The influence of thyroid functional modifications on collagen content of muscular system in broilers

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Thyroid hormones are essential for numerous postnatal developmental processes, including growth and neurogenesis. The collagen content of the adult animal organism is generally constant, but in some physiological states and especially in pathophysiological ones, marked modifications of it take place, both quantitatively and qualitatively. Endocrine glands, through the hormones they produce, influence both the quantity and quality of collagen, thyrotropin stimulates the hyaluronic acid formation in the ground substance, while thyroxine stimulates the connective tissue synthesis in the thyroidectomized animals. Experimental research was conducted on three batches of 40 days old broilers. The first batch consisted of 8 broilers with weight of 837.50±13.98 g. In order to induce the hyperthyroidism, they were fed with combined feed that contained 2 mg Tiroton/5 kg of feed, recipe 21/1. This treatment resulted in a dose of about 40 µg Tiroton per broiler each day. The second batch, consisting of 8 broilers of 677.50±20.68 g were brought into a condition of hypothyroidism by introducing of 5 g Methylthiouracil/5 kg of combined feed, that represented 100 mg Methylthiouracil per broiler daily. The third batch represented the control. After 42 days of feeding, the broilers were slaughtered and their muscular collagen was assayed by an indirect biochemical method based on hydroxyproline determination in accordance with Neuman and Logan indications. Broilers whose feed was supplemented with Tiroton showed a statistically insignificant decrease tendency of the collagen in the pectoral muscle (P>0.05), while broilers in the batch treated with Methylthiouracil showed a statistically insignificant increase tendency (P>0.05). In the thigh muscles, both batches showed an increase tendency, but statistically insignificant (P>0.05). The body weight in both batches was smaller (P<0.01) as compared to the control. The hystopathological examination of the thyroid in the broilers treated with Tiroton revealed the flattening of follicles that are full with colloid. In the broilers treated with Methylthiouracil, the thyroid follicles are unequal, some of them quite large, the walls become thickened, the colloid sometimes having a haemorrhagic aspect owing to their cystization, which characterises the condition of hypothyroidism.

Keywords: collagen content, muscular system, broiler chicken, thyroid
Introduction

Collagen is the most common protein in the animal world, providing the extracellular framework for all multicellular organism (Wick et al., 1989). On the basis of the biochemical composition of the chains that make up the triple helix of the collagen molecule, some 14 types of collagen can be discerned. Changes in hydroxyproline metabolism have been found in endocrine disorders (Bruce et al., 1977; Curcă et al., 1980; Fischer, 1973; Kühn et al., 1986; Merdjev, 1973). Thus, in myxedema there is a decreased urinary hydroxyproline excretion, and in hyperthyroidism there is increased hydroxyproline excretion (Kivirikko et al., 1964).

It was suggested that the effect of thyroid hormone on collagen metabolism is selective and not just the effect of thyroid hormone on metabolism in general. The thyroid hormones exert both coarse and fine control of metabolic rate. Another way to reduce maintenance cost and/or increase growth rate is to manipulate thyroid metabolism. Dietary thiouracil improved feed efficiency. Endocrine glands through the hormones they produce, influence both the quantity and the quality of collagen, thyreothropine is a stimulator mainly for the hyaluronic acid in the ground substance, while thyroxine stimulates the connective tissue in thyroidectomized animals.

Materials and methods

Experimental research was conducted on three batches of broilers aged 40 days. Batch I, consisting of eight broilers having a weight of 837.50±13.98 g, to be brought into a condition of hyperthyroidism, was administered 2 mg Tiroton/5 kg of feed, recipe 21/1, in their combined feed that is about 40 µg Tiroton per broiler each day. Batch II, consisting of eight broilers having a weight of 677.50±20.68 g, to be brought into a condition of hypothyroidism, was administered 5 g Methylthiouracil/5 kg of feed in their combined feed, which means 100 mg Methylthiouracil per broiler daily. Batch III was the control. After 42 days of feeding, the broilers were slaughtered with a view to dosing the collagen in their muscular system by means of the indirect biochemical method which aims at the determination of the hydroxyproline contents according to Neuman and Logan's, 1950; Goll et al., 1963, technical indications.

