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Effects of hyper- or hypothyroid status on growth, adiposity and levels of growth hormone, somatomedin C and thyroid metabolism in broiler chickens

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Summary. Relatively few methods involving dietary manipulation of thyroid status have been used to study the effect of that status on growth and body composition as well as on changes in hormone levels and metabolism. The present work examines the influence of either a hyperthyroid status (induced by feeding triiodothyronine : T_3 or thyroxine : T_4) or a hypothyroid status (induced by feeding methimazole : MMI) on growth, feed consumption and body composition as well as on changes in thyroid hormone levels and metabolism and in hormone levels of the somatotroph axis of broiler chickens.

MMI depressed growth but increased fatness. Long-term administration of thyroid hormones decreased both growth and fat deposition, T_3 being more effective than T_4. The reduced growth of MMI-treated birds was not only related to the hypothyroid state but also to decreased somatomedin C (Sm-C) production, although growth hormone (GH) levels remained high. Thyroid hormone-treated birds showed slightly depressed GH levels but unchanged Sm-C levels. MMI induced an increase in hepatic 5'-monodeiodination activity, while T_3 and T_4 reduced that activity. The T_4 administered was largely eliminated as inactive reverse T_3. Both of the statuses (hypothyroid and hyperthyroid) profoundly changed peripheral thyroid hormone metabolism and influenced GH/Sm-C relationships, but in the opposite way. Hormonal changes could be related to the observed changes in growth and adiposity of broiler chickens and illustrate the negative correlation between T_3 and body fat.

Introduction

Numerous studies have established the importance of thyroid hormones in the metabolism of mammals and birds. However, while various methods are available for the induction of hypothyroidism and hyperthyroidism, relatively few involving dietary manipulation have been used to study the effect of these two statuses on growth and body composition and on changes in hormonal levels and metabolism.

A significant negative correlation between triiodothyronine (T_3) levels and carcass fat has been found within lines in chickens (Stewart and Washburn, 1983) and between lines in pigs (Yen and Pond, 1985), while propylthiouracil (PTU)-induced hypothyroidism in chickens leads to a hepatic glycogen and triglyceride storage syndrome (Raheja *et al.*, 1980). Also, autoimmune thyroiditis is accompanied by obesity (Cole, Kiete and Witebsky, 1968). Buyse *et al.* (1986) however found no differences in mean T_3 and thyroxine (T_4) levels over 24 h between broiler lines selected for high and low concentrations of very low-density lipoproteins (VLDL) (Griffin, Whithehead and Broadbent, 1982). Feeding protamone (thyroactive iodinated casein with 1 % T_4 activity ; 100 mg/kg of feed) to broilers led to a reduction in the abdominal fat pad and feed efficiency and to an increase in carcass protein content, but there was no reduction in 7-week slaughter weight. The same trends were observed with higher doses of protamone, although 7-week body weight was then impaired (Wilson *et al.,* 1983). In broiler chickens fed with 1 ppm of T_3, May (1980) found lower weight gain and feed efficiency than in controls or in broilers fed with 1 ppm of T_4. These data together indicate that thyroid hormones not only affect growth, but also body composition, and that T_3 has a greater effect on these parameters than T_4. The present study examines the influence of an hyperthyroid status (induced by feeding T_3 or T_4) and of a hypothyroid status (induced by feeding methimazole : MMI) on growth, feed consumption and body composition as well as on changes in thyroid hormone levels of the somatotroph axis.

Material and methods

Broilers of the hybro-strain (Euribrid) were housed in flat deck-type cages at 24 ± 1 °C and under 16L : 8 D. Five groups of 20 male birds each in 2 replicates were given either 1 ppm of T_4, 5 ppm of T_4, 1 ppm of T_3 or 0.1 % of MMI (2 mercapto-5-methylimidazole ; Janssens Pharmaceutica), respectively, in the feed from day 1 onwards, or were used as controls. At the start of the experiment, the mean weight of all the groups was 53 ± 4 g. The feed was a commercial all-mash broiler meal (table 1).

TABLE 1

Broiler meal composition.

