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THE EFFECT OF AGE AND FEEDING AN ANABOLIC-ANDROGENIC STEROID (DIMETHAZINE) ON THE BODY AND ORGAN WEIGHTS OF FEMALE CHICKS

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Abstract: A study was undertaken to obtain reference values under local conditions on the effect of age on the organ weights of various internal organs during early ontogenetic development of female chicks (pullets). In addition, the effect of feeding, an anabolic-androgenic steroid, dimethazine, on the growth of chicks was also studied. During the first 100 days of life, the growth of liver, kidney, heart, adrenal, thyroid, ovary, and muscles pectoralis, gastrocnemius, and peroneus longus was different qualitatively and quantitatively. Feeding of anabolic-androgenic steroid increased the appetite of the birds but did not induce any changes in the FCE. The steroid fed groups were heavier ($P<0.05$) than the control groups. Some of the organs studied had heavier weights (statistically significant in certain instances) in the steroid-fed groups when compared to the control groups. The study confirms some earlier reports that androgens can induce positive growth response in birds as they do in mammalian species. .

Key words: Chicks, age, body weight, liver, kidney, heart, muscles, thyroid, adrenal, specific growth rate, and food consuming efficiency.

INTRODUCTION

The subject of growth and development has generated considerable interest among biologists and livestock scientists. Because of these studies, reference values have been reported for different body organs of various animals of economical interest. This type of research has been completely neglected in Pakistan. Although, new breeds have been imported from abroad where these reference values are available for these species. Nevertheless, it has been seen that these values cannot be regarded as representative here in Pakistan, because of many reasons like different physical environmental conditions, or nutritional regimes, etc., which at best are not optimal and normally can be termed poor, in which these animal are kept. In reporting organ weight changes during normal ontogenetic development, one cannot predict whether these differences in weights of internal organs are "normal" under the prevalent conditions or are due to the deficiency of nutritional factors or disease. Furthermore, in the absence of the reference values under local conditions, the researchers cannot make a clear decision as to whether the changes seen in their studies are true effects of growth and development or a combination or interaction of age with other experimental factors. Strain differences in weights of body organs have been reported in broilers and chickens (Hoffman *et al.*, 1953; Al-Dabagh and Abdulla, 1963; Daghir and Pellett, 1967). Similarly, differences in body organ weights of birds have also been reported by various workers under different nutritional regimes (Marion and Edwards, 1963; McCartney and Brown, 1977; Hargis and Creger, 1980; Marks, 1978a,b, 1979; Cornejo *et al.*, 1980).

Androgens, and their derivatives known as anabolic-androgenic steroids, have been shown to stimulate protein anabolism in a variety of laboratory animal species and livestock animals like cattle (Kochakian, 1976; Lone, 1996). Their use as anabolic agents in chicken has not been particularly productive. Evidence to date suggest that androgens neither affect feed efficiency nor growth however, some positive reports also have appeared in this direction (for more details see, Lone, 1996). Dimethazine, a potent anabolic-androgenic steroid used in human medicine, has a very favorable anabolic to androgenic ratio. The experiments done with rats and other mammalian and fish species (Lone and Matty, 1984) always gave positive results as far as positive nitrogen balance and myotropic effects are concerned. Keeping in view its higher anabolic efficiency with comparatively lower androgenic side effects it was decided to study its effects on growth of pullets when fed with diet from age day-one to day 97.

MATERIALS AND METHODS

Newly hatched, three hundred (300) single comb, white Leghorn (layers variety) female chicks (Pullets) were obtained from a local commercial hatchery. The birds were housed in a well aerated and air-conditioned room where the temperature was kept between 25-30 °C. The room floor was thickly covered with sawdust which was changed periodically. Food and water were supplied *ad libitum*. The chicks were fed commercial broiler starter diet (proximate analysis: protein = 16.03%; fat = 4.93%; carbohydrates = 55.53%; moisture = 9.86%; ash = 7.83%). At two weeks of age all chicks were vaccinated against New Castle disease. Before the start of the experiment the chicks were divided into two (2) groups. One group was designated as control and the other experimental. The control group was given a normal diet without any hormone in it while the experimental group was provided with a diet containing dimethazine (2α-17α-dimethyl-17ß-hydroxy-5α-androsten-3,3'-azine; Roxilone, Richter, Italy) at a concentration of 5 mg/kg diet (5 ppm). The steroid was incorporated in to the diet by the method of Lone and Matty (1980). This method essentially means dissolving the steroid in absolute alcohol and then spraying the alcoholic solution on to the diet with the help of a chromatographic sprayer. The volume of alcohol used for the experimental diet is also sprayed on to the control diet but without any steroid. The alcohol is then evaporated in air and the diets are kept in a freezer for future use. For feeding, the chicks were provided diets in an appropriate feeder and the daily intake by the birds was noted. Chicks were weighed individually on the day of hatching and on every alternate day up to 14 days, every four days up to 38 days and weekly thereafter, up to 97 days. Weighing was done to the nearest gram.

