8% |
| Other bromobiphenyls | 11.4% |

It is relatively inert chemically but a study of the photolytic stability of octabromobiphenyl indicates ultraviolet light readily degrades this material under conditions where PCBs are not degraded (Kerst, 1974). The use of PBB has been restricted, ideally, to end-use products that are not exposed to either feed or food. It is also not used in flame retarding fabrics where human exposure might occur

[1]A multicomponent mixture produced by The Michigan Chemical Co., St. Louis, Mi.

(Kerst, 1974). PBB is a relatively new compound with total production estimated at about eleven million pounds up to 1974 (Kerst, 1974).

The literature on PBB metabolism in biological systems is scanty, with avian species receiving very little attention. Lillie et al. (1974) included one level of PBB (BP-6) in their study on reproduction in hens and demonstrated significantly reduced feed intake per hen-day. Egg production was also significantly lower while adult body weight gain, livability, egg weight, egg shell thickness and fertility were not effected at the 20 ppm administered. Source progeny performance tests were also reported showing significantly reduced three week body weight gains in chicks from hens that had received 20 ppm PBB in their diets. Cecil et al. (1975) working with Japanese quail reported reduced phenobarbital sleeping time with a single oral injection of 300 ppm PBB. No data are available on the effects of PBB feeding on organ weights of birds.

Liver enlargement with PBBs has been reported by Aftosmis et al. (1972) and by Norris et al. (1973) both working with rats.

Polychlorinated biphenyls

The chemical and physical characteristics of PBBs and PCBs are similar. Therefore similar results would be expected from the ingestion of the chemicals. PCBs have been widely used in industry in electrical capacitors and in electrical

transformers as well as in heat-exchange fluids. PCB's have been used by the food industry in pasteurization equipment. It was therefore not surprising that this compound found it's way into fishmeal that was to be used in the poultry industry (Harris and Rose, 1971) as feed. The symptoms observed were those similar to chick edema disease (McCune et al., 1965) including varying degrees of liver degeneration, small spleens and muscle incoordination.

Various studies undertaken in chickens have shown increased liver weights with PCB ingestion (McCune et al., 1962; Flick et al., 1965; Platonow and Funnell, 1971). Splenic atrophy has been reported by Flick et al. (1965), while Platonow and Funnell (1971) have indicated that testes and comb size are reduced in chickens given 150 to 250 ppm PCB. Increased thyroid size and weight was reported by Jefferies and Parslow (1972) in the young gull following PCB administration. Rehfeld et al. (1971) using a PCB (Aroclor 2148) at 50 ppm observed in the chick: depressed weight gain; edema; hydropericardial fluid; depression of secondary sexual characteristics; and an increase in liver size as a percentage of body weight. Iturri (1974) also working with the chick demonstrated decreased heart, spleen, testes and comb weights while showing increased liver and thyroid weights with varying levels of PCB.

## Materials and Methods

### Experiment 1.

One-day-old commercial (DeKalb Strain) Single Comb White Leghorn (SCWL) cockerels (180) were reared in conventional electrically heated battery brooders adjusted to 35°C, with raised wire floors. They were randomly divided into six groups of thirty birds each, weighed individually, and wing-banded.

The control group was treated like all others except that they did not receive any PBB in their feed. The five treatment groups received feed with varying amounts of PBB. The amounts added were as follows: 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm.

The experiment was conducted for a period of 4 weeks. Feed and water were provided ad libitum. Body weights and feed consumption of the chicks were recorded weekly. To keep the wastage of feed to a minimum the feeders were half-filled every day.

### Preparation of Feed

Diets containing the different levels of PBB were prepared by adding the respective amount of PBB to a chick starter ration (Table 1).

The chemical was ground to a fine powder with a mortar and pestle and then weighed on a Mettler H10TW (Sargent Welch Scientific Co.) balance. Approximately five hundred grams of the starter ration was then screened (U.S. Standard Sieve

Table 1. Chick starter ration.

| Ingredients | Lbs per ton |
|---|---|
| Corn | 1239 |
| Soybean meal, 49% | 404 |
| Alfalfa meal, 17% | 50 |
| Meat & bone meal, 50% | 60 |
| Fish meal, 60% | 60 |
| Whey, dried | 40 |
| Oats | 100 |
| Salt | 5 |
| Limestone, ground | 15 |
| Dicalcium phosphate | 15 |
| Premix (5003) | 10 |
| Additives | 2 |
| | 2000 |

Series No. 20) three times. This powder was then mixed with the respective PBB. This premix was combined and mixed thoroughly with the basal ration using a mechanical rotary feed mixer. The same procedure was utilized for the control group except that PBB was not added. All rations were stored in 10 gallon metal containers (Reeves, Dover, Ohio) prior to and during their use.

Pair-Fed Experiment

The possibility existed that feed consumption might alter some of the parameters being analyzed. To study this effect, seven groups of thirty SCWL chicks per group were utilized. The experimental groups were given a known amount of feed where PBB had been incorporated and treated as described previously. The levels in the diets were: 50 ppm, 100 ppm, 200 ppm. Feed consumption from these groups was measured on alternate days using a Toledo model 3710 (Toledo Scale Corp.) scale, and an equal amount of the basal ration without PBB was given to the pair-fed group. In this manner the two-day lag allowed the pair-fed group the same amount of feed consumed by the experimental group. This procedure was again followed for a four week period. The seventh group of thirty chicks acted as the control and was fed the chick starter ration *ad libitum*.

Organs

In both experiments, at the end of the four week periods the birds were sacrificed and various organs were dissected

free of fat and surrounding tissue and weighed on a Mettler P1210 (E. H. Sargent and Co.) balance to the nearest 0.1 gram. The organs were kept moist on wet paper towels before the actual weighing. The organs collected were heart, liver, thyroids, testes, comb and spleen. To insure greater accuracy, thyroids were weighed on a Roller-Smith precision balance (Fisher Scientific Co., Pittsburgh, Pennsylvania) to the nearest 0.1 mg.

Body weight

The chicks were weighed at the initiation of the experiments and for the next four weeks at weekly intervals using a Mettler P1210 (E. H. Sargent Co.) balance. They were weighed to the nearest 0.1 gram.

Statistical Analyses

Statistical analyses of the data obtained were accomplished by analyses of variance. Parameters showing significant effects were investigated further by Dunnet's Test.

## RESULTS AND DISCUSSION

### A. Experiment 1.

#### Body Weight

Body weight was significantly reduced by PBB at the 100 ppm dosage as is shown in Figure 1 and in Table 2. It is apparent from these data that with increasing concentrations of PBB there is a concomitant decrease in body weight. At the end of a two week period the birds in the 200 and 250 ppm groups were experiencing increased mortality and were very emaciated and lethargic. The depression in body weight observed is related to a reduction in feed intake. As illustrated in Table 4, by the fourth week the controls were eating 39.2 g/chick/day but the 250 ppm group was consuming only 23.3 g/chick/day, some 40% less. Similar depressions in growth have been reported with PCBs by Flick et al. (1965).

#### Organ Weights

The various organ weights are presented in Tables 2,5,6 and 7 and Figures 2 through 11. Mean heart weights were significantly reduced at the 50 ppm and above dosage. From Fig. 2 it is estimated that a significant decrease in heart weight would be obtained from a dosage between 25 and 30 ppm. Iturri (1974) found a significant decrease in heart weight when feeding PCB 1254 but with no other PCB.

The absolute mean liver weights were not significantly different throughout the range of dosages; however, the relative liver weight increased over controls at all levels. Since no change in actual organ weight was seen, the relative increase could be accounted for by the decrease in body

Figure 1. Body weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E. Y = body weight (grams); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

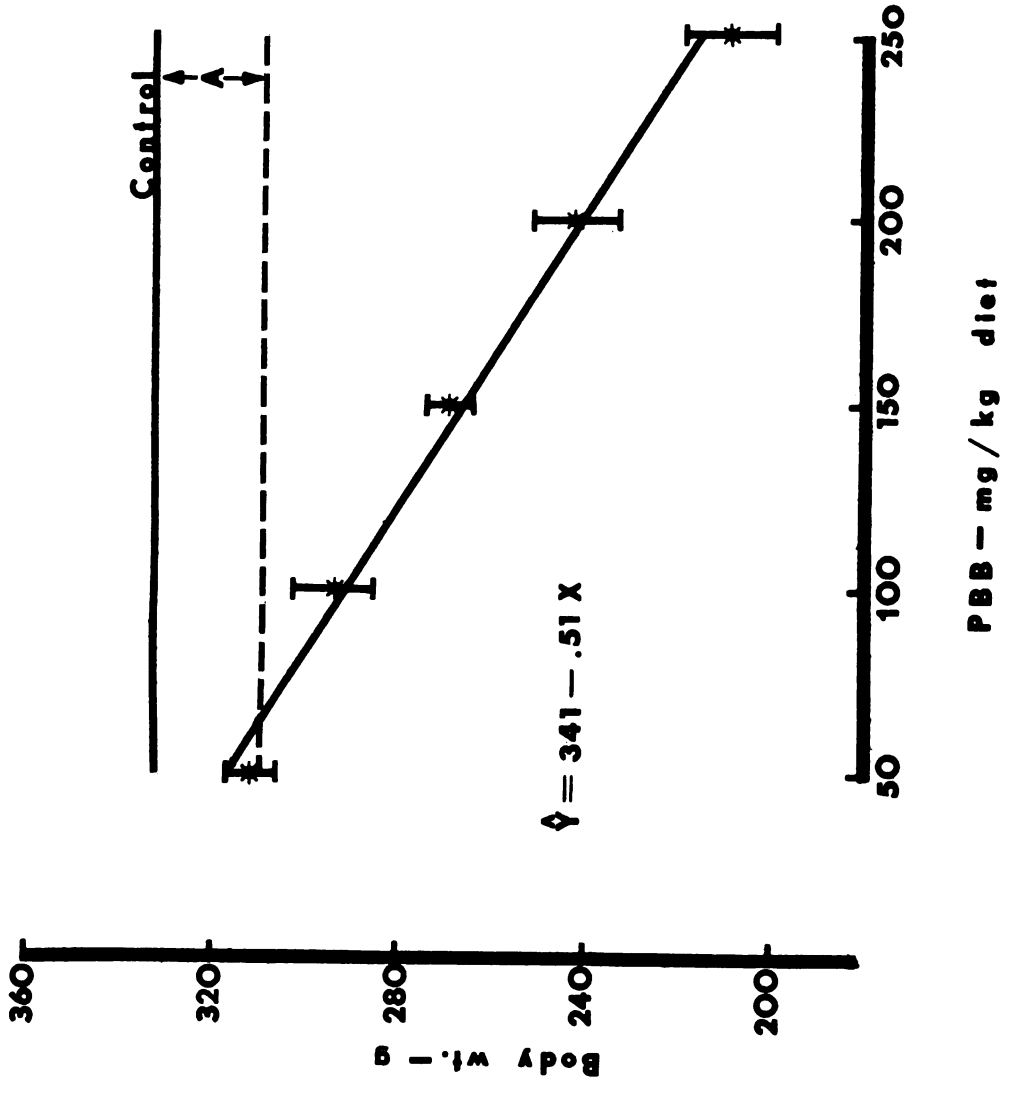

Control
Ŷ = 341 − .51 X
Body wt. − g
360
320
280
240
200
50
100
150
200
250
PBB − mg/kg diet

Table 2. Body and heart weights of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm)[a] | Body wt. (gm) | Heart wt. (gm) | Heart (% b.wt) |
|---|---|---|---|
| 0 | 331.7±5.467[b] (30) | 2.09±0.058 (30) | 0.63±0.016 (30) |
| 50 | 311.2±5.094 (30) | 1.75±0.046 (30) | 0.56±0.011 (30) |
| 100 | 293.3±8.553 (28) | 1.62±0.050 (27) | 0.55±0.013 (27) |
| 150 | 269.1±4.756 (30) | 1.47±0.036 (30) | 0.55±0.013 (30) |
| 200 | 241.9±9.108 (26) | 1.53±0.067 (26) | 0.64±0.031 (26) |
| 250 | 209.3±9.953 (25) | 1.37±0.089 (24) | 0.66±0.033 (24) |

[a]Rations fed up to 4 weeks

[b]Data are reported as Mean ± Standard Error (No. of birds)

Table 3. Average weights of SCWL cockerels in grams fed rations containing PBB.