Metabolisable energy	(Kcal/kg)	3,200
Crude protein	(%)	19
Crude fat	(%)	7
Cellulose	(%)	6.5
Ash	(%)	8
Vitamin A	(I.U./kg)	10,000
Vitamin D3	(I.U./kg)	2,000
Vitamin E	(ppm)	7.5
Avoparcine	(ppm)	15.0

All the animals were weighed each week in the fed state, and food consumption was monitored per cage. The blood of the *ad libitum*-fed chicks was taken every week in heparinized tubes between 09.00 a.m. and 1 p.m. (first sample at the end of the first neonatal week, last sample at slaughter) from a random sample of 8 animals from each group. Plasma samples were stored at −20 °C prior to hormonal assay. At the age of 7 weeks (live body weight : 1.8 to 2 kg), all the birds were killed and their livers were weighed and immediately frozen at −20 °C. Abdominal fat, known to be a good index of total body fat in chickens (Saadoun and Leclercq, 1983) was carefully removed and weighed to 0.1-g accuracy.

Assay procedures. — Commercial kits were used to measure T_4 (T_4 RIA (PEG), Abbott, Diagnostics Division), T_3 (Dac-Cel T_3, Wellcome Reagents Lts.) and rT_3 (Mallinckrodt Diagnostica RIA-mat).

Growth hormone (GH) concentration was measured using an homologous radioimmunoassay (Harvey and Scanes, 1977). Somatomedin C (Sm-C) activity was measured using an heterologous radioimmunoassay (Huybrechts *et al.*, 1985).

Monodeiodination activity was studied in liver samples according to the method of Visser *et al.* (1979) and Decuypere, Scanes and Kühn (1983) with some modifications. These samples were homogenized in 2 ml of a phosphate buffer (0.15 M). After centrifugation, 50 µl of the supernatant were incubated for 1 h in a water bath at 37 °C with 10 µl of T_4 (4 µM) with of without dithiotreitol (DDT ; final concentration : 3 mM). To stop the reaction, 1 ml of ice-cold Brij 35 (1.25 %) was added to each tube, followed by T_3-RIA. Monodeiodinase activity was expressed as ng of T_3 produced per hour per mg of protein. The total, protein-bound and nonprotein sulfhydryl (SH) groups in the liver were estimated according to the method of Sedlak and Lindsay (1968).

The data were analysed by analysis of variance using Statistical Analysis System (SAS) General Linear Model (GLM) procedures (Barr *et al.,* 1979). Hormonal parameters were analysed by non-parametric statistics because of the heteroscedasticity of the data. Overall treatment effect was analysed by the Kruskal-Wallis one-way analysis of variance, while weekly differences between groups were analysed using the Mann-Whitney U-test (Siegel, 1956).

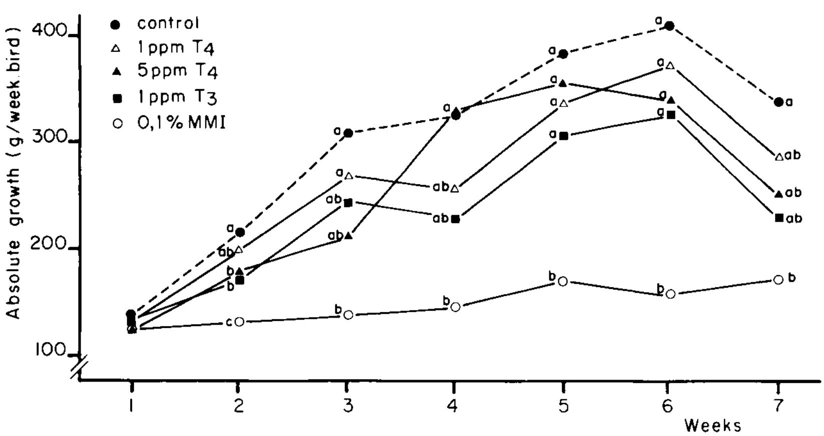

FIG. 1. — *Body weight gain* (g/week bird) ($\overline{X}$, n = 20) *as a function of age in controls* (---- ● ----), *1 ppm T_4-fed group* (— △ —), *5 ppm T_4-fed group* (— ▲ —), *1 ppm T_3-fed group* (— ■ —) *and 0.1 % MMI-fed group* (— ○ —). Different superscripts indicate significant differences between groups (P < 0.05) at the same week (ANOVA).