At each sampling, in addition to taking the total body weight, different body organs like heart, liver, kidney, muscle gastrocnemius, pectoralis major, peroneus longus, thyroid gland, adrenal gland and ovaries were dissected out from eight birds and immediately weighed to the nearest mg. Moreover, comb weight, tibiotarsus weight and length and shank length were also taken. The sampling for these organs was done on the day of hatching and on alternate days up to ten days. Every fourth day up to the age of 38 days, every week up to the age of 52 days and fortnightly thereafter, till the end of the experiment. The weighing of organs was done to the nearest mg.

On the basis of the data obtained on the feed consumption of the control and experimental chicks, feed consumed per chick per week and feed conversion efficiency (weight gain/food eaten X 100) were computed for each group. Specific growth rate (SGR) and tissue-somatic index (relative weight of organ) were also computed according to the following formulae.

$$SGR = \frac{\text{Ln W - Ln w X 100}}{\text{Time (Days)}}$$

where Ln is log natural and W and w are final and initial weights of the bird.

$$\text{Tissue-Somatic Index} = \frac{\text{Weight of the organ}}{\text{Body Weight of the Chick}} \times 100$$

Linear regression equations were also computed for all organs against age while the organs weights of the control and experimental birds were compared by two-tailed Student 't' test according to Sokal and Rohlf, 1973.

RESULTS AND DISCUSSION

The total body weight of each chick was taken according to the weighing schedule detailed in the Materials and Methods above. At hatching mean body weight was 38.37 ± 1.12 g. This weight at the end of the experiment after 97 days was 828.29 ± 34.18 g and 900.85 ± 37.17 g for the control and experimental chicks respectively. The experimental birds were heavier (Fig. 1a) than the control birds and this difference was significant statistically ($P < 0.05$). Fig. 2a presents the weight gain per chick per two weeks. It is clear from the figure that weight gain of the experimental birds was better than the control birds. Theoretically, the specific growth rate (SGR) decreases with age. We saw the same trend in the SGR both in experimental and control groups (Fig.2b). Figure 2c presents data regarding the food consumed by the two groups of the pullets. It appears from it that the experimental birds were eating more (increased appetite) than the control birds. This divergence in food consumed was clearly seen around 52-day of the experiment. For example, the average food consumed by the control birds in the last fortnight of the experiment was 557.44 g while the same amount for the experimental birds was 937.50 g. This increase is around 68 % over the control values. Although, the experimental birds were eating more, their food conversion efficiency (FCE) however, was lower than the control birds. These data are more clearly shown in Fig. 2c and 2d respectively.

It has been shown many times that whereas estrogens may be potent in increasing the total body weight of the birds, anabolic-androgenic steroids did not give similar results (Belloff and Hsu, 1963; Trenkle, 1969; Lone, 1996). We have seen here results with dimethazine which are different as compared with other anabolic-androgenic compounds commonly used as growth promotants. These results however, are not unique in the sense that dimethazine is considered quite potent anabolic steroid in mammalian species. Dimethazine also increased the appetite of the birds which was apparent from the increased food intake however, this increase was not accompanied by an increase in the FCE of the treated birds. Anabolic steroids generally increase the appetite of the animals and this has been reported for birds also. This factor may be the reason for not having any clear cut positive effects on the overall body weight of the birds in the previous studies for it has been reported that the birds which have been selected for higher body weight for a specific age consumed more food and converted this food to flesh more efficiently. Moreover, it has been shown in birds that appetite differences account for most of the genetic differences in growth rate (Sutton and Siegel, 1975; Reddy and Siegel, 1977; Marks, 1979, 1980a, b). In the present study although the hormone was capable of increasing the appetite of the treated birds it could not translate this increase in the appetite into higher FCE.