| PBB Level (ppm)[a] | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| 0 | 38.2 | 78.2 | 148.5 | 240.3 | 331.7 |
| 50 | 36.9 | 78.6 | 143.4 | 226.5 | 311.2 |
| 100 | 37.7 | 74.4 | 129.7 | 210.6 | 292.4 |
| 150 | 36.5 | 72.2 | 123.4 | 197.2 | 269.1 |
| 200 | 36.7 | 68.6 | 111.0 | 175.7 | 241.9 |
| 250 | 36.9 | 66.6 | 107.3 | 160.2 | 209.3 |

[a]Rations fed up to 4 weeks

Table 4. Average weight gain and feed consumption of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm) | Avg. Wt. Gain -g Days 0-7 | Days 8-14 | Days 15-21 | Days 22-28 |
|---|---|---|---|---|
| 0 | 40.5 | 70.7 | 91.8 | 91.9 |
| 50 | 41.6 | 64.8 | 83.7 | 84.8 |
| 100 | 36.7 | 55.3 | 80.5 | 81.8 |
| 150 | 35.7 | 51.2 | 73.8 | 71.9 |
| 200 | 31.9 | 41.2 | 64.7 | 66.2 |
| 250 | 29.6 | 39.8 | 52.9 | 51.5 |
| | Feed Consumption - g/chick/day Days 0-7 | Days 8-14 | Days 15-21 | Days 22-28 |
| 0 | 10.15 | 20.1 | 29.6 | 39.2 |
| 50 | 10.49 | 19.2 | 27.1 | 35.6 |
| 100 | 9.75 | 18.2 | 24.5 | 33.5 |
| 150 | 9.66 | 18.1 | 22.9 | 31.4 |
| 200 | 8.84 | 13.5 | 20.6 | 25.9 |
| 250 | 8.93 | 14.3 | 19.6 | 23.3 |

Figure 2. Heart weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E. Y = heart weight (grams); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

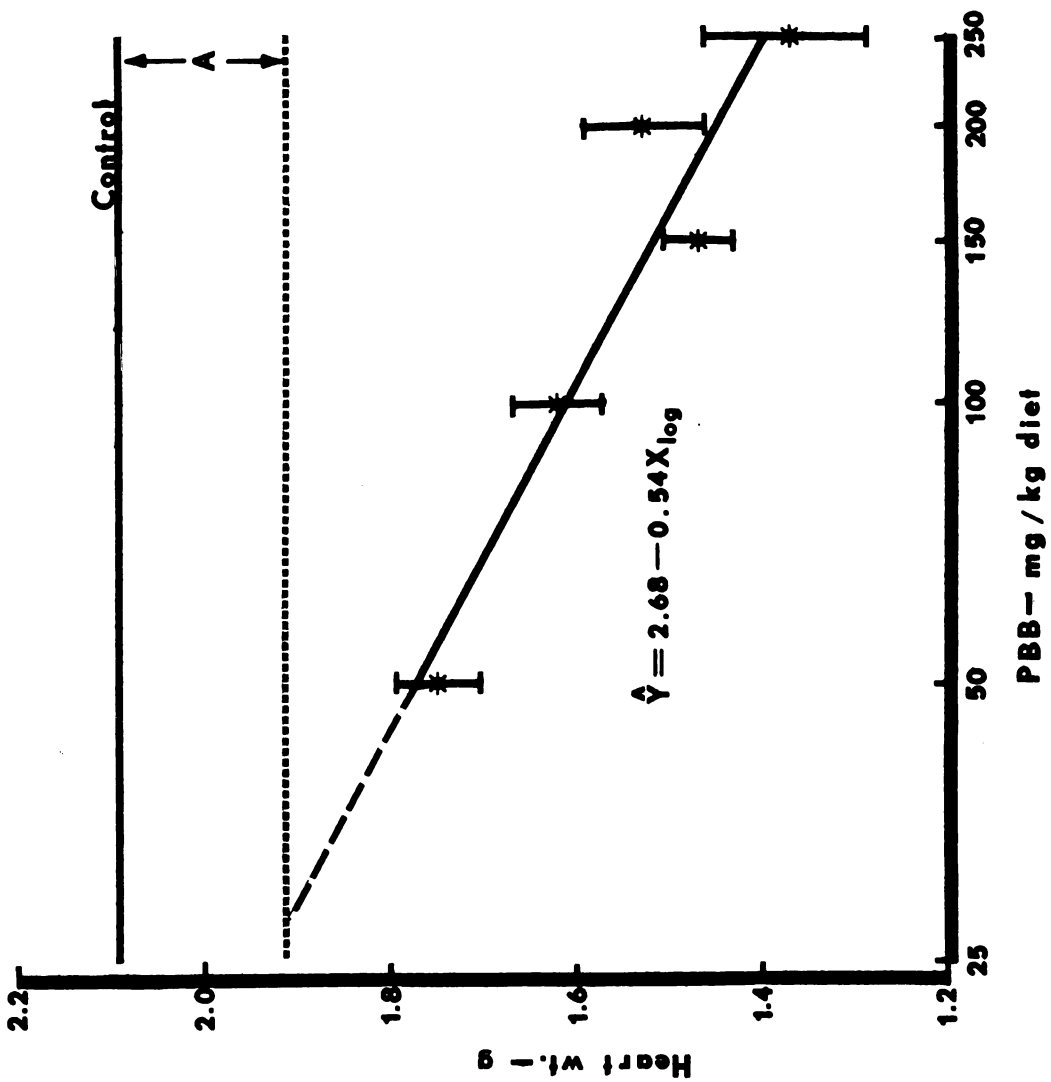

Control
A
$\hat{Y} = 2.68 - 0.54X_{log}$
Heart wt. — g
2.2
2.0
1.8
1.6
1.4
1.2
25
50
100
150
200
250
PBB — mg / kg diet

Figure 3. Liver weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E.

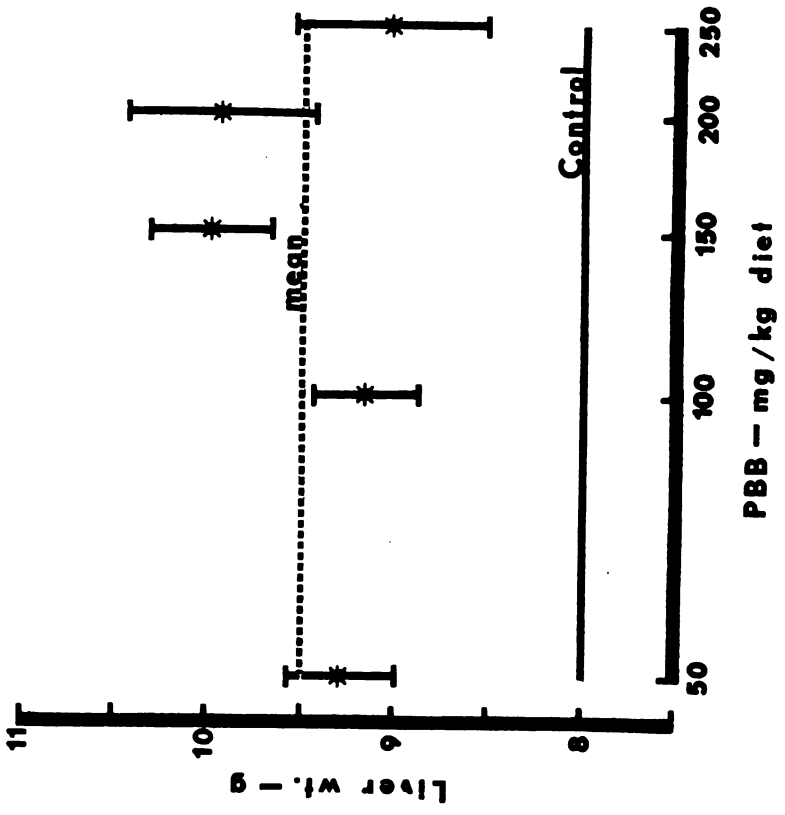
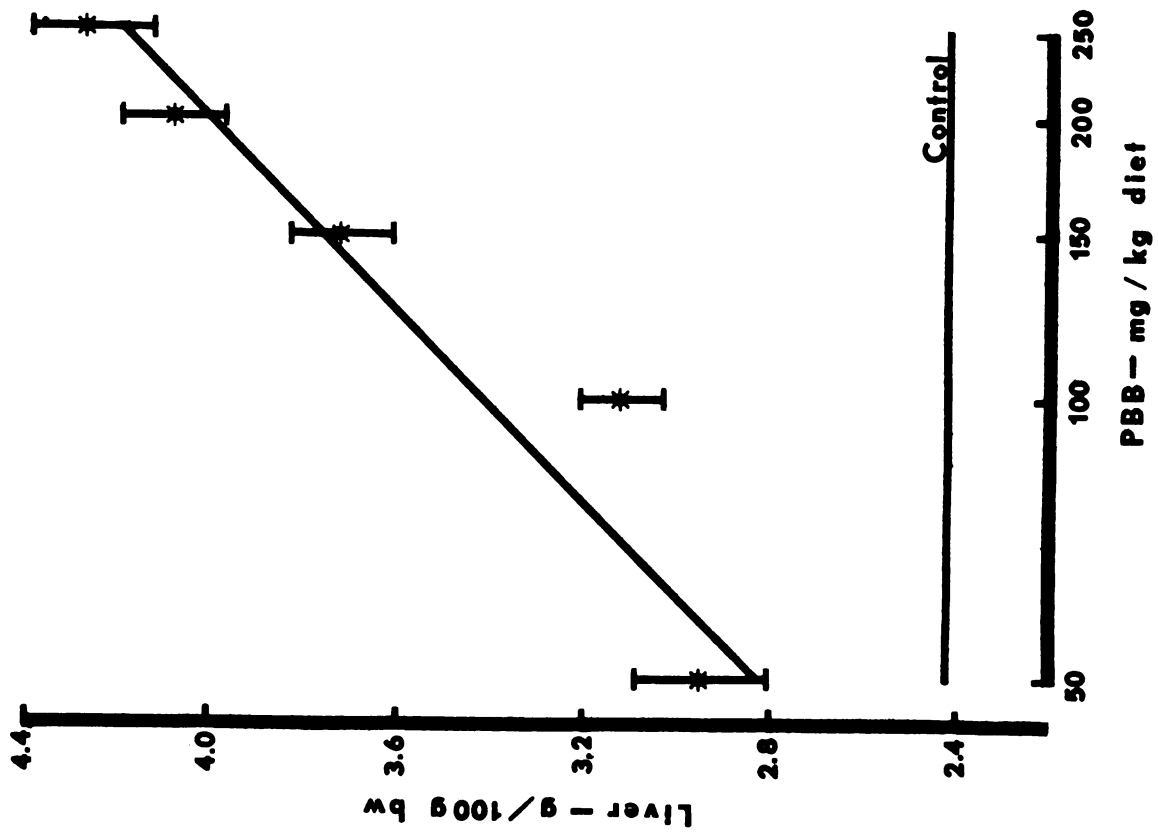

Liver wt. — g
11
10
9
8
mean
Control
50
100
150
200
250
PBB — mg/kg diet

Figure 4. Liver (g/100g bw) weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E.


Liver — g/100g bw
4.4
4.0
3.6
3.2
2.8
2.4
Control
50
100
150
200
250
PBB — mg / kg diet

weight. Similar results with mean liver weights were reported (Rehfeld et al., 1971; Platonow and Funnell, 1971) with PCBs in chicks, however other investigators have found increased weights of the liver with increasing concentrations of PCB (Flick et al., 1965; Grant et al.; 1971). Iturri (1974) also demonstrated increased liver weights in chicks with PCB 1242 and 1260.

Spleen weights were significantly reduced at levels $\geq$ 100 ppm. As a percentage of body weight they also displayed a decreasing trend. This seems to be consistent with the data for PCBs in chickens (Flick et al., 1963; Iturri, 1974) and in rats (Grant et al., 1971). No explanation has been given for the decreased size of the spleen and further histological studies will no doubt be extremely helpful.