Results

Performance. — As expected, MMI depressed broiler growth to a large extent, but long-term administration of both thyroid hormones also slightly decreased growth, T_3 being more effective than T_4 (fig. 1). Liver weight was not markedly affected by thyroid hormone treatment. On the other hand, MMI greatly increased liver weight (4.9 in MMI-treated *vs* 2.1 % of body weight in controls), while a pale orange-yellow colour indicated increased fat content (fig. 2).

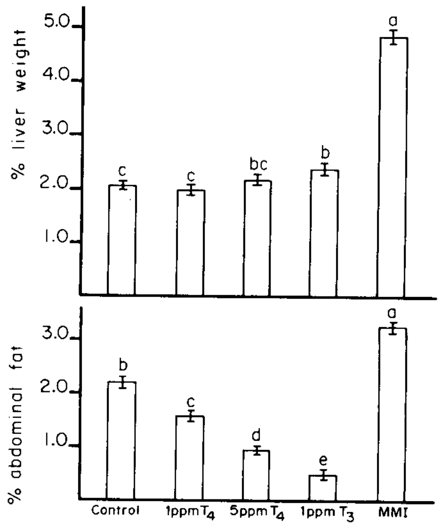

FIG. 2. — *Procentual liver- and abdominal fat weight of the experimental groups.* $\bar{X} \pm$ S.E.M. (n = 20). Different superscripts indicate significant differences between groups ($P < 0.05$) with ANOVA.

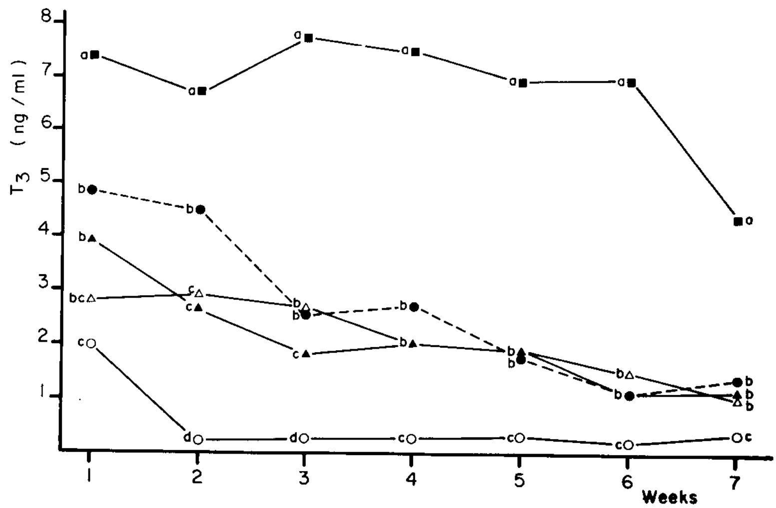

FIG. 3. — *T3 plasma concentrations* (median values) *as a function of age in controls* (----●----), *1 ppm T_4-fed group* (—△—), *5 ppm T_4-fed group* (—▲—), *1 ppm T_3-fed group* (—■—), *and 0.1 % MMI-fed group* (—○—). Different superscripts indicate significant differences between groups ($P < 0.05$) at the same week (Mann-Whitney U test).

Abdominal body fat decreased in a dose-dependent way after T_4 treatment, while T_3 was even more effective in reducing broiler fat content (fig. 2). In contrast, the hypothyroid MMI-treated birds showed a higher relative abdominal fat content than any of the other groups.

Hormonal parameters. — MMI treatment greatly decreased the levels of both circulating T_3 and T_4 over the entire period (figs. 3, 4). Sm-C production was also significantly reduced in this group (fig. 5), while GH fluctuated considerably but was not different than in the controls over the whole period (fig. 6).

