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The Effect of Clomid (Clomiphene Citrate) on Chickens

III. Sexual Characteristics in the Male

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ABSTRACT

The effects of daily force-feeding 2 mg/bird of clomid (clomiphene citrate)[2] were investigated using sixty, 12-week old, Single Comb White Leghorn-type cockerels.

Force-feeding of clomid significantly improved body weight gain and feed efficiency of the cockerels. Between the treatment means no significant differences were found in respect to feed consumption. Clomid severly decreased comb and testis weights. Also daily injection of 20 ug dehydro-epi-androsterone to cockerels fed clomid reduced comb and testicular weight significantly but to a lesser degree than clomid alone. On the other hand, adrenals and thyroids weights were significantly increased. Though liver weight was not significantly affected in treated cockerels, the cytological studies using PAS and osmic acid indicated a reduction in liver glycogen accompanied by an increase in fat deposition. Additionally, the spectrophotometric determination of plasma glucose showed that clomid treated cockerels had significant lower glucose levels.

INTRODUCTION

In a previous paper, the same authors (13) showed that the administration of clomid increased egg production in laying pullets.

Scommegna and Lash (11) reported that clomiphene has no apparent progestational, androgenic or anti-androgenic activities. However, McGinnis and Wallace (9), using one-day-old male chicks, reported that clomiphene possesses some anti-androgenic activity whether applied topically on the chick comb or injected intramuscularly.

The objective of the work reported herein was to ascertain the influence of the drug on the male sexual characteristics.

MATERIALS AND METHODS

A total of sixty Single Comb White Leghorn-type cockerels, 12 weeks of age, were used in the present study. They were housed in wire cages, two birds per cage. Each cage was 23 cm × 46 cm with a height of 40 cm.

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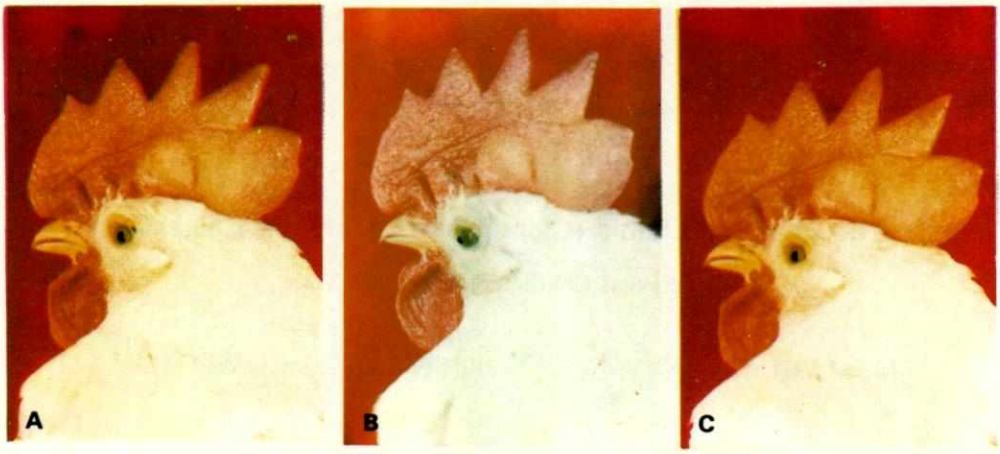

Fig. 1. A. Comb of a control bird; B. Comb of a clomid + testesterone treated bird; C. Comb of a clomid treated bird.