Comb and testes weights will be considered together(Figures 7,8, and 9, Tables 6 and 7) because of their obvious relationship. Comb weight was significantly reduced at all levels tested therefore the dosage at which PBB has a significant effect on the comb would be approximately 20 to 25 ppm. It is interesting to note that although comb weight was significantly reduced at 50 ppm, testes weight was not depressed until the 200 ppm level was utilized. As can be seen in Fig. 7 the birds fed the 50 and 100 ppm diet have testes actually weighing more. This could be a drug effect caused perhaps by increasing the amount of adipose tissue in the testes, or it could be experimental error. Whichever the case, the testosterone is not being produced and/or not having a phys-

Figure 5. Spleen weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E. Y = spleen weight (mg); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

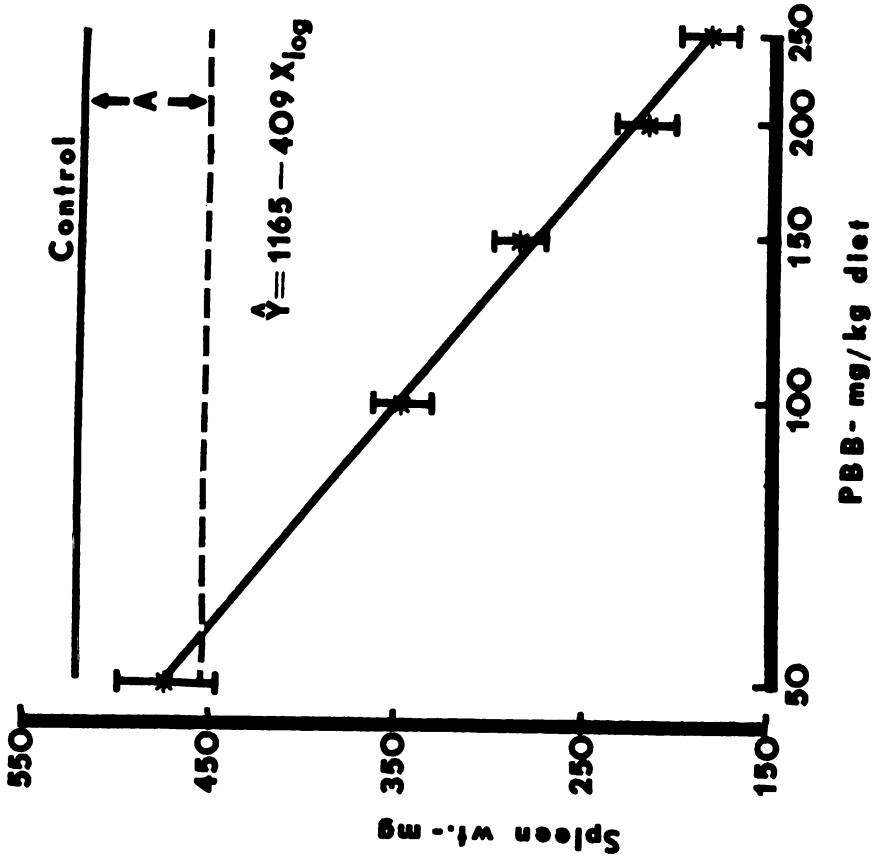

Control
A
$\hat{Y} = 1165 - 409\,X_{log}$
550
450
350
250
150
Spleen wt.- mg
50
100
150
200
250
PBB- mg/kg diet

Figure 6. Spleen (mg/100g bw) weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E.

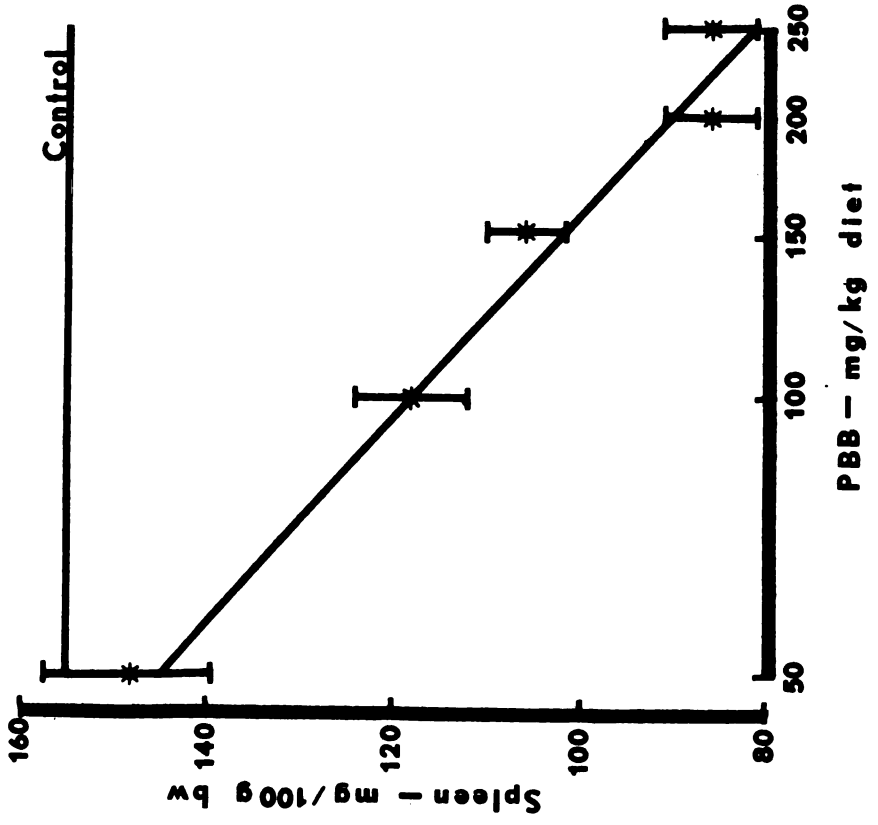

Control
160
140
120
100
80
Spleen — mg/100g bw
50
100
150
200
250
PBB — mg/kg diet

Table 5. Liver and spleen weights of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm)[a] | Liver wt. (gm) | Liver (% b.wt) | Spleen wt. (mg.) | Spleen (% b.wt) |
|---|---|---|---|---|
| 0 | 7.99±0.274[b] (30) | 2.42±0.084 (30) | 519±32.7 (30) | 155±9.8 (30) |
| 50 | 9.29±0.294 (30) | 2.99±0.102 (30) | 472±25.9 (30) | 148±8.8 (30) |
| 100 | 9.17±0.293 (27) | 3.12±0.094 (27) | 347±16.2 (27) | 118±5.8 (27) |
| 150 | 9.99±0.330 (30) | 3.72±0.107 (30) | 286±14.3 (30) | 106±4.3 (30) |
| 200 | 9.94±0.485 (26) | 4.08±0.108 (26) | 213±15.9 (26) | 86±4.7 (26) |
| 250 | 9.03±0.513 (24) | 4.26±0.133 (24) | 184±15.2 (23) | 86±4.7 (23) |

[a]Rations fed up to 4 weeks

[b]Data are reported as Mean ± Standard Error (No. of birds)

Figure 7. Testes weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E. Y = testes weight (mg); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

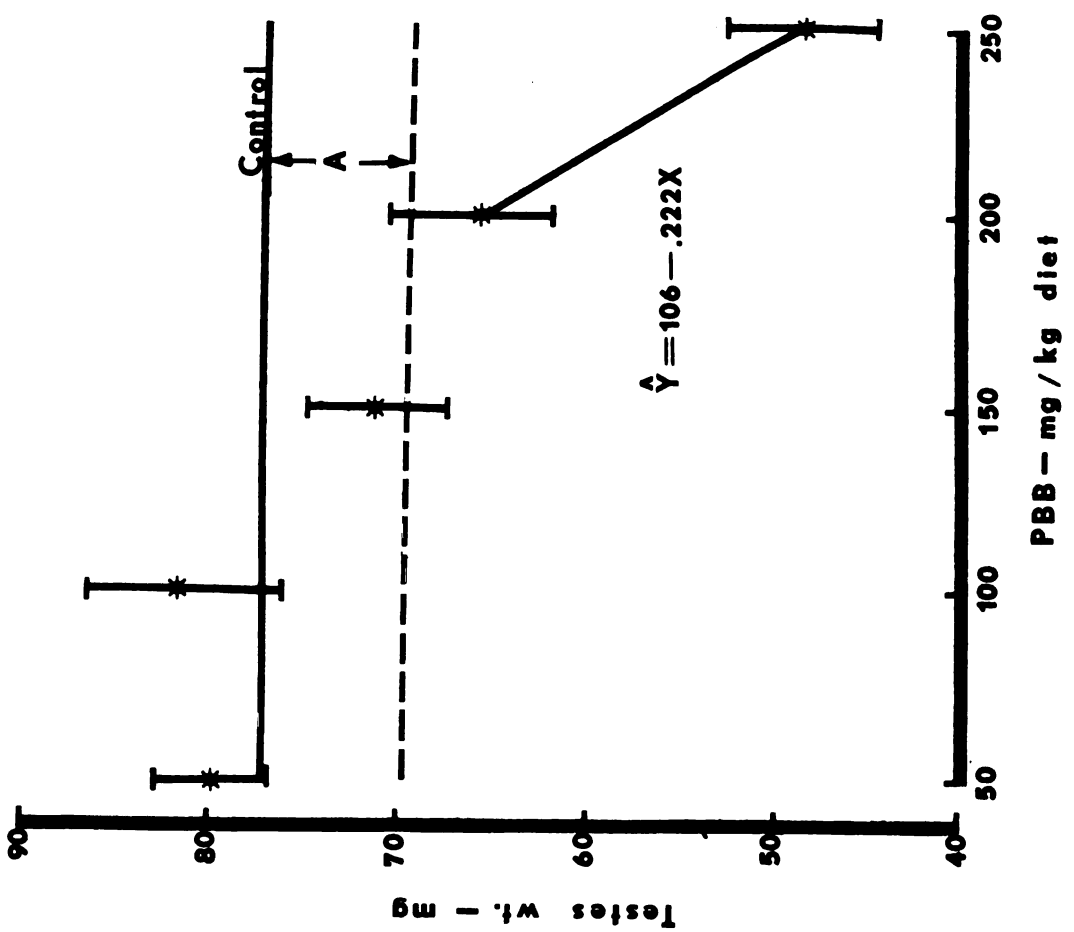

Testes wt. — mg
90
80
70
60
50
40
Control
A
$\hat{Y}$=106—.222X
50
100
150
200
250
PBB — mg / kg diet

Figure 8. Comb weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E. Y = comb weight (mg); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

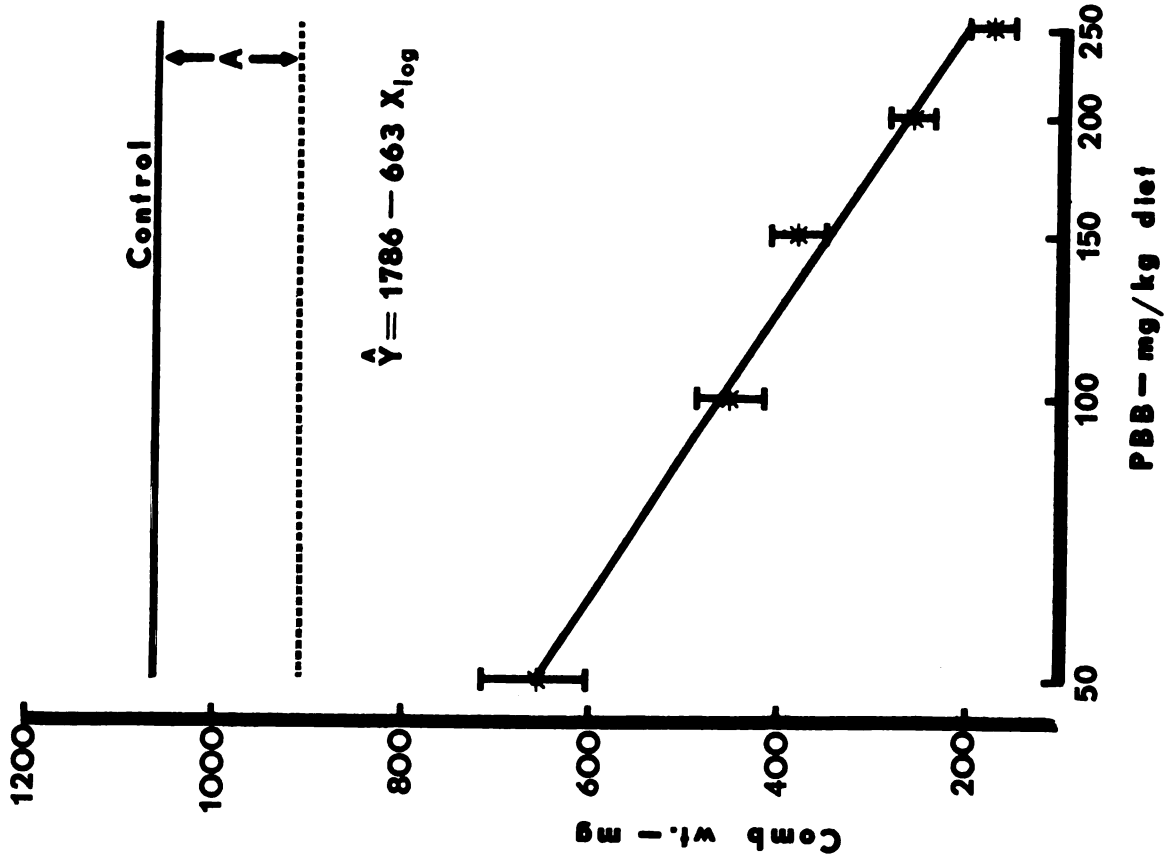

Control
A
$\hat{Y} = 1786 - 663\ X_{log}$
Comb wt. - mg
1200
1000
800
600
400
200
50
100
150
200
250
PBB - mg/kg diet