As expected, circulating levels of T_4 were high and dose-dependent in T_4-fed chicks, while in T_3-fed birds T_4 levels were slightly reduced (fig. 3). On the other hand, circulating T_3 levels were not increased but were similar to control animals in T_4-fed birds, but highly increased in T_3-fed ones (fig. 4). Concentrations of plasma rT_3 were significantly increased in a dose-dependent way in the T_4-fed groups but were not different from the controls in the T_3-fed group. In MMI-fed

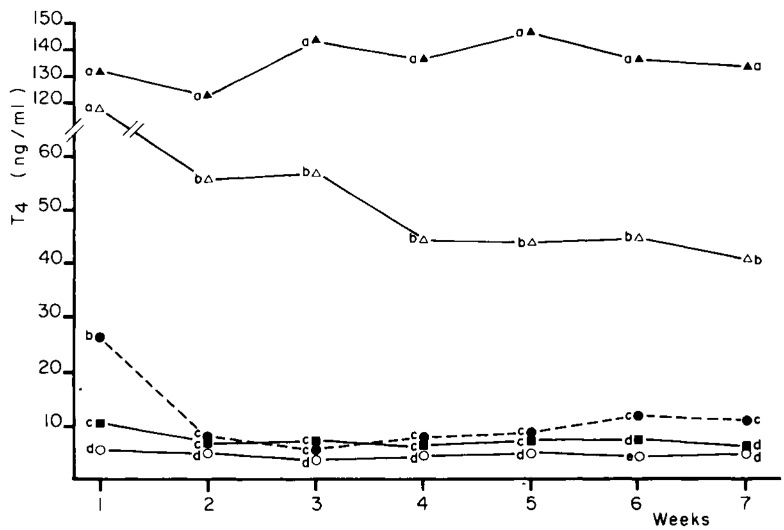

FIG. 4. — *T4 plasma concentrations* (median values) *as a function of age.* For legend, see Fig. 3.

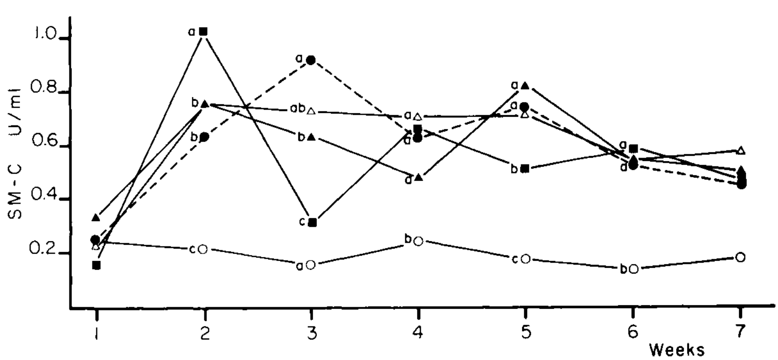

FIG. 5. — *Sm-C activity* (median values) *as a function of age.* For legend, see Fig. 3.

birds, however, plasma rT_3 levels were frequently reduced in comparison to controls (fig. 7).

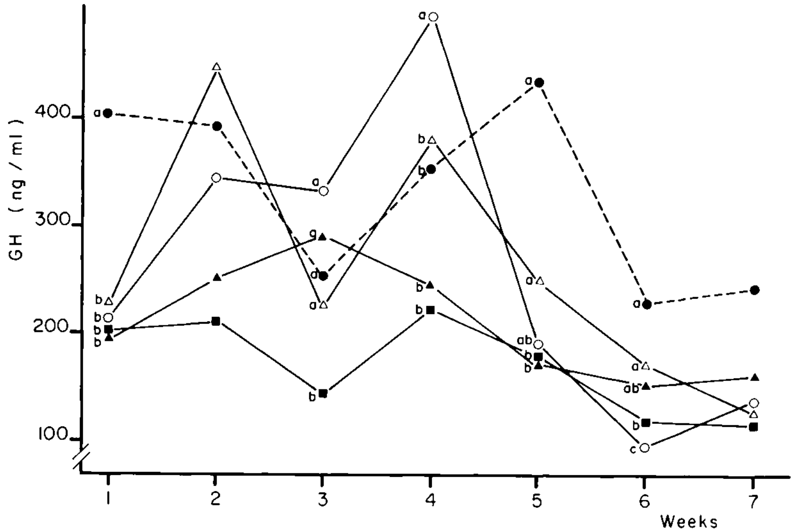

FIG. 6. — *GH plasma concentrations* (median values) *as a function of age.* For legend, see Fig. 3.