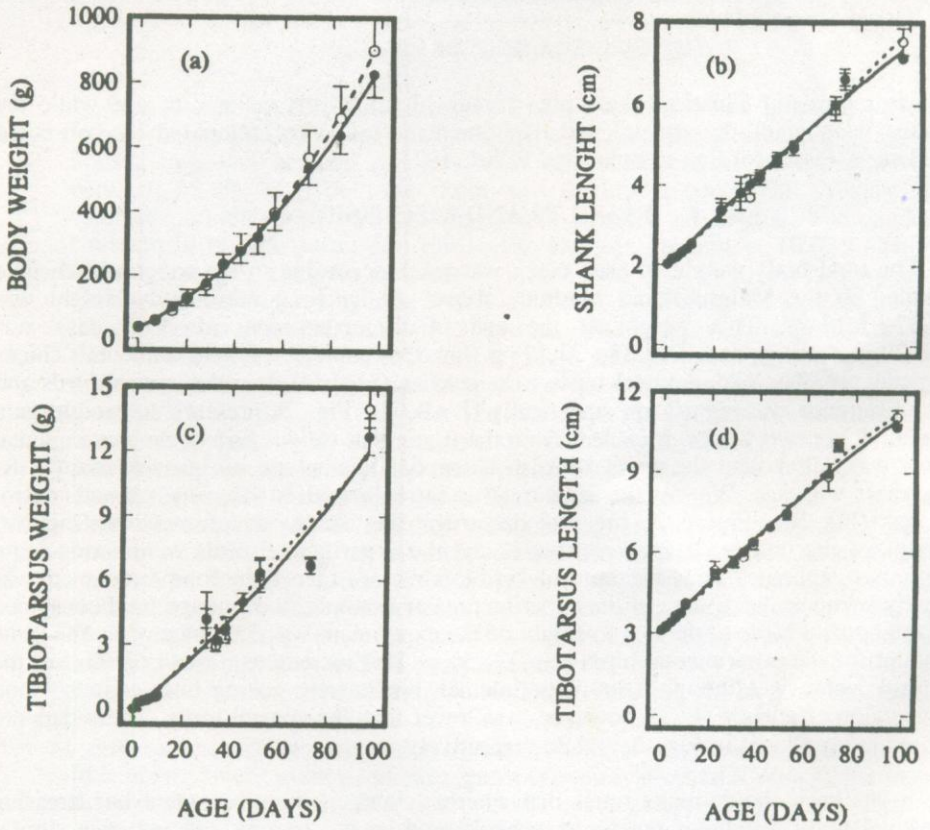

Fig. 1. Effect of feeding dimethazine on the body (a) and tibiotarsus (c) weight (g) and shank (b) and tibiotarsus (d) length (cm). Solid circles represent controls while open circles show the experimental data. Values given are mean ± standard deviation of at least 4 animals each.

There was no difference between the shank length (Fig. 1b) and tibiotarsus weight and length (Fig. 1c, 1d) of the control and treated birds. These parameters were studied in order to observe the effect of the steroid on the bone growth and height of the birds.

In addition to the body weight and other nutritional parameters, different body organs were removed from the slaughtered birds. Three muscles, i.e., pectoralis major, gastrocnemius and peroneus longus were removed and their total weights determined. The weight changes of these muscles are given in Fig. 3a, b, c, d and 4a, b. Whereas the body weight increased from zero-day to 97 days 21.39 times, the pectoralis, gastrocnemius and peroneus longus grew during the same time around 117, 34 and 58 times respectively. This means that these muscle were growing much faster than the body weight during the time of the experiment. Here, the steroid fed animals always

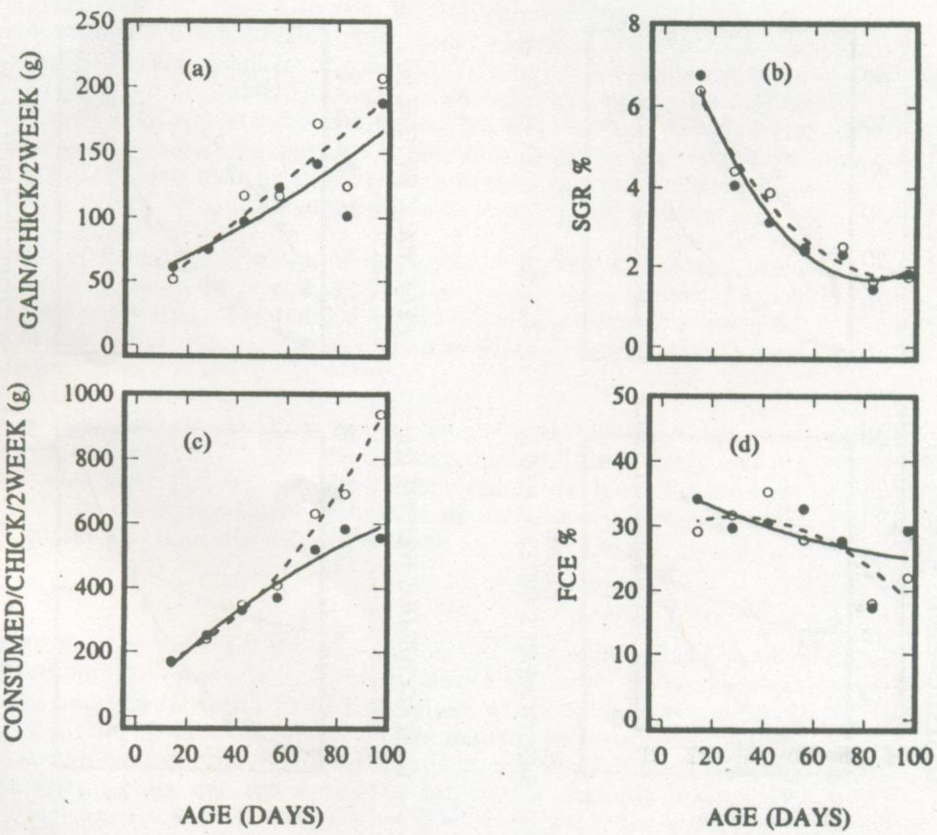

Fig. 2. Effect of feeding dimethazine on the weight gain (a), specific growth rate (g%) (b), feed consumed (c) and feed conversion efficiency (d) of female chicks. For more details please see Fig. 1.

