Effect of dietary supplementation level of carbimazole on physiological responses of male Bovans chicks during Summer conditions

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Summary
This study was performed to validate the use of the antithyroid drug carbimazole in the control of thyroid function in chicks and to establish the dose (0.03, 0.057, 0.08, 0.081 and 0.1 g/bird/day) response relationship in male Bovans chicks for 21 days. The rectal temperature ($T_r$) decreased ($P<0.05$) with the increase in carbimazole dose. The mean food intake did not maintain a consistent pattern and the mean body weight (B.W) was not affected significantly by the increase in carbimazole dose. The total leukocyte count (TLC) decreased ($P<0.05$) with the increase in carbimazole dose. The ratio of lymphocytes decreased ($P<0.05$) while the ratio of heterophils increased ($P<0.05$) on days 14 and 21 following supplementation of carbimazole. The serum cholesterol concentration increased ($P<0.05$) with the increase in carbimazole dose. The absolute weights of the thyroid glands, the thymus and the spleen showed a biphasic pattern of response to the level of carbimazole. The absolute weight of the bursa of Fabricius decreased ($P<0.05$).

Introduction
Thyroid hormones are known to be potent mediators of many aspects of avian physiology, particularly growth and oxidative metabolism (Wentworth and Ringer, 1986). Various studies have indicated that thyroid hormones maintain important effects on thermoregulation in birds by modulating metabolic heat production (Freeman, 1971; Klandorf et al., 1981; Hwang et al., 1990). Evidence of a direct relationship between thyroid function and heat
tolerance of chicks was reported by Bowen and Huston (1980). In broiler chickens exposed to heat stress, Fox (1980) reported an increase in survival time when treated with propylthiouracil (PTU) and a decrease in survival time when thyroxine (T₄) was administered for 3 days.

Various techniques have been adopted to induce hypothyroidism in birds. Earlier studies utilized radiothyroidectomy using I¹³¹ to induce hypothyroidism in chickens (Mellen and Wentworth, 1962; Snedecor, 1964; Bowen et al., 1984). Other workers resolved to surgical removal of the thyroid gland in chickens (Fabris, 1973; Mashaly et al., 1983; Kai et al., 1987). Several studies adopting the chemical control investigated the effect of antithyroid drugs on the physiological responses in chickens (Kai et al., 1988, 1993; Fowles et al., 1997). Thiocarbamide drugs including carbimazole, methimazole and PTU are used for the treatment of hyperthyroidism in humans (Genter, 1998). Carbimazole is an antithyroid drug commonly used for the treatment of hyperthyroidism in humans, and in high doses it causes hypothyroidism. The established mode of action of carbimazole is reduction of formation of iodotyrosines by inhibition of the iodination of thyroglobulin and reduction of the synthesis of triiodothyronine (T₃) and T₄ via inhibition of coupling reaction between iodotyrosines (Kampmann and Hansen, 1981). Studies on cats showed that during absorption, carbimazole is completely converted to methimazole (Peterson and Aucoin, 1993). In this study, carbimazole was used to induce experimental hypothyroidism in euthyroid chicks for the first time.

Materials and Methods

36-day-old chicks were obtained from a commercial supplier and kept in an animal house (5x3x2.5 m) with concrete floor and sufficient ventilation. Appropriate cages were designed for individual accommodation of the birds with dry wood shavings as litter. The light was provided for 24hr (natural and/or artificial light). The chicks were randomly assigned to six
groups of 6 each (A, B, C, D, E and F). The control group (A) was fed the standard commercial diet while the treated groups (B, C, D, E and F) were fed the commercial diet supplemented with increasing levels (0.03, 0.057, 0.08, 0.081 and 0.10 g/bird/day) of carbimazole (Remedica Ltd.-Limassol-Cyprus).