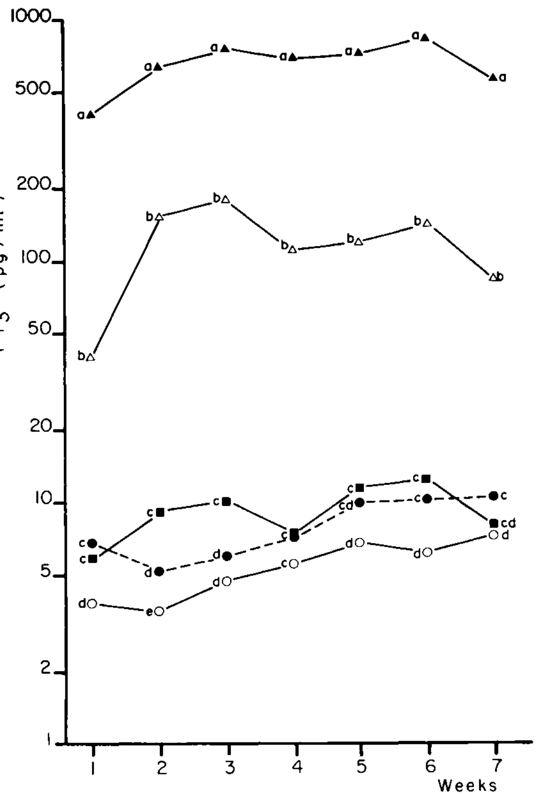

FIG. 7. — *rT3 plasma concentrations* (median values) *as a function of age.* For legend, see Fig. 3.

Plasma concentration of GH was slightly depressed after T_3 treatment and also after T_4 treatment (fig. 6). Sm-C activity, however, was not influenced by thyroid hormone feeding in contrast to MMI-fed animals (fig. 5), in which Sm-C activity decreased from the second week on throughout the experimental period.

Liver 5'-monodeiodinase activity, expressed as ng of T_3/hour x mg of liver protein, was decreased in a dose-dependent way after T_4 treatment, with again the 1 ppm dose of T_3 being as effective as 5 ppm of T_4 in reducing deiodinase activity *in vitro* in the presence of DTT. After MMI feeding, this activity was greatly increased in the presence of DDT (fig. 8).

The SH groups, necessary for reactivating the deiodinase enzyme *in vivo,* were similar in all groups, except in the MMI group where increased levels were found (fig. 9).

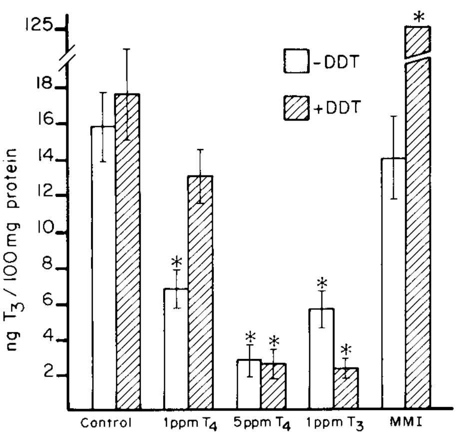

FIG. 8. — *5'-monodeiodinase activity in the different groups measured as the amount of T_3* (ng) *produced after 60 min in 50 μl of liver homogenate to which 10 μl of T_4* (4 μM) *was added in the presence or absence of dithiothreitol (DTT).* $\overline{X} \pm$ S.E.M. (n = 20). Differences ($P < 0.05$) from controls are indicated with * (ANOVA).