Table 6. Testes and thyroid weights of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm)[a] | Testes wt. (mg) | Testes (% b.wt) | Thyroid wt. (mg) | Thyroid (% b.wt) |
|---|---|---|---|---|
| 0 | 76.9±3.0[b] (30) | 23.4±1.0 (30) | 17.3±0.7 (30) | 5.19±0.20 (30) |
| 50 | 79.8±3.0 (30) | 25.7±0.9 (30) | 20.2±1.0 (30) | 6.46±0.29 (30) |
| 100 | 81.7±4.8 (26) | 27.5±1.4 (26) | 23.5±1.4 (27) | 7.94±0.42 (27) |
| 150 | 71.0±3.8 (30) | 26.3±1.2 (30) | 25.6±1.5 (30) | 9.53±0.52 (30) |
| 200 | 66.1±4.4 (26) | 27.0±1.2 (26) | 25.6±1.6 (25) | 10.74±0.52 (25) |
| 250 | 48.5±4.1 (24) | 22.6±1.5 (24) | 22.8±1.5 (24) | 10.74±0.50 (24) |

[a]Rations fed up to 4 weeks

[b]Data are reported as Mean ± Standard Error (No. of birds)

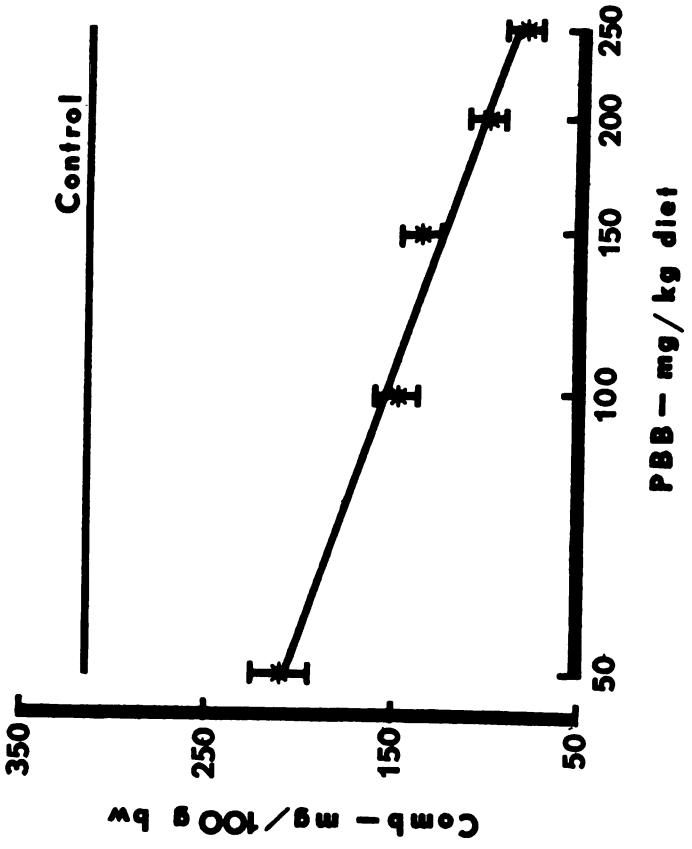
Table 7. Comb weights of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm)[a] | Comb wt. (mg) | Comb (% b.wt) |
|---|---|---|
| 0 | 1061±78.0[b] (30) | 324±25.3 (30) |
| 50 | 656±56.1 (30) | 209±16.3 (30) |
| 100 | 447±38.5 (27) | 148±10.4 (27) |
| 150 | 379±26.7 (30) | 136±9.6 (30) |
| 200 | 258±23.8 (26) | 104±8.2 (26) |
| 250 | 176±24.5 (24) | 80±9.2 (24) |

[a]Rations fed up to 4 weeks

[b]Data are reported as Mean ± Standard Error (No. of birds)

Figure 9. Comb (mg/100g bw) weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E.


Control
350
250
150
50
Comb – mg/100 g bw
50
100
150
200
250
PBB – mg/kg diet

iological effect on the comb. Platonow and Funnell (1971) in a 13-week study demonstrated similar weights in testes at the end of a six week period between controls and PCB treated cockerels but by nine weeks there was a significant decrease. They also noted a decreased comb growth appearing before a similar decrease in size of the testes.

Jefferies and Parslow (1972) demonstrated increased thyroid weights in the gull with the administration of PCB. They described the condition as being similar to that of simple goiter, and suggested interference with the iodine trap mechanism or decreased thyroid stimulating hormone quantities. In this study thyroid weight was significantly increased at all levels of PBB above 50 ppm, Fig. 10. Interestingly there appeared to be a leveling off of thyroid weight between 150 and 200 ppm PBB and a decrease below the 200 ppm weight, by the group fed 250 ppm. This indicated that the toxicity was too great at the 250 ppm level and that some form of degeneration was taking place overcoming the stimulatory effect seen at lower levels. Plasma thyroxine concentration in PCB treated birds (Iturri, 1974) was significantly increased by PCBs 1254 and 1260. Why the thyroxine concentration is augmented and the mechanism by which the treated thyroids increase in size is not clear.

B. Experiment 2 (Pair-Fed Experiment).

Body Weight

The object of a pair-fed study is to eliminate the in-

Figure 10. Thyroid weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E. Y = thyroid weight (mg); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

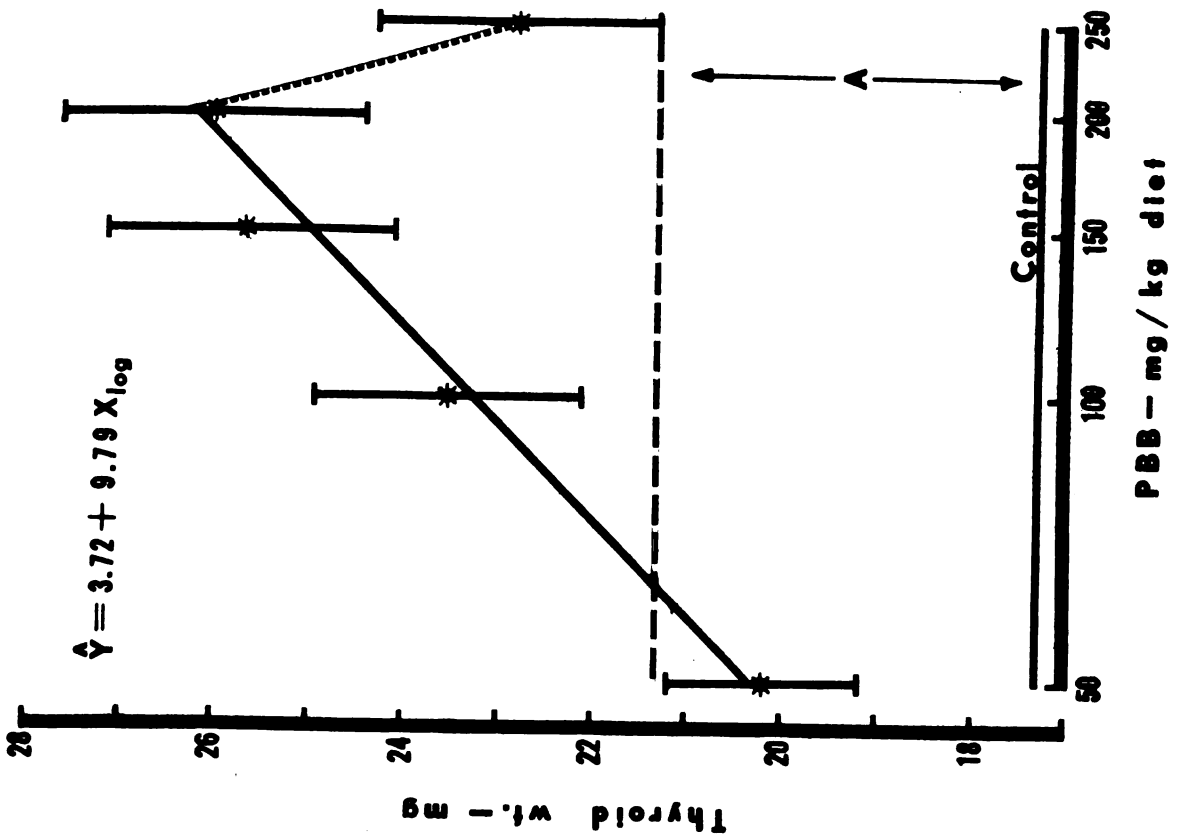
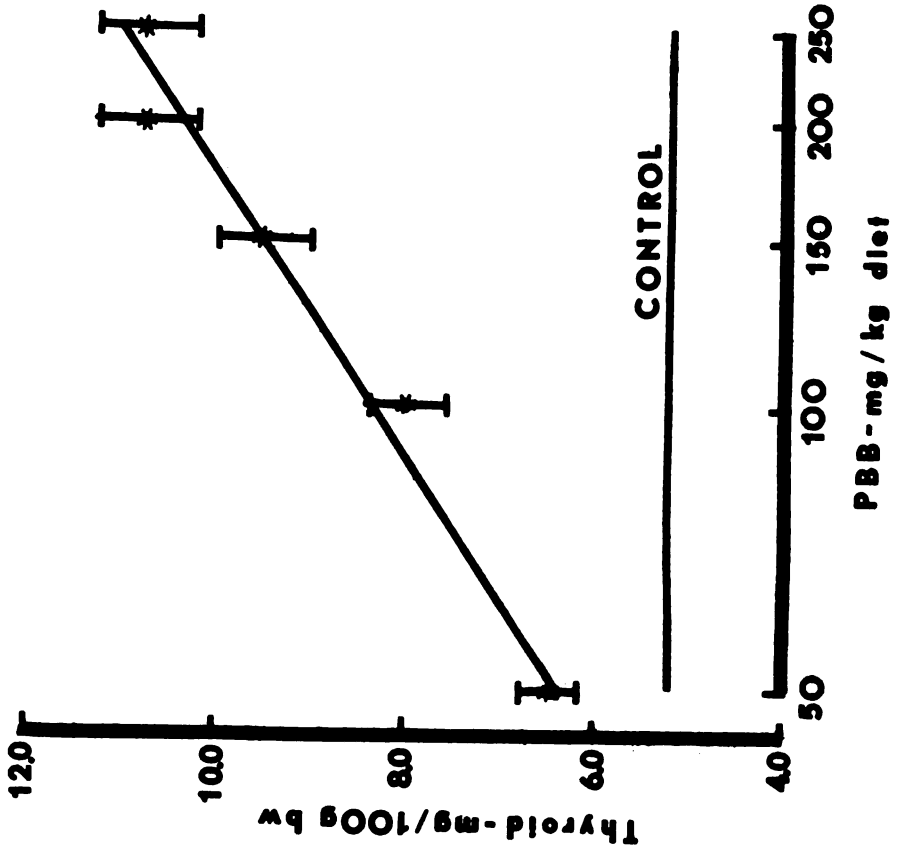

Thyroid wt. — mg
28
26
24
22
20
18
$\hat{Y} = 3.72 + 9.79\, X_{log}$
A
Control
50
100
150
200
250
PBB — mg/kg diet

Figure 11. Thyroid (mg/100g bw) weight of SCWL cockerels fed rations containing PBB. Mean values ± S. E.