Food intake was measured daily at 8:00 a.m while rectal temperature (T_r) and body weight (B.W.) were recorded at intervals of 3 days at 8:00 a.m. Blood samples were drawn from the wing vein weekly at 8:00 a.m for haematology and serum analysis. Total leukocyte count (TLC) was determined by the method of Natt and Herrick (1952). Blood smears stained with Giemsa-May-Grünwald were used for differential leukocyte count (DLC). Serum cholesterol concentration was determined using a commercial kit (Randox Laboratory Ltd., London). After 21 days, 4 chicks from each group were decapitated. Thyroid glands, thymus, spleen and bursa of Fabricius were dissected out carefully and weighed accurately.

The data for climatic conditions prevailing during the experimental period in summer were obtained from the local meteorological station at Shambat (mean ambient temperature 35.6±1.5°C, mean relative humidity 20.0±7.5%) Statistical analysis was performed using the procedure of the SAS programme (SAS, 1988). Analysis of variance (ANOVA) test was carried out to examine the effects of the treatment. Mean separation was performed using Duncan Multiple Range Test.

Results

The thermoregulatory pattern (Fig.1) illustrates a gradual increase in body core temperature (T_r) until day 6. Then for all groups, T_r was maintained at this high level until day 15. There was a significant (P<0.05) decrease in T_r value in treated groups on days 12 and 21 following supplementation of carbimazole. The mean food intake decreased sharply
with the lowest carbimazole dose, 0.03g (Fig. 2), but it did not maintain a consistent pattern. Following day 9, the mean food intake did not reflect an organized pattern. However, the food intake decreased (P<0.0001) on days 12 and 21 in all treated groups. During the experimental period, the mean body weight (B.W) increased almost linearly with time (Fig. 3). The mean B.W. of the control group was slightly higher than the respective values for the treated groups.

Fig 1. Effect of dietary supplementation of carbimazole (g/bird/day) on the mean rectal temperature (Tr) in male Bovans chicks during summer conditions.

Fig 2. Effect of dietary supplementation of carbimazole (g/bird/day) on the mean food intake (g/bird/day) in male Bovans chicks during summer conditions.
Fig. 3. Effect of dietary supplementation of carbimazole (g/bird/day) on the body weight (B.W) in male Bovans chicks during Summer conditions.

![Graph showing the effect of dietary supplementation of carbimazole on body weight.]

Fig. 4. Effect of dietary supplementation of carbimazole (g/bird/day) on total leukocyte count (TLC) in male Bovans chicks during Summer conditions.

![Graph showing the effect of dietary supplementation of carbimazole on total leukocyte count.]

TLC for the treated groups decreased (P<0.5-0.01) on days 14 and 21 (Fig 4). The ratio of lymphocytes decreased (P<0.05) on day 14 in the treated groups receiving 0.03, 0.057, 0.08 and 0.081 g of carbimazole but it was not
Table 1. Effect of dietary supplementation level of carbimazole on differential leukocyte count of male Bovans chicks during Summer conditions.

<table>
<thead>
<tr>
<th>Carbimazole dose (g)</th>
<th>7 (Experimental period)</th>
<th>14 (Experimental period)</th>
<th>20 (Experimental period)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>H</td>
<td>M</td>
</tr>
<tr>
<td>0.00</td>
<td>65.00± 10.67</td>
<td>27.50± 4.8</td>
<td>1.83± 0.6</td>
</tr>
<tr>
<td>0.03</td>
<td>70.00± 5.7</td>
<td>28.00± 4.8</td>
<td>1.17± 0.6</td>
</tr>
<tr>
<td>0.057</td>
<td>58.83± 9.8</td>
<td>35.83± 5.6</td>
<td>2.17± 1.3</td>
</tr>
<tr>
<td>0.08</td>
<td>64.67± 9.8</td>
<td>33.17± 5.6</td>
<td>0.6± 0.8</td>
</tr>
<tr>
<td>0.081</td>
<td>67.33± 9.8</td>
<td>32.17± 5.6</td>
<td>1.5± 1.3</td>
</tr>
<tr>
<td>0.10</td>
<td>68.33± 9.8</td>
<td>29.33± 5.6</td>
<td>0.8± 0.8</td>
</tr>
</tbody>
</table>