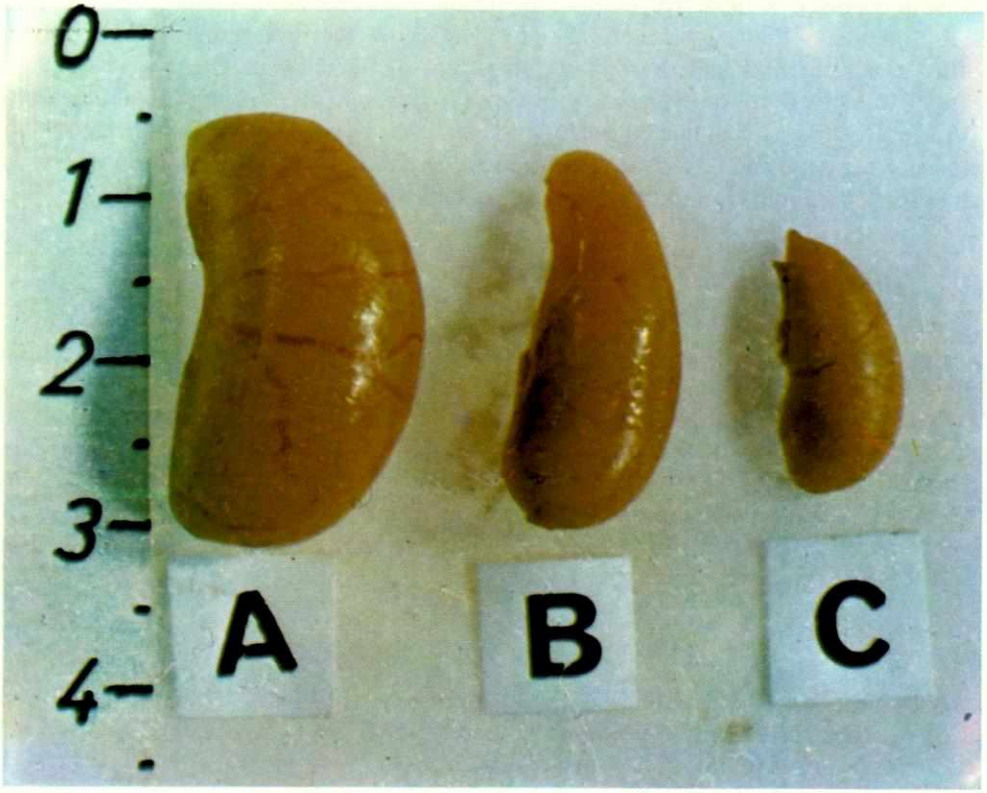

Fig. 2. A. Testis of a control bird; B. Testis of a clomid plus Testesterone treated bird; C. Testis of a clomid treated bird.

The birds were divided into four equal groups of 15 birds. Each bird in the first group was orally given 2 mg daily of clomid suspended in 1 ml sterile saline; each bird in the second group was orally given 1 ml clomid plus 20 ug testestrone (dehydro-epi-androsteron) given intramuscularly in the peronus longus muscle; in the third group, acting as a control for the first group, each bird was orally given 1 ml daily of sterile saline; in the fourth group, acting as a control for the second group, each bird was given 2 ml of sterile saline — 1 ml orally and 1 ml intramuscularly.

All birds were fed a standard growing diet containing 17% protein. Feed and water were available to birds *ad libitum.*

Feed consumption and body weights were recorded daily for each of the four groups. At the end of the experiment, 8 birds randomly taken from each group were killed by dislocating the cervical vertebra. Testes, thymi, adrenals, thyroids, spleens, livers and combs were then removed from each of these birds and weighed individually.

A small part of each liver was kept in 1% Aqueous Osmic Acid solution for 3 days and was then prepared for cytological fat detection. Another part of each liver was kept in absolute methyl alcohol for 24 hours and was then prepared and stained with Periodic Acid Schiff (PAS) for cytological glycogen detection.

Blood samples from the wing vein were drawn using heparinized syringes and needles. The plasma was separated by centrifugation then stored at −20 C pending analysis. Only one blood sample was taken from each bird. Plasma glucose was measured at the end of the experiment, which lasted 15 days, by the anthrone method described by Seifter *et al.* (12) using a spectrophotometer at wave length 560 u.

Data obtained were analysed by the analysis of variance as described by Snedecor and Cochran (14).

RESULTS AND DISCUSSION

Comb weight

Comb weight data is presented in Table 1. The comb weight was significantly reduced by the administration of clomid whether alone or with testesterone. Clomid administration severely reduced the comb weight to nearly $\frac{1}{7}$ of its weight in the control group. However, the addition of testesterone tended to alleviate the effect of clomid, as the comb weight was only reduced to $\frac{1}{3}$ of its weight in the control group.