Thyroid - mg/100g bw
12.0
10.0
8.0
6.0
4.0
CONTROL
50
100
150
200
250
PBB - mg/kg diet

fluence that increased or decreased feed consumption might have on experimental results. In Experiment 1 PBB depressed feed consumption; therefore a pair-feeding study was conducted to test the effects of the drug per se. Since the treated and the pair-fed group received equal amounts of feed it was presumed the effects seen were due only to the action of the drug. As seen in Fig. 12 it would appear that PBB acted as a growth stimulant at low dietary levels. This is in conflict with a pair-fed study with PCBs by Iturri (1974) who demonstrated no significant difference between pair-fed and treated birds. It was felt that the chicks that received 50 and 100 ppm pair-fed levels were far less effecient than the controls. The pair-fed 50 group consumed 99.5% of the control intake (Table 9) yet were some 40 grams lighter in body weight at the termination of the experiment. As will be pointed out later, Fig. 16, the spleens of this group were greatly enlarged, indicating other types of problems. These problems have precluded the use of the data as a percent of body weight and these are included only for the sake of completeness. As seen in Table 11 the treated 50 ppm and 100 ppm PBB groups consistently outgained their pair-fed counterpart on an average weight gain basis, so it would appear that there was a problem from the beginning.

Organ Weights

Heart weights were significantly reduced in the $\geq$ 100 ppm groups. There were, however, no significant differences within the respective groups, for this reason a single slope

Figure 12. Body weight of SCWL (pair-fed) cockerels fed rations containing PBB. Mean values ± S. E. Y = body weight (grams); X = PBB level (mg/kg) in the diet.

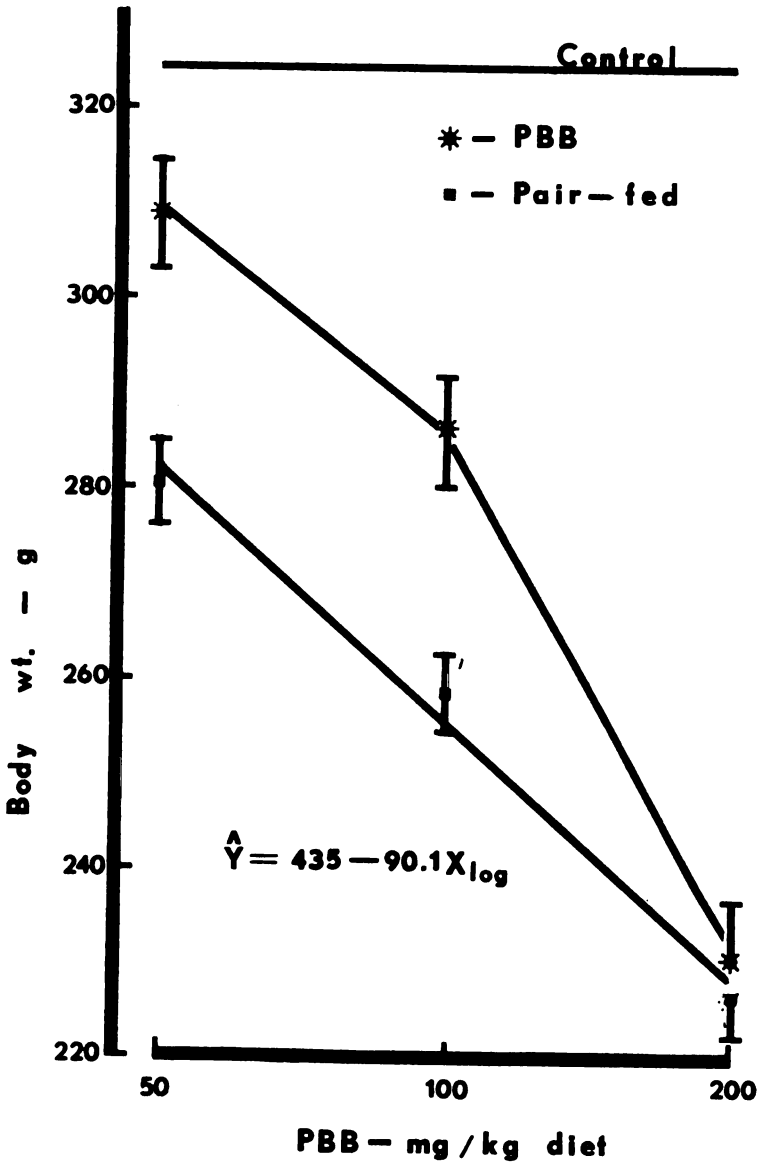

Control
* — PBB
▪ — Pair—fed
320
300
280
260
240
220
Body wt. — g
$\hat{Y} = 435 - 90.1X_{log}$
50
100
200
PBB — mg / kg diet

Table 8. Pair-fed study of body and heart weights of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm)[a] | Body wt. (gm) | Heart wt. (gm) | Heart (g/100g bw) |
|---|---|---|---|
| 0 | 324.4±5.9[b] (30) | 2.11±0.07 (30) | 0.65±0.02 (30) |
| Pair-Fed 50 | 280.7±4.4 (29) | 2.07±0.07 (29) | 0.74±0.02 (29) |
| PBB 50 | 309.1±5.7 (29) | 1.99±0.07 (29) | 0.64±0.02 (29) |
| Pair-Fed 100 | 258.6±3.9 (30) | 1.87±0.04 (30) | 0.72±0.02 (30) |
| PBB 100 | 285.9±5.9 (29) | 1.85±0.07 (28) | 0.64±0.02 (28) |
| Pair-Fed 200 | 226.5±3.7 (29) | 1.59±0.05 (29) | 0.70±0.02 (29) |
| PBB 200 | 230.7±5.8 (28) | 1.71±0.07 (28) | 0.74±0.03 (28) |

[a]Rations fed up to 4 weeks

[b]Data are reported as Mean ± Standard Error (No. of birds)

Table 9. Pair-fed study of feed consumption of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm) | Feed Consumption - g/chick/day 1st Week | 2nd Week | 3rd Week | 4th Week |
|---|---|---|---|---|
| 0 | 9.8 | 17.8 | 25.8 | 32.9 |
| Pair-Fed 50 | 10.2 | 18.0 | 25.1 | 32.6 |
| PBB 50 | 10.2 | 18.0 | 25.1 | 32.6 |
| Pair-Fed 100 | 10.1 | 17.3 | 23.0 | 29.1 |
| PBB 100 | 10.1 | 17.3 | 23.0 | 29.1 |
| Pair-Fed 200 | 9.9 | 15.9 | 20.7 | 21.8 |
| PBB 200 | 9.9 | 15.9 | 20.7 | 21.8 |

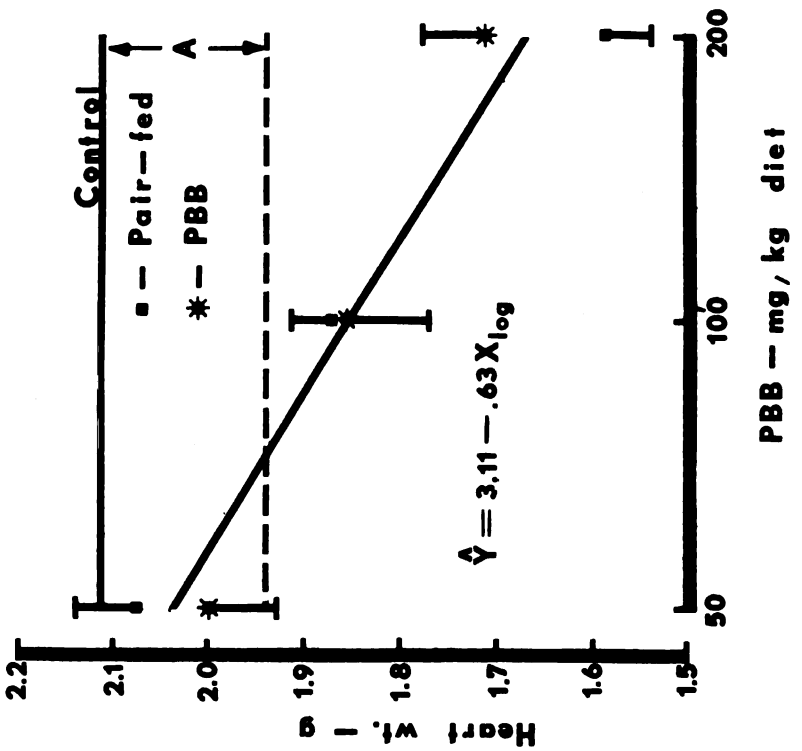
Table 10. Pair-fed study of average SCWL cockerel body weights (on a weekly basis) fed rations containing PBB.

| PBB Level (ppm) | Avg. Chick wts. - g Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| 0 | 49.6 | 83.9 | 148.8 | 234.5 | 324.4 |
| Pair-Fed 50 | 48.0 | 73.0 | 132.7 | 205.5 | 280.7 |
| PBB 50 | 47.7 | 82.9 | 144.1 | 223.0 | 309.1 |
| Pair-Fed 100 | 49.6 | 72.6 | 126.6 | 193.1 | 258.6 |
| PBB 100 | 49.1 | 78.4 | 132.9 | 206.3 | 285.9 |
| Pair-Fed 200 | 49.6 | 70.5 | 117.5 | 176.0 | 226.5 |
| PBB 200 | 48.9 | 75.8 | 121.3 | 180.4 | 230.7 |

Table 11. Pair-fed study of average SCWL cockerel body weight gain (on a weekly basis) fed rations containing PBB.

| PBB Level (ppm) | Avg. Wt. Gain -g Days 0-7 | Days 8-14 | Days 15-21 | Days 22-28 |
|---|---|---|---|---|
| 0 | 34.3 | 64.9 | 86.0 | 98.7 |
| Pair-Fed 50 | 25.0 | 59.7 | 72.8 | 73.8 |
| PBB 50 | 34.8 | 61.3 | 78.9 | 86.1 |
| Pair-Fed 100 | 22.9 | 54.0 | 66.5 | 65.5 |
| PBB 100 | 29.3 | 54.6 | 73.4 | 78.3 |
| Pair-Fed 200 | 20.8 | 47.0 | 59.4 | 49.1 |
| PBB 200 | 26.9 | 45.5 | 59.0 | 48.9 |

Figure 13. Heart weight of SCWL (pair-fed) cockerels fed rations containing PBB. Mean values ± S. E. Y = heart weight (grams); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).


Control
■ — Pair-fed
* — PBB
A
$\hat{Y} = 3.11 - .63 X_{log}$
Heart wt. — g
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
50
100
200
PBB — mg/kg diet

is provided. The results indicate that the drug has no effect on heart weight. The hearts from the PBB-treated groups throughout appeared to be more flaccid and not as thick-walled as the hearts of controls and on a size basis they seemed to be equal to, or larger than the controls.

Mean liver weights were significantly increased at ≥ 50 ppm (Fig. 14), while the pair-fed groups showed a decreasing trend with the pair-fed 200 group having significantly lower liver weights. This demonstrates that PBB has a definite effect on liver weight. This effect was potentiated as the level of PBB was increased as indicated by the difference between the groups at the 200 level. On a relative basis the pair-fed birds show essentially no change with starvation, the treated, on the other hand, increased dramatically. Results from this pair-fed study is in agreement with another pair-fed study (Iturri, 1974) in which PCB 1242 was incorporated at 100 ppm. In Iturri's study the treated group was not significantly different from the control, but there was a significant decrease in the pair-fed liver weight. This demonstrated that PCB maintained the liver weight while it should have decreased.