had slightly heavier muscles than the respective control animals. For example, the pectoralis ($P<0.05$) and gastrocnemius ($P<0.001$) were 14 % and peroneus longus ($P<0.01$) was 22 % heavier than their respective controls at the end of the experiment. When seen in terms of the relative weight (tissue-somatic index) of the individual muscle, the growth pattern of the two leg muscles was different from the breast muscle (compare Fig. 3a and 4a). The growth pattern of the breast muscle was exponential while the growth of the leg muscles was linear in nature. Moreover, both leg muscles also differed in their growth characteristics. The growth of the peroneus longus was quite slow during the first month of life but became quite faster afterwards. These differences probably stem from the different function of the specific muscle and cellular events which are triggered because of the function. This can also be seen from the response of these muscles under experimental conditions of restricted nutrition or

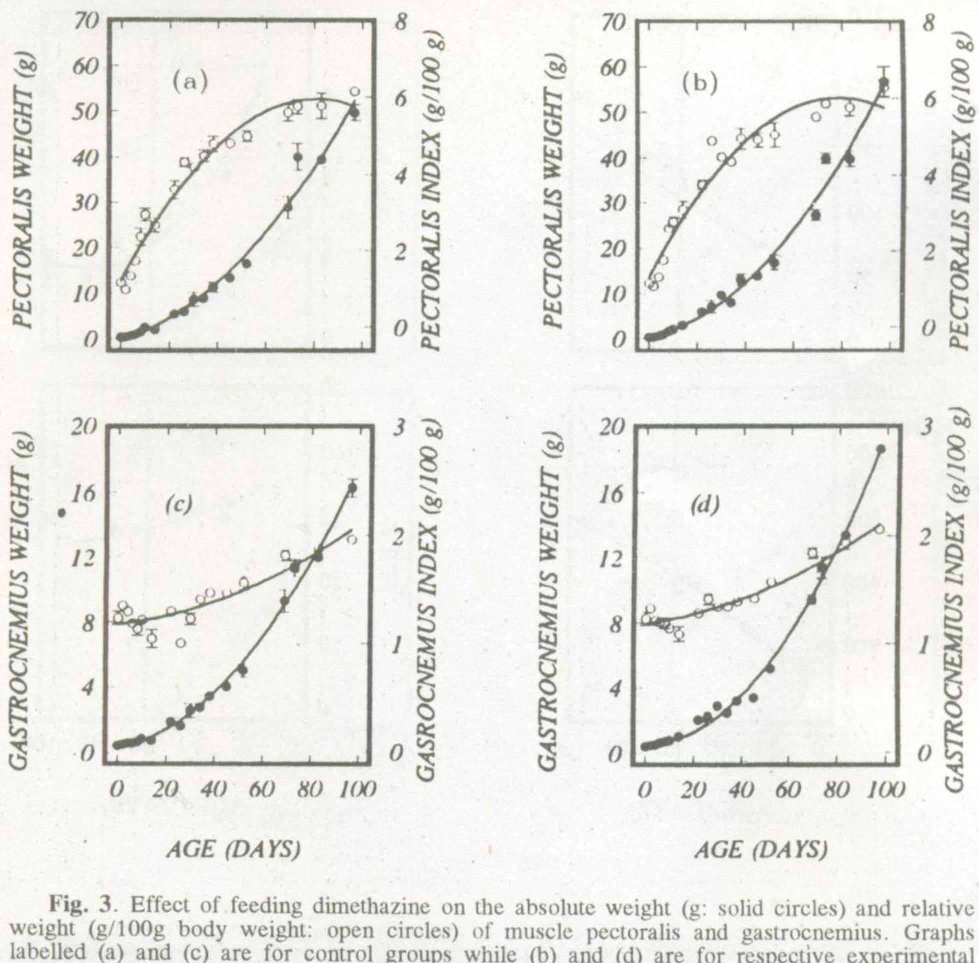

Fig. 3. Effect of feeding dimethazine on the absolute weight (g: solid circles) and relative weight (g/100g body weight: open circles) of muscle pectoralis and gastrocnemius. Graphs labelled (a) and (c) are for control groups while (b) and (d) are for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

starvation. The muscle which perform more active function, for example, leg muscles, are affected most by the energy or feed restriction than those muscles which are less active functionally or metabolically (Burger *et al.*, 1962; Daghir and Pellett, 1967; Cornejo *et al.*, 1980).