L: Lymphocytes, H: Heterophils, M: Monocytes, E: Eosinophils, B: Basophils. Values are means ±SD. Means within the same row bearing different letters are significantly different at (P<0.05).
affected significantly in the group receiving 0.10 g. of the drug (Table 1). On day 21, the ratio of lymphocyte showed a significant decrease (P<0.05) in the treated groups receiving 0.03, 0.057 and 0.081 g of carbimazole. However, in the treated groups receiving 0.08 and 0.1 g of the drug there was no significant effect. The ratio of heterophils showed an increase (P<0.05) in the treated groups receiving 0.057, 0.08 and 0.081 g of carbimazole. The ratio of heterophils was not affected as carbimazole dose was increased to 0.08 and 0.10 g on days 14 and 21.

Fig.5. Effect of dietary supplementation of carbimazole (g/bird/day) on serum cholesterol concentration in male Bovans chicks during Summer conditions.

The serum cholesterol concentration decreased sharply on day 14 in all treated groups except in treated group receiving 0.081 g of the drug there was a slight increase. On day 21, the cholesterol increased (P<0.01) in all treated groups except in the group receiving 0.081 g of carbimazole (Fig.5).

The absolute weight of the thyroid glands was increased (P<0.01) as carbimazole dose was increased to 0.03 and 0.057 g (Table .2). At doses of 0.08 and 0.081 g, the absolute weight of the thyroid glands was decreased. In carbimazole- dosed group at 0.10 g, there was a slight increase in the weight of thyroid glands. The absolute weight of the thymus was decreased sharply as carbimazole dose was increased to 0.03 g and the organ weight increased
as the dose was increased to 0.057g (Table.2). At doses of 0.08 and 0.081g, there was a progressive decrease, but this pattern of change was not significant. The absolute weight of the spleen decreased (P<0.01) as carbitazole dose was increased to 0.03g (Table.2). However, as the dose of the drug was increased to 0.057g, a sharp increase in the absolute weight of the spleen was noticed. There was a progressive decrease in the absolute weight of the spleen with the increase in the dose of carbitazole. Although the absolute weight of the bursa of Fabricius was decreased (P<0.05) as carbitazole dose was increased to 0.03 and 0.08g (Table.2), the absolute weight of the bursa of Fabricius was in general, not affected significantly by the higher doses of carbitazole.

Table 2. Effect of dietary supplementation level of carbitazole (g/bird/day) on the weight of thyroid and lymphoid organs (mg) in male Bovans chicks during Summer conditions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g)</th>
<th>Thyroid</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Bursa of Fabricius</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.00</td>
<td>9±0.05b</td>
<td>38±0.1a</td>
<td>24±0.6a</td>
<td>1466±0.2a</td>
</tr>
<tr>
<td>B</td>
<td>0.036</td>
<td>23±0.01ab</td>
<td>23±0.1a</td>
<td>20±0.6b</td>
<td>1239±0.2b</td>
</tr>
<tr>
<td>C</td>
<td>0.057</td>
<td>46±0.02a</td>
<td>46±0.1a</td>
<td>27±0.5a</td>
<td>1386±0.2a</td>
</tr>
<tr>
<td>D</td>
<td>0.08</td>
<td>41±0.02a</td>
<td>35±0.2a</td>
<td>25±0.5a</td>
<td>1189±0.2b</td>
</tr>
<tr>
<td>E</td>
<td>0.081</td>
<td>25±0.02ab</td>
<td>32±0.2a</td>
<td>22±0.5b</td>
<td>1209±0.3b</td>
</tr>
<tr>
<td>F</td>
<td>0.1</td>
<td>31±0.02ab</td>
<td>28±0.20a</td>
<td>17±0.40b</td>
<td>1287±0.3b</td>
</tr>
</tbody>
</table>

Means within the same column bearing different letters are significantly different at (P<0.01). Values are means±SD

Discussion

The induction of hypothyroidism in Bovans chicks was evidenced mainly by the increase in the weight of the thyroid gland. The results showed a specific pattern of change in T3 values for all groups of chicks with time. The increase in T3 with time during the experimental period could be related to the increase in metabolic activity associated with growth. Previous studies showed an elevation of T3 with the increase in metabolic rate in growing chicks (Freeman, 1970; Yahav et al., 1996). The results indicated that there was a
progressive increase in Tc until day 15, then Tc declined. This might suggest a response to changes in environmental temperature. When the ambient temperature increases, the thermal gradient between the body and the surroundings is reduced and consequently the rate of heat loss by sensible channels, radiation and convection is reduced. Various workers have reported previously an increase in body core temperature in chicks exposed to high ambient temperature (Weiss et al., 1963; Ward and Peterson, 1973; Altan et al., 2000).