Figure 16 depicts the results of PBB ingestion on mean spleen weights. A significant difference was seen at 100 and 200 ppm PBB. This was in contrast to their unchanged pair-fed groups. The pair-fed 50 group had a very high weight. Considered with the decreased body weight of this group an infection or some other disorder might be indicated. The

Figure 14. Liver weight of SCWL (pair-fed) cockerels fed rations containing PBB. Mean values ± S. E. Y = liver weight (grams); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

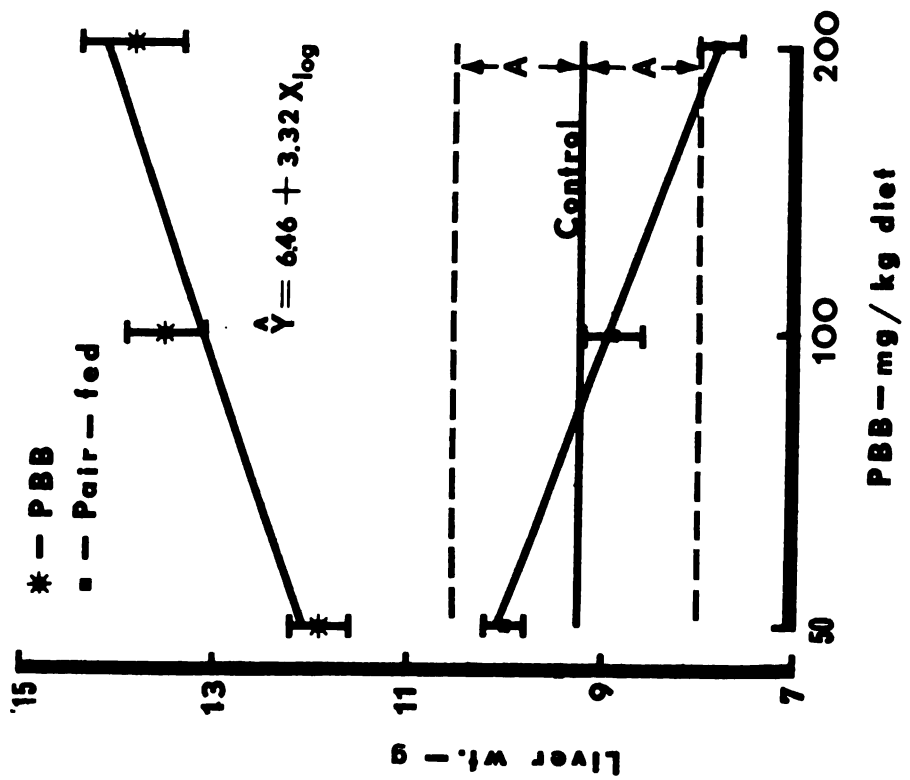

* — PBB
▪ — Pair — fed
$\hat{Y} = 6.46 + 3.32 X_{log}$
Control
A
A
Liver wt. — g
15
13
11
9
7
50
100
200
PBB — mg / kg diet

Figure 15. Liver weight (g/100g bw) of SCWL cockerels fed rations (pair-fed) containing PBB. Mean values ± S. E.

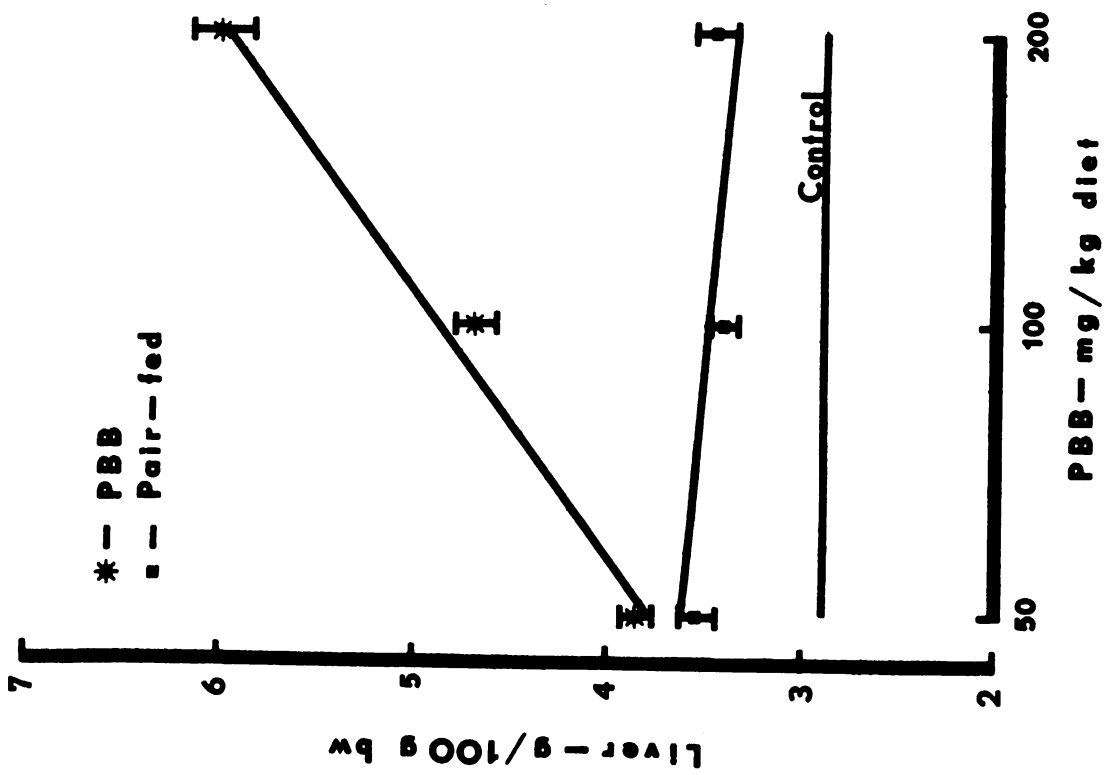

* — PBB
▪ — Pair-fed
Control
Liver — g/100 g bw
PBB — mg/kg diet
7
6
5
4
3
2
50
100
200

Figure 16. Spleen weight of SCWL (pair-fed) cockerels fed rations containing PBB. Mean values ± S. E. Y = spleen weight (mg); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

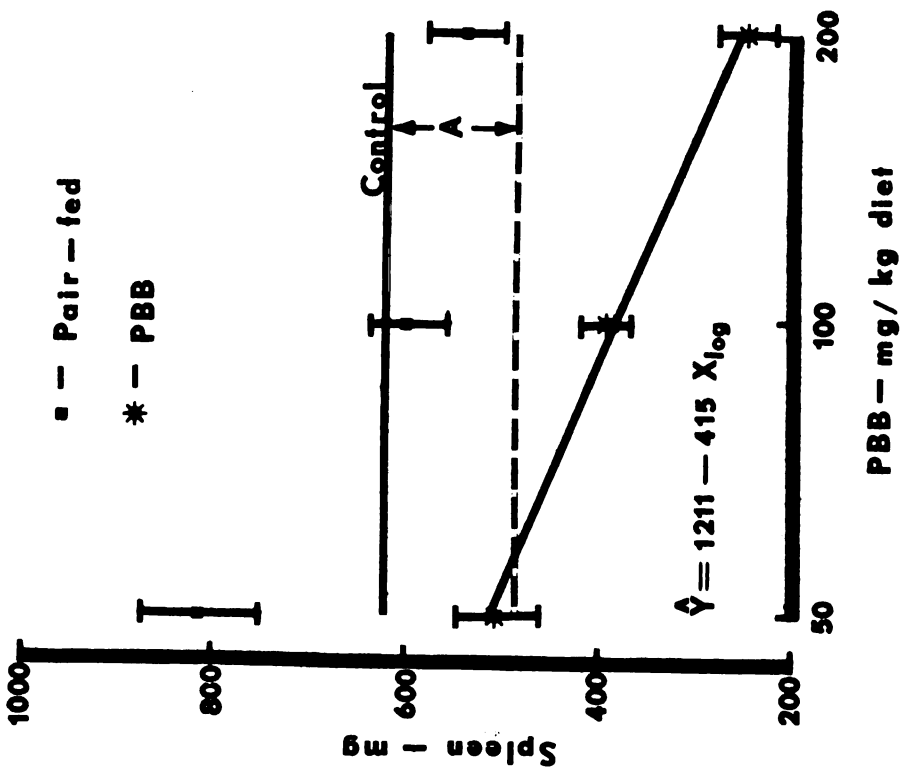

Pair-fed
PBB
Control
A
$\hat{Y} = 1211 - 415\ X_{log}$
Spleen - mg
1000
800
600
400
200
50
100
200
PBB - mg/kg diet

Table 12. Pair-fed study of liver and thyroid weights of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm)[a] | Liver wt. (gm) | Liver (g/100g bw) | Thyroid wt. (mg) | Thyroid (mg/100g bw) |
|---|---|---|---|---|
| 0 | 9.30±0.24[b] (30) | 2.87±0.06 (30) | 16.45±1.09 (20) | 5.00±0.30 (20) |
| Pair-Fed 50 | 9.97±0.24 (29) | 3.53±0.09 (29) | 17.42±1.21 (20) | 6.16±0.42 (20) |
| PBB 50 | 11.87±0.30 (29) | 3.84±0.07 (29) | 21.45±2.03 (20) | 6.69±0.56 (20) |
| Pair-Fed 100 | 8.90±0.28 (30) | 3.41±0.09 (30) | 15.52±0.95 (20) | 6.00±0.38 (20) |
| PBB 100 | 13.49±0.40 (28) | 4.69±0.10 (28) | 23.90±1.35 (20) | 8.43±0.47 (20) |
| Pair-Fed 200 | 7.81±0.22 (29) | 3.45±0.09 (29) | 13.31±0.85 (20) | 5.85±0.34 (20) |
| PBB 200 | 13.86±0.55 (28) | 5.98±0.16 (28) | 21.82±1.62 (20) | 10.82±0.84 (20) |

[a]Rations fed up to 4 weeks

[b]Data are reported as Mean ± Standard Error (No. of birds)

Table 13. Pair-fed study of spleen weights of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm)[a] | Spleen wt. (mg) | Spleen (mg/100g bw) |
|---|---|---|
| 0 | 618.9±33.4[b] (30) | 191.5±10.2 (30) |
| Pair-Fed 50 | 812.6±59.4 (29) | 292.8±23.1 (29) |
| PBB 50 | 504.6±42.6 (29) | 161.0±12.0 (29) |
| Pair-Fed 100 | 599.3±41.1 (29) | 231.1±14.2 (29) |
| PBB 100 | 394.5±23.5 (28) | 136.2± 6.8 (28) |
| Pair-Fed 200 | 541.6±45.0 (29) | 236.8±18.7 (29) |
| PBB 200 | 254.1±23.9 (28) | 107.7± 8.6 (28) |

[a]Rations fed up to 4 weeks

[b]Data are reported as Mean ± Standard Error (No. of birds)

exact reason for this increase is not evident. Utilizing the 100 and 200 ppm groups a definite effect can still be discerned by PBB administration on the spleen. A decrease in relative spleen weights over pair-fed and controls is also illustrated (Fig. 17).

As was shown in Fig. 7, there was first an increase in weight of the testes at lower levels (Fig. 18) and then a steady decrease to a significant level at 200 ppm. The drug effect was already seen by 50 ppm and as the PBB level is increased, more of an effect is seen from starvation as is demonstrated by the converging lines. The comb effect elicited in Experiment 1 (Fig. 8) was also evident in the group fed 50 ppm. It would appear then that this would be a consistent effect of the drug. Similar results were found by using PCBs in chickens (Platonow and Funnell, 1971). Testes and comb weights on a percentage basis (Fig. 20 and 21) were also shown to decrease with increasing concentrations of PBB, with the maximum effect seen at 50 ppm.

Mean thyroid weights are interesting (Fig. 22). There was a significant increase in thyroid weight in all PBB levels utilized while the pair-fed displayed a downward trend, though not statistically significant. The treated groups displayed first an upward, then downward trend (Fig. 10). This research does not presume what might happen to thyroid weight at a higher level of drug, 300 ppm for example, but Figures 10 and 22 indicate that there is some toxic effect occuring at 200 to 250 ppm PBB. The relative thyroid weights (Fig. 23) gave a misleading pattern for although at the 200 ppm level

the absolute weight was decreased, the relative weight showed an increase.

Figure 17. Spleen weight (mg/100g bw) of SCWL cockerels fed rations (pair-fed) containing PBB. Mean values ± S. E.