Determination of the collagen content in pectoral and thigh musculature in broilers, expressed in g of collagen/100 g of protein points to changes depending on to age, sex, anatomic region (Curcă et al., 1980; Smith et al., 1976 and 1977; Nakamura et al., 1975), antibiotics in food (Ladetto et al., 1971) and inorganic and organic bioelements (Curcă et al., 2001a and 2001b).

Results and discussions

Following the 42 day Tiroton administration, the average collagen levels in the pectoral muscles amounted to 222.57±33.94 mg/100 g of fresh tissue; these values were considerably lower that those in the control batch yet statistically insignificant (P > 0.005) (Table 1). However, we need to observe the wide value range that is 170.37 – 355.25 mg/100g of fresh muscular tissue, together with a rather high range quotient, that is 34.10%.

The average collagen contents in the thigh/shank muscles was recorded at 417.60 ± 60.45 mg/100g of fresh tissue, a value much higher than the quantity found in the pectoral muscles (Table 2). In comparison with the control batch the differences are statistically insignificant (P > 0.05); at thigh/shank level the collagen-recorded values ranged from 333.50-to 652.50-mg/100 g of fresh tissue. Clinical research conducted by Kivirikko, 1964 and 1965, on human subjects has shown a rise in hydroxyproline secretion following the administration of thyroidal hormone as well as in the clinical cases of hyperthyroidism. The hyperthyroidism is characterized by a low collagen synthesis accompanied by an intense degradation of both the soluble and insoluble collagen.
The type of thyroidal hormone administered is particularly important, thus having been proved that thyroxine incorporates amino acids more intensely than tri-iodothyonin does, whereas the acetylated derived triiodothyonine stimulates this processes to a greater extent than thyroxine.

Initially, the chickens weighed 837.50 ± 13.98 g and following the 42 day Tiroton administration their weight has increased to 1840.00 ± 93.25 g, less than in the control batch, with a statistically significant difference (P < 0.01) (Table 3).

May et al., 1980a and 1980b, present interesting observations in this respect that show the negative impact of T₃ (tri-iodothyonin) in a dose of 1 ppm on weight gain and efficient feed consumption. Under normal conditions the concentrations of thyroidal hormones is optimal for collagen synthesis stimulation, while in a condition of hyperthyroidism the catabolism of both soluble and insoluble collagen increases, whereas the synthesis decreases (Kivirikko, 1964). There are however observations which emphasize the fact that both synthesis and collagen degradation are intensified, the latter prevailing. Changes in collagen degradation speed are reflected in levels of urinary hydroxyproline that are high in hyperthyroidism (Kivirikko, 1964 and 1965) and low in hypothyroidism (Kivirikko, 1965). On the other hand the thyroidal hormones do not impact all tissues and organs with the same intensity. Even if there is a tight relationship between changes in collagen levels in tyropathies and specific tests carried out to diagnose thyroid malfunctions, there are cases when this connection between thyroidism intensity shown by sample I¹³¹ and collagen modifications are not recorded. This inconsistency suggests that changes in collagen levels depend rather on the status of the general metabolism than on hyperthyroidism intensity while it still differs from a case to another.
Table 3 The dynamics of body weights (g)