Kidney, liver and heart weights were taken as representative of the most important internal organs. The growth pattern of these organs is presented in Fig. 4c and d and 5a, b, c and d. The weight increase of these vital organs seem to be more or less linear with age over the entire range of the experimental period of 97 days. As reported above the total body weight increase during the experiment was 21 times, the weight increase of the liver and kidney during this time was 20 and 19 respectively, showing that these two organs were still growing directly proportional to the body weight. The heart weight increase during this time was 15.5 times, exhibiting a slowing of growth with

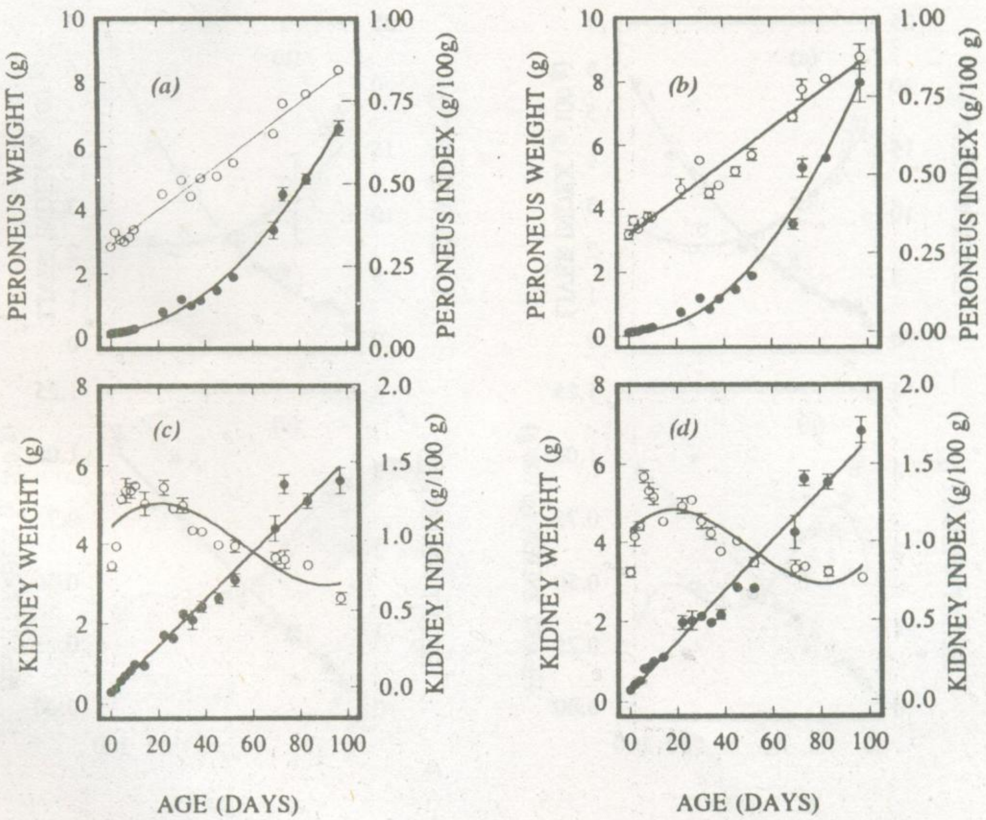

Fig. 4. Effect of feeding dimethazine on the absolute weight (g: solid circles) and relative weight (g/100g body weight: open circles) of muscle peroneus longus and kidney. Graphs labelled (a) and (c) are for control groups while (b) and (d) for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

age. Feeding of dimethazine caused the weight of these organs to increase (13.65, 21.89, and 15.49% increase in the final weight of the liver, kidney and heart respectively after 97 days of steroid feeding). This increase in weight over controls was significant ($P<0.01$) statistically. When the weight of these organs were calculated as the percentage of the body weight then it appears that the growth pattern of these three organs was different. The relative maximum weight of these organs in comparison to body weight (tissue-somatic index) was seen on 4th, 10th and 4th day for liver, kidney and heart. The experimental groups showed this maxima slightly earlier than the control values given above. After this maxima, the relative weight of these organs decreased steadily with increasing age. The qualitative pattern in this connection was similar between kidney and heart (compare Fig. 4c and 5c). The liver, on the other hand, showed a pattern similar to first order decay reaction. In this respect, our results are