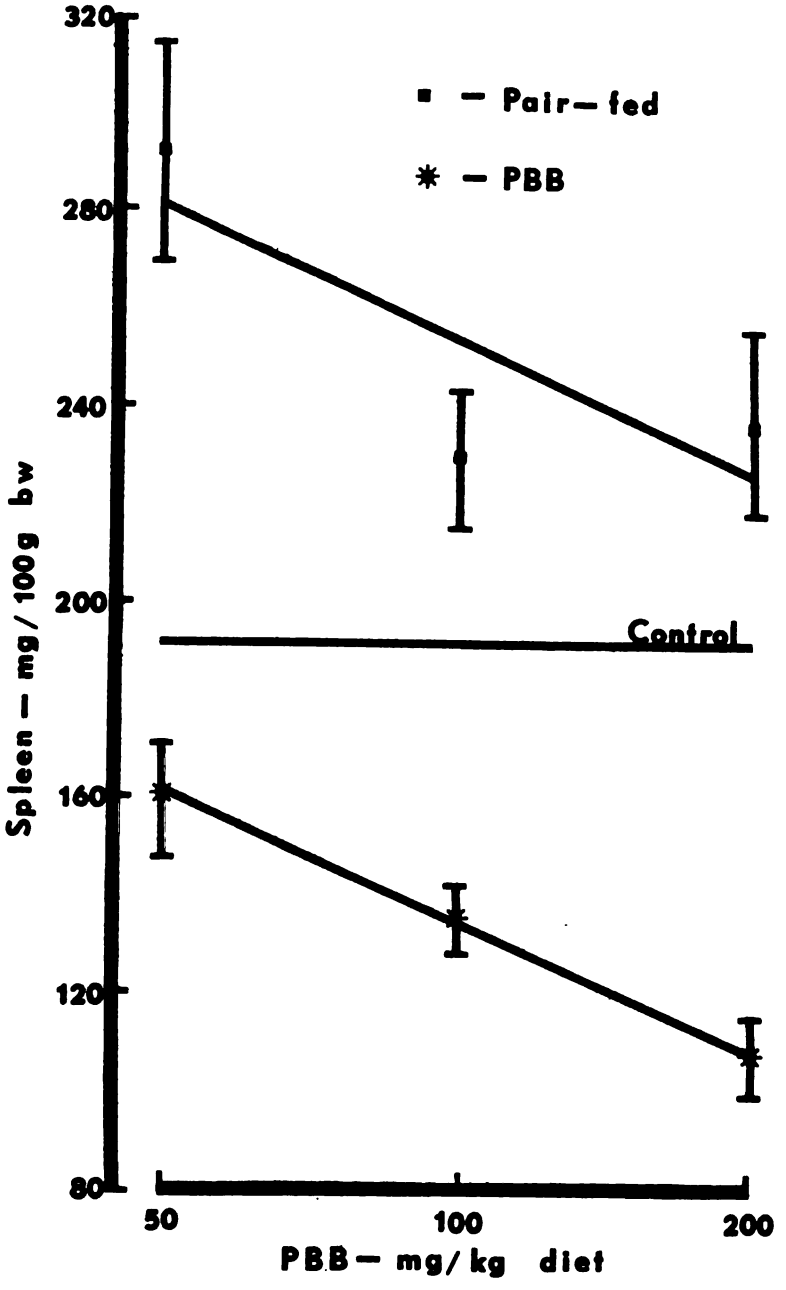

320
280
240
200
160
120
80
Spleen – mg/100g bw
■ – Pair–fed
✱ – PBB
Control
50
100
200
PBB – mg/kg diet

Figure 18. Testes weight of SCWL (pair-fed) cockerels fed rations containing PBB. Mean values ± S. E. Y = testes weight (mg); X = PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

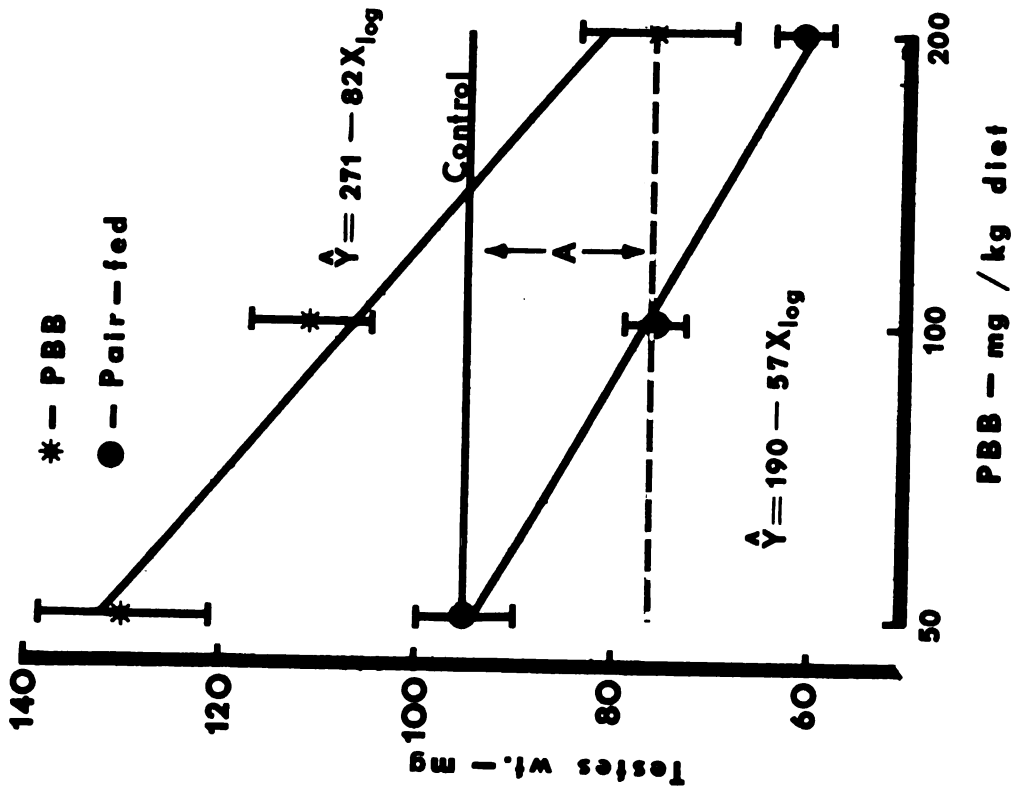

* - PBB
● - Pair-fed
$\hat{Y} = 271 - 82X_{log}$
Control
A
$\hat{Y} = 190 - 57X_{log}$
Testes wt. - mg
140
120
100
80
60
50
100
200
PBB - mg / kg diet

Figure 19. Comb weight of SCWL (pair-fed) cockerels fed rations containing PBB. Mean values ± S. E. Y = comb weight (mg); X= PBB level (mg/kg) in the diet; A indicates a non-significant change ($p<0.05$).

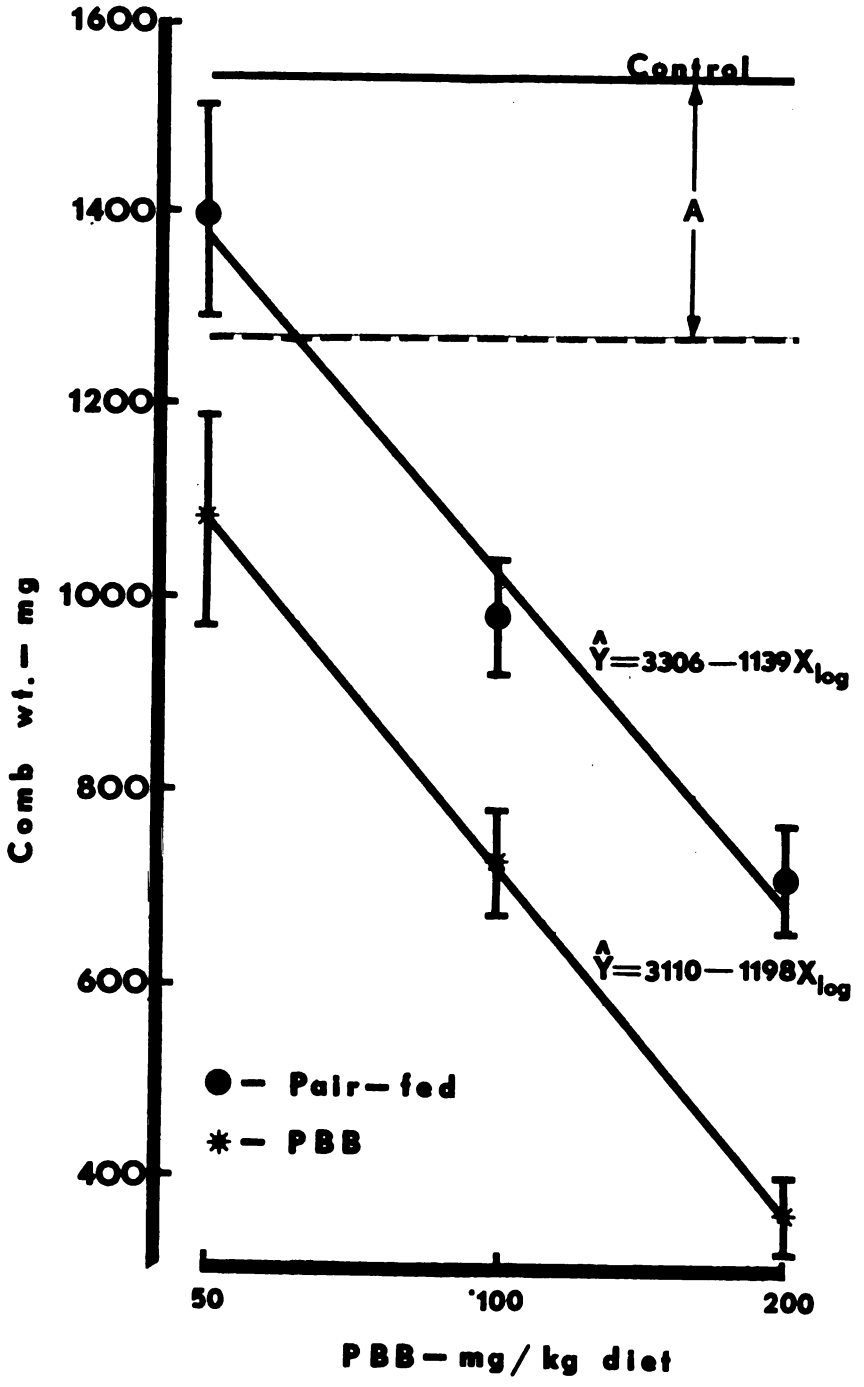

1600
1400
1200
1000
800
600
400
Comb wt. – mg
Control
A
$\hat{Y}=3306-1139X_{log}$
$\hat{Y}=3110-1198X_{log}$
● – Pair–fed
✱ – PBB
50
100
200
PBB – mg / kg diet

Figure 20. Testes weight (mg/100g bw) of SCWL cockerels fed rations (pair-fed) containing PBB. Mean values ± S. E.

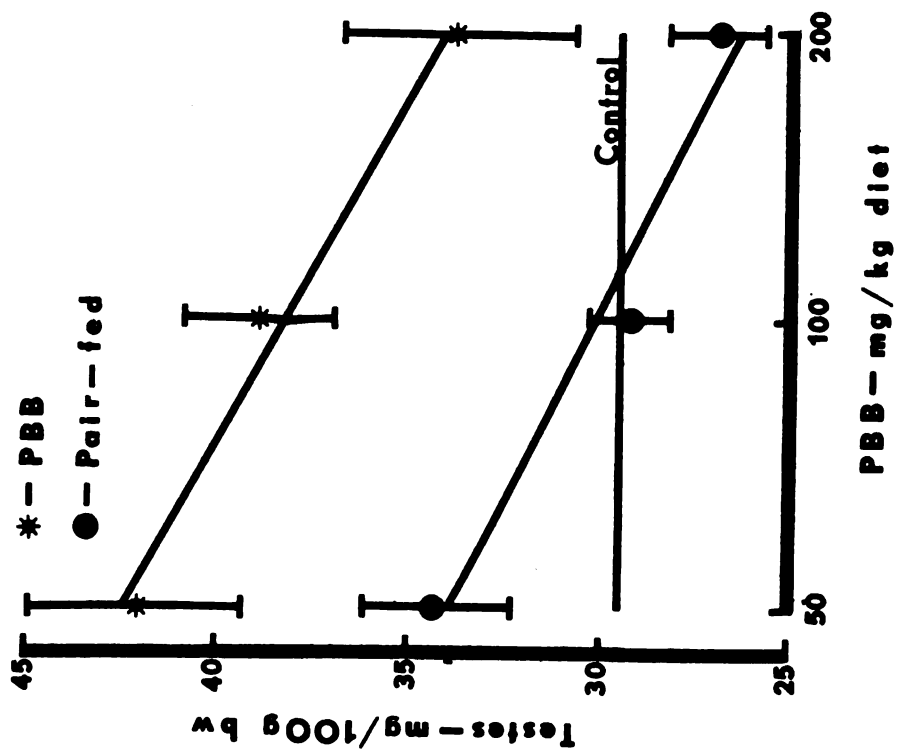

* — PBB
● — Pair-fed
Testes — mg/100g bw
45
40
35
30
25
Control
50
100
200
PBB — mg/kg diet

Figure 21. Comb weight (mg/100g bw) of SCWL cockerels fed rations (pair-fed) containing PBB. Mean values ± S. E.