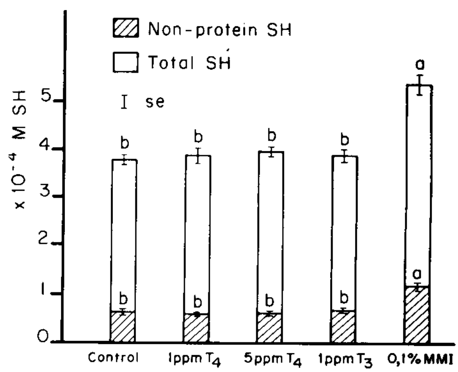

FIG. 9. — *Non-protein and total SH groups in liver homogenates of the different experimental groups.* $\overline{X} \pm$ S.E.M. (n = 20). Different superscripts indicate significant differences between groups ($P < 0.05$) with ANOVA.

Discussion

Our data on depressed growth rate due to dietary T_3 confirms previous results (May, 1980). However, while 0.1 or 1 ppm of T_4 in the studies of May had no effect on growth, the higher dose of T_4 used in our study indicates that dietary

T_4 also depressed growth in a dose-dependent way, although it was far less effective than an equivalent amount of T_3. The observation of decreased abdominal fat content in T_3-treated birds provides direct evidence of a thyroidal link with adiposity. This is in agreement with the negative correlation found between T_3 levels and carcass fat in chickens (Stewart and Washburn, 1983) and pigs (Yen and Pond, 1985). However, T_4 treatment also resulted in a dose-dependent decrease of the percentage of abdominal fat, with no concomitant increase in plasma T_3 levels, suggesting that T_4 (or other factors) is probably also involved in adiposity. The leaner body composition in thyroid hormone-treated birds may be related to higher metabolic energy loss or to decreased digestibility of food due to decreased retention time (Robertshaw, 1981). Hyperthyroidism induced by dietary T_4 in turkeys is indeed associated with an elevation in basal body temperature (Bilezikian, Loeb and Bammon, 1980).

The depressed growth in the hypothyroid birds is in agreement with previous findings (Scanes *et al.*, 1976 ; Chiasson *et al.,* 1979). The observation that MMI treatment reduced the plasma Sm-C values in these birds suggests that growth reduction in the MMI-treated groups was due to Sm-C deficiency and not only to hypothyroidism. The occurrence of a higher percentage of abdominal fat and the fatty appearance of the liver in these birds confirm the hepatic lipid storage syndrome in PTU-induced hypothyroid chikens (Raheja *et al.*, 1980).

Data on circulating T_3 and T_4 levels in T_3 and T_4-fed broilers confirm the earlier data of May (1980) in chickens and of Bilezikian, Loeb and Gammon (1980) in turkeys. The observed increases of rT_3 in T_4-fed birds, where there is no influence on T_3 levels, can be explained by a switch from 5′ to 5-deiodination activity. This is illustrated in our study by the dose-dependent reduction in liver 5′-monodeiodinase activity. With respect to this reduction in enzyme activity, T_3 administration seemed more effective than giving T_4. However, in the light of current knowledge of the deiodination enzyme system, we cannot explain the absence of a DTT effect, or even the inhibitory effect of DTT in the hyperthyroid state while on the other hand, reducing agents have a potentiating effect on enzyme activity in the hypothyroid state.

The availability of SH groups *in vivo,* on which 5′-monodeiodinase depends (Visser *et al.,* 1976), was not altered in any of the thryoid hormone-treated groups, indicating that the different amount of available enzyme was responsible for the decreased conversion of T_4 to T_3. Since both thyroid hormones depressed circulation GH levels (confirming the results of Harvey, 1983), while exogenous GH is a potent stimulator of liver 5′-monodeiodinase in chickens (Kühn *et al.,* 1985), the decrease found in peripheral T_4 conversion can be related to the decreased GH levels after thyroid hormone treatment. The lower GH levels in birds treated with thyroid hormones may be regulated by an increased negative feedback mechanism on the release of thyrotropin-releasing hormone (TRH), which is known to be a potent GH-secretagogue (Scanes *et al.,* 1984). Also, the rather high GH levels in the MMI-fed broilers can be related to the diminished negative feedback of circulating thyroid hormones on TRH release in these birds.