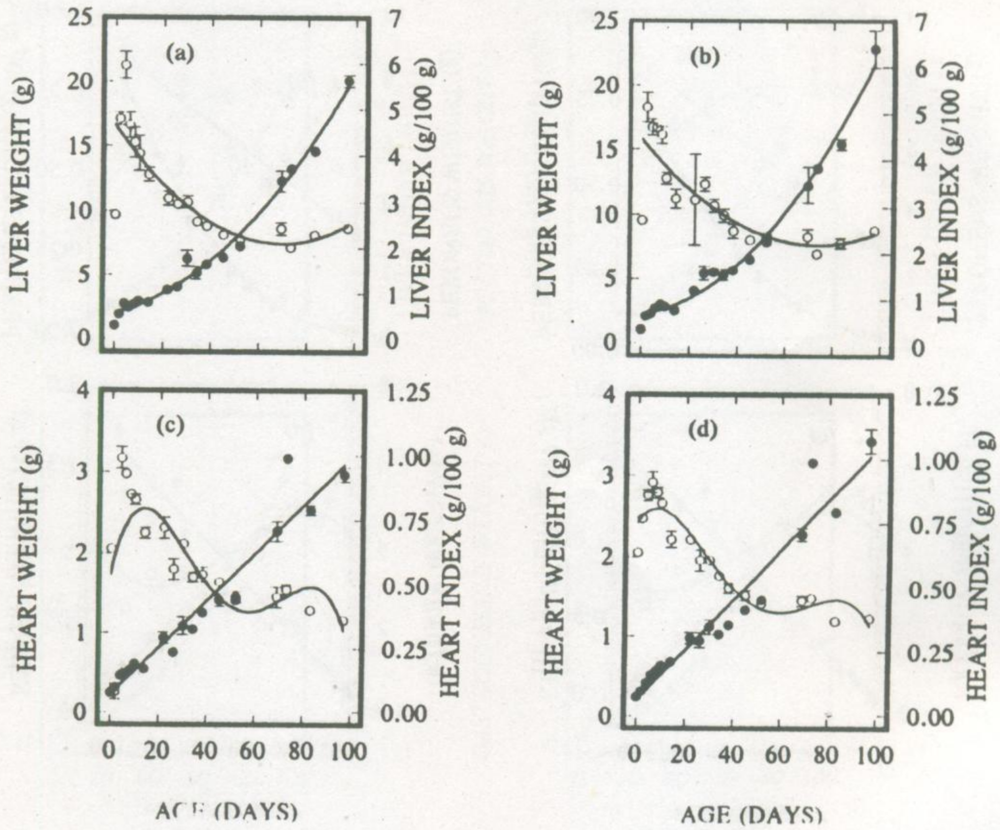

Fig. 5. Effect of feeding dimethazine on the absolute weight (g: solid circles) and relative weight (g/100g body weight: open circles) of liver and heart. Graphs labelled (a) and (c) are for control groups while (b) and (d) are for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

similar to the earlier published reports in terms of qualitative (Burger *et al.*, 1962; Al-Dabagh and Abdulla, 1963; Daghir and Pellett, 1967; Cornejo *et al.*, 1980) changes but strain, nutritional and environmental differences caused changes in the quantitative pattern of the growth of these organs when compared with earlier published values.

In addition to the muscle and other vital body organs, some endocrine organs like adrenal, thyroid and ovary and a non-endocrine tissue, comb were also dissected out from the slaughtered birds and their growth in terms of organ weights was studied. The growth pattern of adrenal gland is presented in Fig. 6a, and b. The adrenal gland weight at hatching was 4.53±0.3 mg. At the end of the experiment, at age 97 days, this weight was 67.38±3.13 mg, i.e., an increase of some 15 times the weight at hatching. The weight increase was uniform over time and the hormone fed groups also

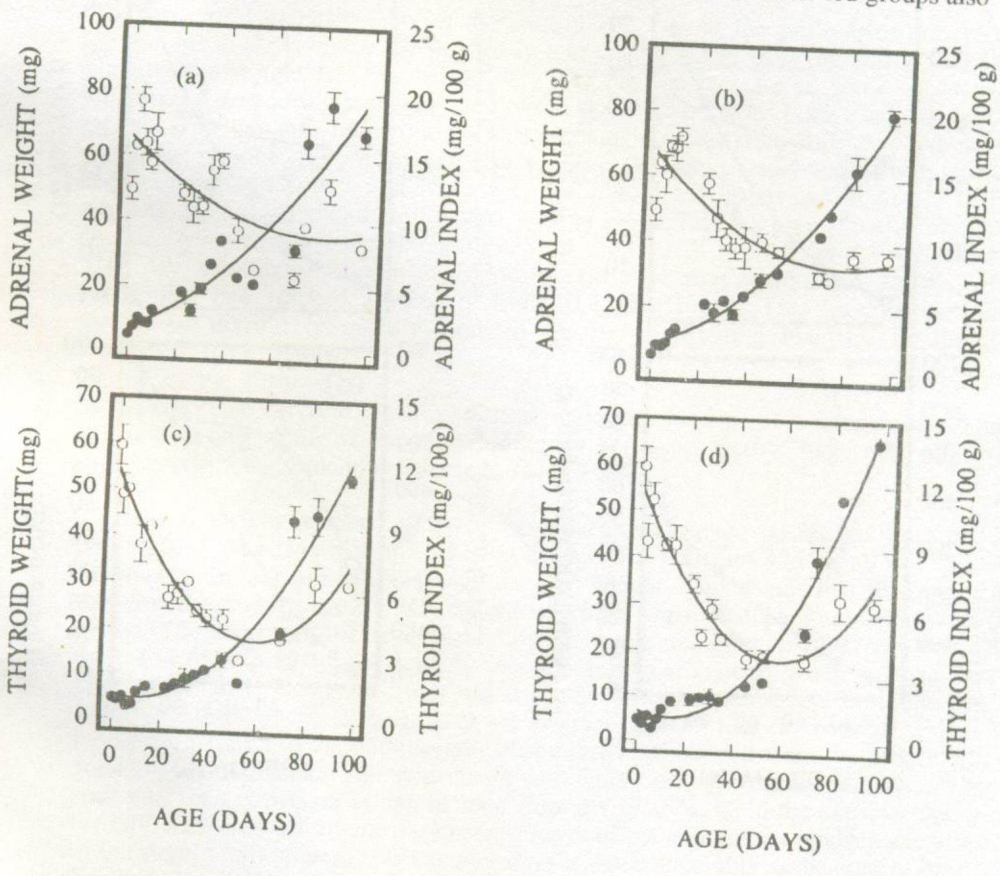

Fig. 6. Effect of feeding dimethazine on the absolute weight (mg: solid circles) and relative weight (mg/100g body weight: open circles) of adrenal and thyroid. Graphs labelled (a) and (c) are for control groups while (b) and (d) are for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