The results of the present study clearly indicated that carbimazole had a biphasic effect on the thyroid glands weight. This response could be related to the increase in the secretion rate of thyroid stimulating hormone (TSH) from the anterior pituitary in response to decline in the circulating thyroid hormones (Hood et al., 1999). In rats, dietary supplementation of PTU caused a significant increase in the weight of the thyroid gland. The treatment showed that very low doses of PTU effectively stimulated TSH secretion and consequently caused enlargement of the thyroid gland (Mannisto et al., 1979). The progressive decrease in Tc with increase in carbimazole dose is related to thyroid hypofunction associated with a reduction in metabolic heat production. The mean food intake did not reflect an organized pattern. This could be attributed to changes in environmental temperature. The pattern of response to carbimazole dose could be related to the effect of thyroid hormones on the appetite. Hypothyroidism is usually associated with a decrease in energy utilization by the hypothalamus which in turn inhibits the satiety centre (Guyton, 2000). The decline in food intake obtained influenced the metabolic activity associated with growth and body weight changes. Previous studies showed that dietary supplementation of antithyroid drugs caused a significant decrease in body weight of chickens (Yam et al., 1981; Bachman and Mashaly, 1986; Kai et al., 1987).

The marked decrease in TLC associated with an increase in carbimazole dose could be related to the reduction in the weights of the lymphoid organs.
The decrease in TLC for higher carbimazole doses could also be related to the increase in the rate of secretion of corticosterones from the adrenal cortex. Kai et al. (1993) reported an increase in the level of corticosterones in chicks receiving high level of PTU. Corticosterones are known to decrease the number of circulating leukocytes in chickens (Gross and Siegel, 1983). The present results are in agreement with the findings reported by Kai et al. (1988) who showed that TLC decreased progressively as the dose of PTU was increased. The decrease in the ratio of lymphocytes in the treated groups might be related to the induced hypothyroidism and decline in the secretion rate of thyroid hormones. These hormones are known to play a major role in the growth and maturation of lymphoid organs (Hadded and Mashaly, 1990).

The increase in the ratio of heterophils in the treated groups could be attributed to the increase in the secretion rate of corticosterones in hypothyroid chicks. The present results are in agreement with the findings reported previously in chicks by other workers (Raheja and Snedecor., 1970; Kai et al., 1988, 1993). Conversely, Bux et al. (2000) reported that treatment with carbimazole caused neutropenia in humans.

The progressive increase in serum cholesterol concentration as the dose of carbimazole was increased was probably associated with a decrease in low density lipoprotein (LDL) receptors in response to the lack of thyroid hormones. LDL receptors are known to regulate cholesterol transport through hepatic cell membrane (Guyton, 2000). However, Fowles et al. (1997) reported that in mallards the changes in serum cholesterol level were not consistent with the increase in the dose of methimazole dose. The results indicated that the increase in carbimazole dose had a biphasic effect on the absolute weights of the thymus and the spleen. This could be related to the increase in the secretion rate of corticosterones at high doses (Kai et al., 1988, 1993). The decrease in the absolute weight of the bursa of Fabricius following administration of the antithyroid drug obtained could be related to
the lack of the thyroid hormones. Generally, the decrease in the absolute weight of the lymphoid organs obtained is in agreement with the findings reported previously by many workers (Yam et al., 1981; Mashaly et al., 1983; Martin et al., 1988; Kai et al., 1987).

We conclude that carbimazole induces hypothyroidism in growing Bovans chicks, and that the dose response relationship can be established for some basic physiological responses.

References