The increased liver 5′-monodeiodinase activity *in vitro* in MMI-treated chicks, together with an increased availability of SH groups, compared to controls, is in agreement with a stimulated conversion of exogenously administered T_4 in surgically thyroidectomized animals (Rudas and Pethes, 1984). This points to the importance of a peripheral regulation system that acts together with the hypothalamo-hypophyseal control of thyroid gland activity in controlling the level of biologically active T_3.

The reduced T_4 levels in T_3-fed chicks are probably due to the negative feedback action of the high circulating T_3 levels in these birds, as already observed by May (1980).

Although GH induces Sm-C production in the liver (Scanes *et al.,* 1984), there is no clear relationship between circulating levels of the two hormones. Low Sm-C values, together with high circulating GH levels in the MMI-treated groups, can be related to the low circulating levels of thyroid hormones since T_4/T_3 have been reported to stimulate Sm-C production (Chockinov and Daughaday, 1976). However, it may also be suggested that the effect of MMI on Sm-C is not directly related to thyroidal influences in MMI-treated birds. In view of the liver's presumed role as the major source of circulating Sm-C, it is possible that MMI treatment exerts a toxic effect on the liver, decreasing Sm-C secretion due to impairment of liver functioning. Indeed, in the present study, MMI treatment was observed to affect liver weight and appearance.

On the other hand, the low GH levels after thyroid hormone treatment did not result in lower Sm-C levels. These results indicate that thyroid hormones are necessary to, or at least influence, GH-induced Sm-C production in the liver, probably by affecting the GH receptors in liver tissue.

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Résumé. *Effets d'un état hyper- ou hypothyroïdien sur la croissance, la quantité de graisse et le niveau plasmatique de l'hormone de croissance, la somatomédine C et le métabolisme thyroïdien chez des poulets de chair.*

Relativement peu de méthodes entraînant une manipulation nutritionnelle de l'état thyrodien ont été utilisées dans l'étude de cet état en relation avec la croissance pondérale et proportionnelle ainsi qu'avec la dynamique des taux et du métabolisme hormonaux. Dans le présent travail nous examinons l'influence d'un état hyperthyroïdien (induit par une administration de triiodothyronine (T3) ou de Thyroxine (T4) dans les aliments) ou hypothyrodien (induit par une administration similaire de methimazol (MMI)) sur la croissance pondérale et proportionnelle, la consommation alimentaire, ainsi que sur les taux des hormones somatotropes et thyroïdiennes et sur le métabolisme de ces dernières.

La croissance pondérale allait en baisse, mais l'adiposité augmentait suite à l'administration de MMI, tandis que la croissance pondérale et adipeux diminuait lors d'une administration prolongée d'hormones thyrodiennes, la T3 ayant un effet plus prononcé que la T4. La réduction de la croissance des poulets traités au MMI était dès lors mis en rapport avec une hypothyroïdie provoquée, mais celle-ci marquée également par une production de somatomédine-C (Sm-C) en décroît, malgré le maintien du taux de l'hormone de croissance (GH) à un niveau élevé. Chez les animaux traités aux hormones thyrodiennes les taux de

GH allaient légèrement en baisse alors que les taux de Sm-C demeuraient inchangés. Suite au MMI l'activité hépatique de la 5'-monodéiodinase allait en hausse alors qu'elle diminuait par la T3 et la T4. Après administration la T4 se trouvait largement éliminée en tant que rT3 inactive. Les états hypothyroïdien tout comme hyperthyrodien avaient une répercussion profonde sur le métabolisme thyrodien périphérique en sens opposé toutefois, et également sur le rapport entre l'hormone de croissance et la somatomédine-C. Nous avons été en mesure de mettre un lien entre les changements hormonaux d'une part et les changements constatés au niveau de la croissance pondérale et de l'adiposité des poulets et d'illustrer la corrélation négative entre la T3 et la graisse corporelle.

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