showed similar growth pattern, however, the experimental birds had their adrenals 18% heavier ($P<0.01$) than the control birds at 97 days of life. The relative mass of adrenal in control birds was maximum(19.13 ± 0.93) at day-4 of the post-natal development while this maximum weight (17.97 ± 0.65 mg/100 g) in dimethazine fed birds was seen after 10 days. This means that the dimethazine inhibited the growth of adrenal during early days of life, however, later on this inhibition is eliminated and adrenal weight catches up and becomes higher than the control values. After these maximum values the adrenal weights of both groups declined over time. At 97 days of life the relative mass of the adrenal in control birds was around 34% less than the hatching values of this parameter.

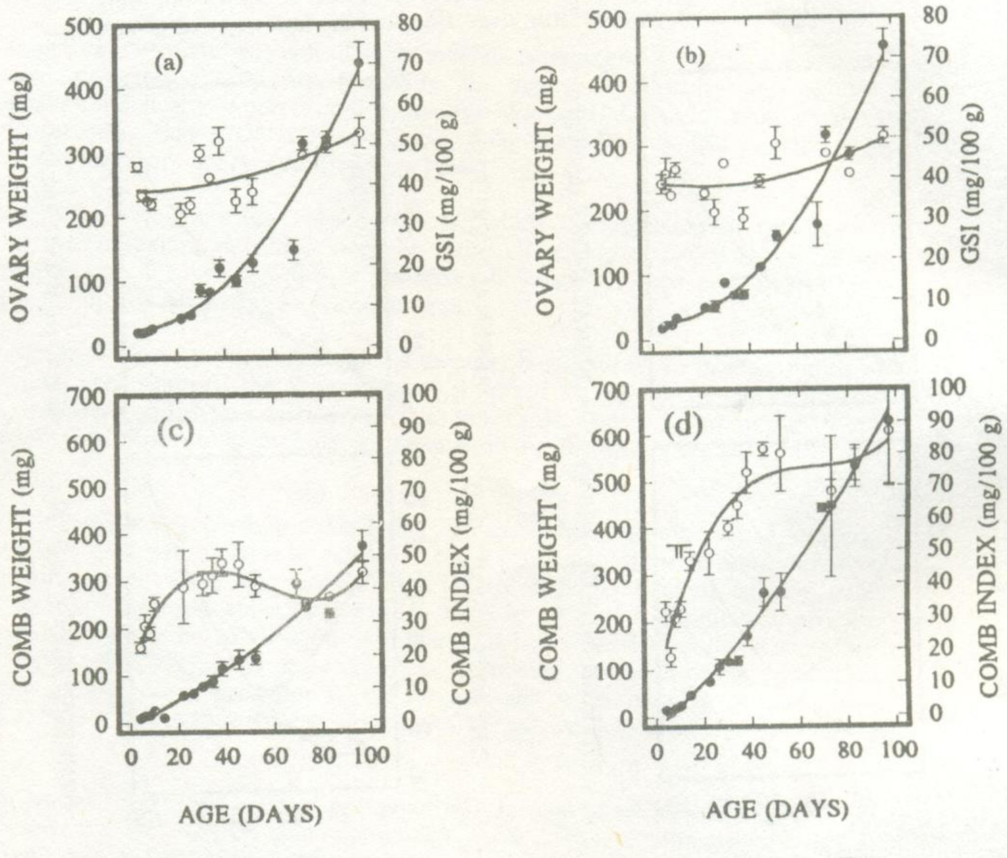

Fig. 7. Effect of feeding dimethazine on the absolute weight (mg: solid circles) and relative weight (mg/100g body weight: open circles) of ovary and comb. Graphs labelled (a) and (c) are for control groups while (b) and (d) are for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

It has been shown in many birds species that during the first few weeks after hatching the absolute weight of the adrenal gland increases but the body weight increases are proportionally more. As a result, a sharp decline in the relative mass of the gland occurs during post-natal development. An observation also seen in the present study. It may be mentioned here that once birds mature, a constancy in the relative mass of the adrenal weight is also achieved. However, in mature birds, increase or decease in adrenal weight frequently occur in response to seasonal and environmental changes and these changes reflect the functional attributes of the gland. Chronic stress and social standing of the organism also cause a change in the adrenal weight of the birds (Holmes and Cronshaw, 1980). In the present study, the birds didn't reach maturity, which is generally achieved at the age of 18-20 weeks (Gilbert, 1971) therefore, the changes reported above in the relative weights of adrenal were not observed.