Table 1 The effect of clomid and clomid plus testesterone on comb, testis, adrenal and thyroid weights; and plasma glucose of White Leghorn cockerels.

		Gland Weight[1]			
Treatments[2]	Comb weight	Testis	Adrenal	Thyroid	Plasma glucose
	gm	mg	mg	mg	mg/100 ml
Clomid	5.4[a](8)	350[a](8)	220[a](8)	154[a](8)	150[a](6)
Clomid + testesterone	10.9[b](8)	1090[b](8)	130[b](8)	80[b](7)	225[b](7)
Control[3]	32.6[c](16)	2440[c](16)	160[b](15)	109[b](11)	210[b](12)

[1] Average weight of two glands.
[2] Number of birds used is shown in brackets.
[3] Average means of the two control groups.

This effect of clomid on the comb resembles that of castration of cockerels which was well established after the classical work of Berthold (1) who was the first to note that castration causes atrophy of the comb and that the transplantation of testicular tissue into a castrated rooster prevented that effect. Since then, numerous evidence has been accumulating confirming the sensitivity of the cock's comb to castration and to the administration of androgen.

Recently Dickson (3) reported that the castration of the cockerel is followed by atrophy of the comb and wattles with a loss of red colour, a loss of courtship behaviour, and a loss of pugnacity. It has been observed in the present experiment that the clomid treated birds exhibited poor plumage.

The decrease in comb weight which resulted from the administration of clomid (Fig. 1) could be due to the anti-androgenic effect of the drug. A further proof is the opposing effect of dehydro-epi-androsterone injected into the clomid fed group in which the comb weight was slightly less affected.

Testis weight

The administration of clomid alone dramatically reduced testicular weight and size as compared with either the control group or the group receiving clomid plus testesterone (Table 1). Despite the fact that testicular weight of birds receiving clomid olus testesterone was greatly heavier than that of birds receiving clomid alone, it was nevertheless significantly lower than the control (Fig. 2). Several hormones may be involved in this large reduction of testicular weight.

It has been reported that the administration of prolactin in birds causes: atrophy of the testis, a reduction in androgen secretion and a concomitant decrease in size of the comb (Bates *et al.*, 1937; Breneman, 1942; Nalbandov, 1945; as reported by Lake and Furr, 8). Similar results have been obtained on the effect of adrenocorticotrophic hormone (ACTH) on the fowl testis. This hormone, given to 26-week-old cocks caused a decrease in testis weight and disruption of spermatogenesis (Flickinger, 5). Dulin (4) has shown that the administration of cortisone decreased testis weights in 6-week-old cocks.

It is almost certain that the LH of the gonadotrophic complex is responsible for the stimulation of interstitial leydig cells which respond by secretion of androgen. Larse and Furr data (8) shows that there is a strong correlation between testis weight, rate of testesterone production, and the weight of androgen-dependent end organs. Nalbandov (10) has reported that it appears virtually certain that both FSH and LH are essential to induce full and complete spermatogenesis. Experimental evidence suggests that androgen does stimulate spermatogenesis but does not simply maintain sperm cells that have differentiated while the testes were under gonadotrophic stimulation.

Adrenal and thyroid weights

In the clomid treated cockerels both adrenal and thyroid weights were significantly greater than those of the control group and those receiving clomid plus testesterone ($P < 0.05$). From the data shown in Table 1 it appears that clomid causes over-stimulation of the adrenal and thyroid glands. Whether this effect occurs directly or indirectly through the hypothalamus has not been ascertained.

Flickinger's studies (5, 6) on the effect of ACTH revealed that the administration of ACTH results in adrenocortical hyperplasia and that this may be accompanied by an increase in adrenal weight. The increased steroid output stimulated by

ACTH caused a linear reduction in the size of the testes (Flickinger, 5). Nalbandov (10) reported that the response of the seminal vesicles to adrogen is reduced in the hyperthyroid condition.