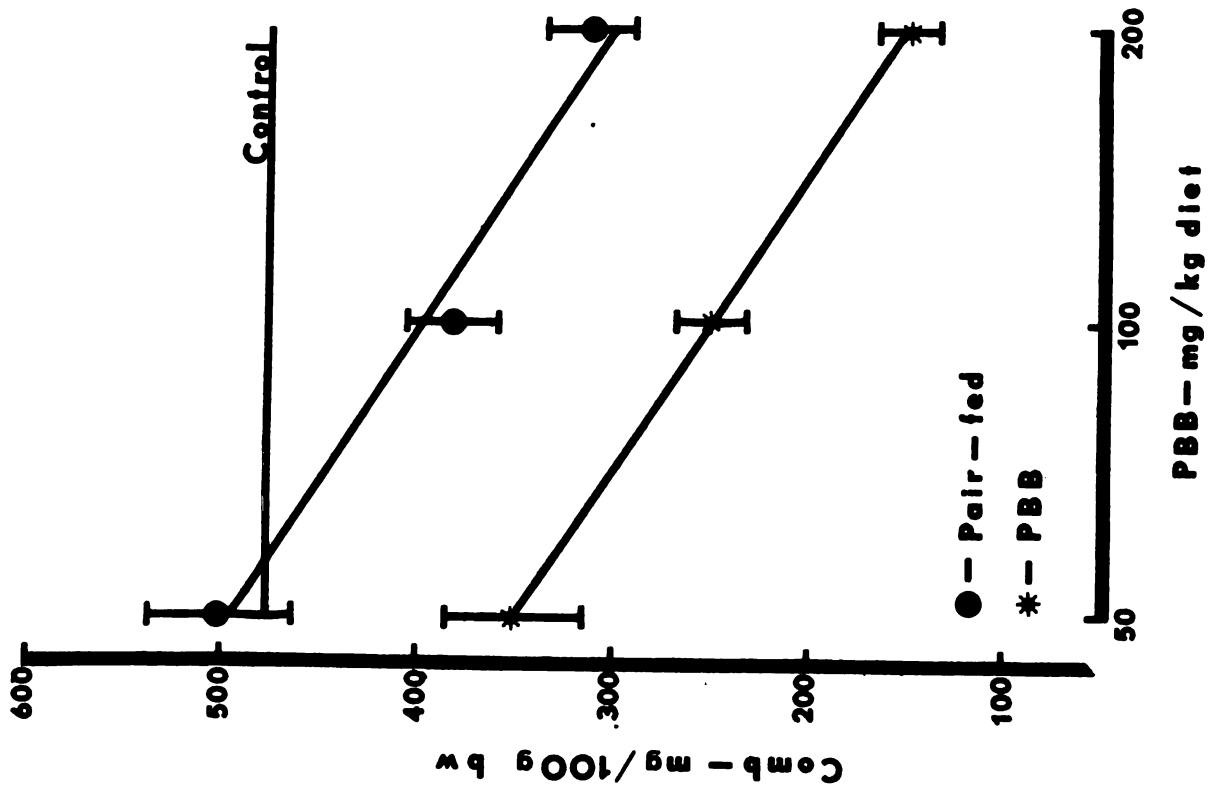

Control
600
500
400
300
200
100
Comb – mg/100g bw
50
100
200
PBB – mg/kg diet
● – Pair-fed
* – PBB

Table 14. Pair-fed study of testes and comb weights of SCWL cockerels fed rations containing PBB.

| PBB Level (ppm)[a] | Testes wt. (mg) | Testes (mg/100g bw) | Comb wt. (mg) | Comb (mg/100g bw) |
|---|---|---|---|---|
| 0 | 95.23±4.57[b] (30) | 29.34±1.29 (30) | 1542.0±111.6 (30) | 475.5±33.4 (30) |
| Pair-Fed 50 | 95.00±4.80 (29) | 34.23±1.89 (29) | 1395.7±108.4 (29) | 500.0±37.0 (29) |
| PBB 50 | 129.72±8.79 (29) | 41.95±2.58 (29) | 1080.6±108.8 (29) | 350.8±34.3 (29) |
| Pair-Fed 100 | 75.57±3.08 (29) | 29.18±1.08 (29) | 984.6±62.6 (30) | 382.4±24.3 (30) |
| PBB 100 | 111.38±5.93 (28) | 38.85±1.90 (28) | 725.1±57.5 (28) | 249.9±18.2 (28) |
| Pair-Fed 200 | 61.01±3.02 (29) | 26.88±1.24 (29) | 709.2±55.8 (29) | 310.6±23.2 (29) |
| PBB 200 | 79.96±8.14 (28) | 33.68±3.03 (28) | 357.9±40.6 (28) | 149.2±14.7 (28) |

[a]Rations fed up to 4 weeks

[b]Data are reported as Mean ± Standard Error

Figure 22. Thyroid weight of SCWL (pair-fed) cockerels fed rations containing PBB. Mean values ± S. E. A indicates a non-significant change ($p<0.05$).

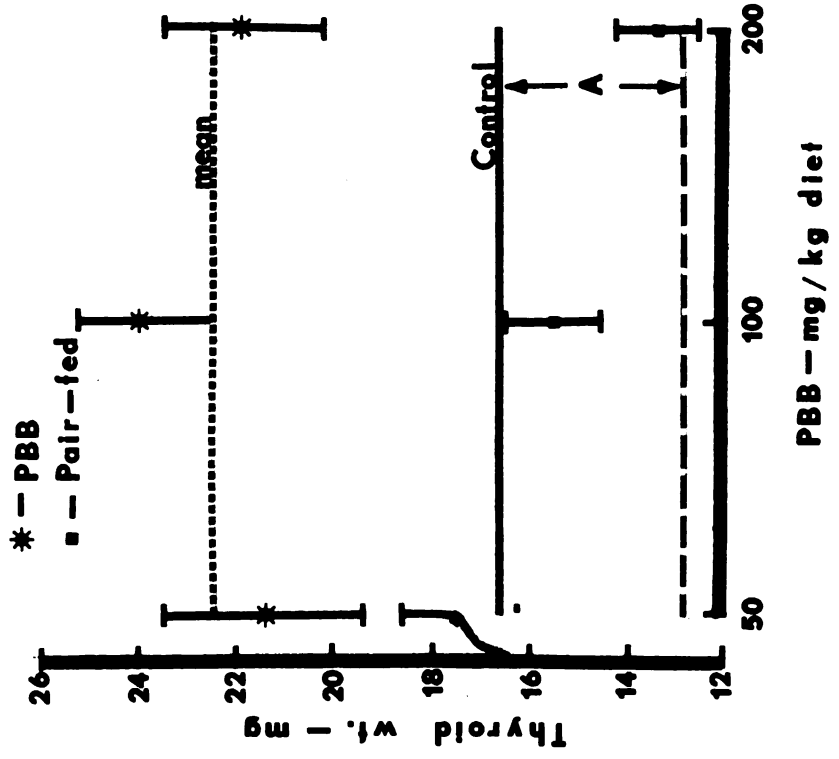
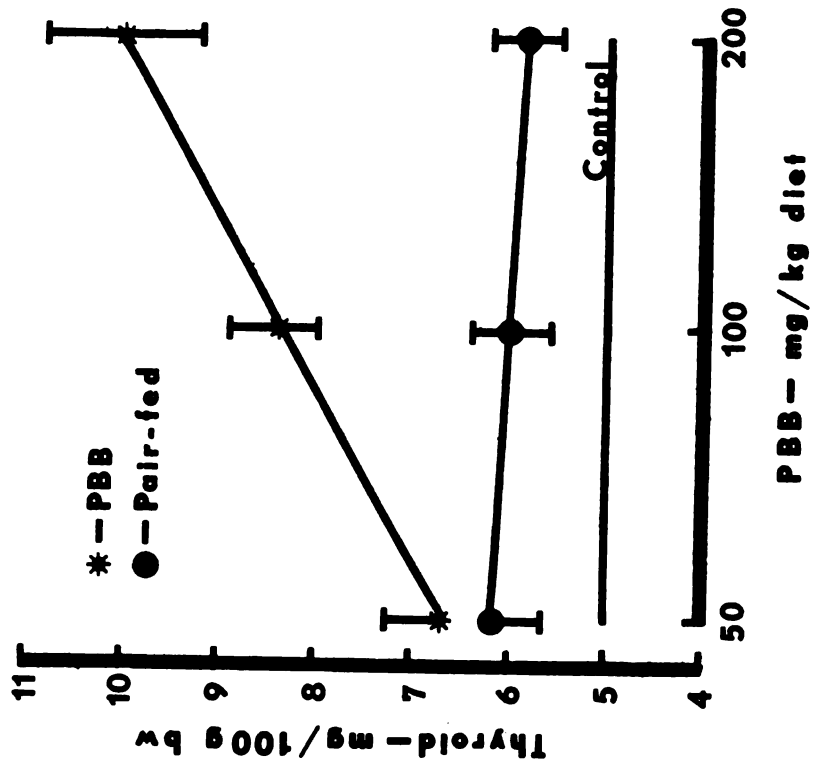

* — PBB
▪ — Pair-fed
mean
Control
A
Thyroid wt. — mg
26
24
22
20
18
16
14
12
50
100
200
PBB — mg/kg diet

Figure 23. Thyroid weight (mg/100g bw) of SCWL cockerels fed rations (pair-fed) containing PBB. Mean values ± S. E.


Thyroid — mg/100g bw
11
10
9
8
7
6
5
4
*—PBB
●—Pair-fed
Control
50
100
200
PBB— mg/kg diet

# GENERAL DISCUSSION

Toxicity symptoms after oral administration of PBB were similar to those described for PCBs by other workers. A very evident symptom was decreased body weight. Comparing PCB pair-fed work (Iturri, 1974) and the present PBB study it was evident that the decreased feed intake was responsible for the lower body weights and not an effect of the drug *per se*. The drug could be unpalatable to the bird or may act as a depressant on the appetite center in the hypothalamus.

The heart weight was significantly reduced in both experiments when PBB was fed. However, the pair-fed study demonstrated that the decrease was due to the lowered feed intake and not due to the drug directly. As was pointed out previously, although there was no change in weight there seemed to be morphological changes in the heart of treated birds. The hearts appeared to be thin-walled and flaccid.

The effects of PBB on the liver in Experiment 1 are misleading on an absolute weight basis unless they are considered with the pair-fed study. This study demonstrated that in a feed-reduction condition the liver would decrease in size, but in Experiment 1 they remained the same and in the pair-fed the mean weights increased. These results provide evidence that PBB does cause definite changes in the liver.

Mean spleen weights in treated groups were decreased in both experiments. Although the high spleen weight in the pair-fed 50 group precludes making a definite statement, it would appear from this and similiar studies with PCBs (Flick et al., 1965; Iturri, 1974) that the drug does have an effect on the spleen and that this is not the result of decreased feed intake, but an action on the spleen itself or the immunological system.

Testes and comb weights show an interesting and consistent pattern in both studies. Comb weight is significantly reduced at the 50 ppm level but the testes weight at this level is equal to or above the weights of testes of controls. The effect on the comb is therefore elicited before a similiar effect is seen in the testes. Decreased secondary sexual characteristics is indicative of interference in the testosterone production or utilization mechanism. The specifics of these results are not clear.

The significant increase in weight of the thyroids, the leveling off and the decrease in weight at the 250 ppm level indicate a stimulatory action at lower levels. As the dosage in the feed is increased a toxic effect seems to be demonstrated and finally a decreasing trend is noted. Similiar results were seen in the pair-fed study.

# SUMMARY AND CONCLUSIONS

Symptoms observed after chronic administration (0-250 ppm) of PBB to 180 chicks and then again to 210 chicks in a pair-fed study (0-200 ppm) included 1) depressed body weight as a result of decreased feed intake, 2) decreased comb, testes and spleen weights, 3) decreased heart weight as a result of decreased feed intake, 4) increased liver and thyroid weights.

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# BIBLIOGRAPHY


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