The thyroid gland weights of the control and steroid fed pullets are presented in Fig. 6c and d. At hatching the thyroid weight was 12.32 ± 0.89 mg and 97 days after hatching the weight has reached 53.08±1.42 mg, an increase of some 11.34 times. This means that during this time the thyroid gland growth was nearly half of the body growth. This can also be seen in the relative weight of the control birds which showed maximum relative thyroid mass at hatching. At 97 days of age the relative mass has decreased some 49.49 %. At this stage, the steroid fed group had higher (23.40 %) absolute thyroid weights than the control birds ($P<0.001$) but there relative mass were similar. After reaching a minimum value of 2.83±0.23 mg/100 g of body weight at 52nd day, the relative weight of thyroid started increasing again and at the end of the experiment had reached a value of 6.42±0.22 mg/100 g. This increase in relative weight of thyroid is probably related to the approaching maturity of the birds (Falconer, 1971, 1984).

Like adrenal, thyroid gland also show variations because of season, iodine content of the diet and strain of the birds. Maturity and reproductive stage also affects the thyroid weight and function (Falconer, 1971, 1984).

Ovarian weight at 4 days of age (when ovaries were removed for the first time) was 20.65±0.55 mg. This weight reached at the end of 97 days to 436.68±33.81 mg. This amounts to an increase of 21 times the initial weight. This means that ovarian growth was directly proportional to body weight which also increased 21 times during this time period. The ovaries of the experimental birds (receiving 5 ppm dimethazine) at this time were 454.65±25.19 mg, a 4.12 % increase from the control group's organ weight. This means that during the first 100 days the anabolic-androgenic hormone did not affect ($P>0.05$) the total weight of the ovary. The fact that the ovarian weight was increasing parallel to the body weight of the birds can be seen from the progress of the gonado-somatic index. The gonado-somatic index did not change much during the course of the experiment as can be seen from the Fig. 7a, b. In the experimental birds, the GSI decreased a little during the early days of the experiment (androgenic effect (?) of the steroid fed) however, in the later days it was comparable to the control birds.

The comb of chicken is considered a very sensitive target organ for the androgens. This organ was included in the present study in order to know whether the birds were getting enough steroid from the food and also to know its androgenic potential. As can be seen from the weight increase and the relative mass of the comb (Fig. 7c and d) that the birds fed dimethazine with the food had absorbed it from the gut. The experimental birds always had higher comb weights from the corresponding control birds at any time. The comb growth was linear during the course of the experiment in both groups however, the slope of the curve for the two groups was different. The relative mass of the comb was maximum in the control birds on 38th day of the experiment while this maxima was not achieved in the experimental birds till the end of the experiment. At the end of the experiment the comb weight was around 70 % higher ($P<0.05$) than the control birds. Another observation made during the study was that the steroid-fed groups had bigger wattles than the control birds. This again show that the steroid was present in the body and that androgen sensitive tissue did respond to it.

Table 1: Linear regression equations ($Y=a+bX$) of age versus organ weights of control and dimethazine (5mg/kg food) fed pullets during the first 97 days of life.

Parameter	Control $Y=a+bX$	r^2	Dimethazine $Y=a+bX$	r^2
Body weight	-26.39+8.04	0.975	-43.71+8.77	0.965
Shank length	2.03+0.06	0.990	1.972+0.06	0.993
Tibiotarsus weight	-0.299+0.12	0.959	-0.462+0.13	0.932
Tibiotarsus length	3.164+0.08	0.990	3.13+0.09	0.990
Weight gain	43.39+1.24	0.745	33.41+1.61	0.820
SGR (%)	6.15-0.05	0.794	6.27-0.05	0.880
Feed consumed/chick/2weeks	104.17+5.24	0.949	-13.21+8.99	0.959
FCE (%)	34.82-0.12	0.346	35.22-0.14	0.564
Muscle Pectoralis	-4.13+0.51	0.942	-4.34+0.53	0.930
Muscle Gastrocnemius	-1.06+0.15	0.930	-1.275+0.17	0.905
Muscle Peroneus longus	0.46+0.06	0.920	-0.64+0.07	0.881
Kidney	0.280+0.06	0.974	0.217+0.06	0.967
Liver	0.632+0.17	0.943	0.412+0.19	0.926
Heart	0.220+0.03	0.936	0.209+0.03	0.939
Adrenal	2.42+0.67	0.838	2.649+0.67	0.942
Thyroid	-1.51+0.47	0.809	-1.83+0.55	0.832
Ovary	-34.12+4.07	0.880	-34.91+4.11	0.886
Comb	-23.06+3.06	0.943	-57.12+6.86	0.980

In the end we can conclude that the birds did absorbed the steroid from the food and dimetazine was able to induce significant somatic growth in the pullets. This study generated data on the ontogenetic development of the pullets and provides data during the first 100 days of life on the weight of the internal organs. These weights can be used as reference values for the determination of either the organ weight or the total body weight or age if either of the two is available by using the regression equations provided in the paper (Table I).

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