Plasma glucose

Plasma glucose levels are shown in Table 1. Between the birds of the control group and those receiving clomid plus testesterone there was no significant difference in the level of plasma glucose. The administration of clomid alone significantly reduced plasma glucose (hypoglycaemia) which may be due to the direct or indirect effect of the drug on the adrenal glands.

Liver

Liver weight of the treated groups was not significantly affected as shown in Table 2 ($P < 0.05$). Cytological studies revealed that the glycogen in the liver of the clomid treated birds was less than that of the control, whereas the amount of fat was much higher than either that of the control group or the clomid plus testesterone treated birds. The increase in fat deposition in the liver may be due to the conversion of glucose to fat, indicated by the depression in plasma glucose level of the clomid treated birds.

Table 2 The effect of clomid and clomid plus testesterone on liver weight, feed intake, body gain and feed efficiency of White Leghorn cockerels.

Treatments[1]	Liver weight	Feed intake	Body gain	Feed efficiency
	(gm)	(gm/bird/day)	(gm/day)	(kg feed/kg body gain)
Clomid	31.9^{a}(8)	114^{a}(15)	18.75^{a}(15)	6.08^{a}(15)
Clomid + Testesterone	29.4^{a}(8)	107^{a}(15)	17.25^{a}(15)	6.20^{a}(15)
Control[2]	29.3^{a}(14)	106^{a}(30)	12.16^{b}(30)	8.72^{b}(30)

[1] Number of birds used is shown in brackets.
[2] Average means of the two control groups.

Other parameters studied

Feed consumption, body weight gain and feed efficiency data are shown in Table 2. Neither the administration of clomid nor the administration of clomid plus testesterone affected feed consumption ($P < 0.05$). These results differ from those obtained by the same authors (13, I) using 6 months White Leghorn pullets. This may be due to the difference in age and reproductive status of the birds.

Expressed as gm/day, clomid alone and clomid plus testesterone had no significant effect on body gain ($P < 0.05$). However, when compared with the control, the body weight gain of the treated groups was significantly increased. Subsequently, the administration of both clomid alone and clomid plus testesterone had significantly improved feed efficiency ($P < 0.05$).

CONCLUSION

Clomid may depress the male sexual characteristics in White Leghorn cockerels through 3 possible routes:

The first is that the drug may have an influence on the arcute nucleus of the

hypothalamus causing a severe decrease of GnRH which in turn suppresses LH secreted by the adenohypophysis. This leads to a marked decrease in the number of leydig cells of the testis with a subsequent reduction in the production of androgen to such a degree that it causes comb atrophy, poor plumage with a concomitant impaired spermatogenesis.

The second may arise through a direct effect of the drug on the adrenal cortex. This may occur through activating the ventromedial nucleus of the hypothalamus to increase CRH causing release of ACTH which leads to:

a) activation of the adrenal glands, indicated by the increase in their weight, observed in the present experiment, with a subsequent increase in the adrenocortical hormones.

b) a decrease in the testicular weight and disruption of spermatogenesis, as reported by Flickinger (1966).

Dulin (4) and Kudiza and Champion (7) reported that cortisone has an inhibitory effect on the reproductive system of the cockerel, mouse and rat respectively.

The third possibility is that the drug may act directly on the testis causing a decrease in the leydig cell number which would eliminate androgen secretion hence causing the previously mentioned sexual insufficiency.

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تأثير عقار الكلوميد على الدجاج
٢ — الصفات الجنسية

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المستخلص

إن عقار الإخصاب المعطى لذكور طيور اللجهورن الأبيض ، انقص من وزن الأعراف والخصاه ، وكذلك انقص من مستوى الغشاء الحيواني المخزون في الكبد ومستوى الجلوكوز في الدم .

أما وزن الغدد الكظرية ، والدرقية فقد زاد بمقارنته بالطيور التي لم تُعْطَ عقار الإخصاب أو التي أعطيت عقار الإخصاب ممزوجا مع الهرمون المذكور .