<table>
<thead>
<tr>
<th>Specification</th>
<th>$\bar{X} \pm S_{\bar{X}}$</th>
<th>DS (s)</th>
<th>Range</th>
<th>V%</th>
<th>t</th>
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</thead>
</table>
| Lot 1 treated with TIROTON
-Initial                                 | 837 ± 13,98                 | 39,55  | 770,00 – 900,00                | 4,72| ***     |
| -At 42 days                           | 1840,00 ± 93,25             | 263,76 | 1400,00 – 2320,00              | 14,33|         |
| Lot 2 treated with METHYLTHIOURACIL
-Initial                                 | 677,50 ± 20,68              | 58,49  | 590,00 – 760,00                | 8,63| ***     |
| -At 42 days                           | 1831,00 ± 90,19             | 238,64 | 1580,00 – 2140,00              | 13,03|         |
| Lot 3 CONTROL BATCH
-Initial                                 | 1031,00 ± 51,40             | 162,55 | 890,00 – 1100,00               | 16,05|         |
| -At 42 days                           | 2462,22 ± 136,26            | 408,77 | 1700,00 – 2950,00              | 16,60|         |

Differences: $t_{1,2} =$ nonsignificant difference ($P>0.05$); $t_{2,3} =$ distinctive significant difference ($P<0.01$); $t_{3,4} =$ distinctive significant difference ($P<0.01$); *** = highly significant difference ($P<0.001$).

The specific literature allows us to draw valid conclusions such as: under normal circumstances, thyroidal hormones stimulate collagen synthesis, while a hyperthyroidism condition leads to its accelerated degradation and low synthesis.

The hystopathologycal examination of the thyroid in chickens that received Tiroton combined feed has shown the flattening of the ephytelium in thyroidal follicles which are of different size and full of colloid. In most cases the ephytelium of thyroidal follicles was uniform and the cells were iso-diametrical with ovoid nucleuses. The nucleuses are relatively rich in chromatin with a compact appearance and the cytoplasm is granular. The follicles are generally different in terms of size the big one seem to suggest cystization and other areas show a hemorrhagic aspect in the perifollicles.

Chickens treated with Methylthiouracil showed an average of collagen content in the pectoral muscles of $306.86 \pm 38.12 \text{ mg/100 g of fresh tissue}$, a much higher value than the ones in the control batch and the one in chickens treated with Tiroton, but the difference was statistically insignificant ($P>0.05$), (see Table 1).

The average collagen levels in thigh/shank muscles was $417.78 \pm 29.61 \text{ mg/100 g fresh tissue}$, higher than that in the control batch and equal to that in chickens treated with Tiroton, but the differences were not statistically different ($P>0.05$), (see Table 2).

Following the 42 day Methylthiouracil administration the average weight was $1831.00 \pm 90.19 \text{ g}$, significantly lower than that of the control batch chickens ($P<0.01$), (see Table 3). Clinical research conducted by Kivirikko, 1964 and 1965, on human subjects has shown a decrease of hydroxyproline secretion in hypothyroidism conditions and research conducted with $\text{C}^{14}$ in experimental hypothyroidism have led to the conclusion that the rate of collagen synthesis as well as that of soluble and insoluble collagen degradation are low. Thyroidal hormones administered in doses, which correct a hypothyroidism condition, stimulate the rate of collagen synthesis but inhibit it in doses that produce hyperthyroidism.

Observations recorded by May et al., 1980a and 1980b, have shown that Thiouracil improved the fat deposit together with the commercial quality of the carcass but it also reduced the growth rate. Domestic bird thyroid does not vary significantly with age but increases its absolute weight. This thyroid growth rate is almost constant from hatching until approximately the 15$^{th}$ day of life, after which it accelerates up to 100-120 days followed by significant slow down (Sturkie, 1965).
There is a positive correlation between thyroid and body weight, so that in New Hampshire 4-week old chickens this was +0.59 and the linear regression quotient was 9.85 mg of thyroid/100 g body weight. Feeding regime influences the thyroid size and weight so that any iodine deficiency in chickens leads to an increase in thyroid volume and weight. The season influences thyroid weight in the way that during autumn and winter time it is bigger than during summer time which points to an intensified metabolism during cold seasons. However, an increase in thyroid volume may also reflect both a hyper function and a hypo function. TSH (thyroid stimulating hormone) stimulates the thyroid and produces both hypertrophy and hyperplasia together with an intensified thyroxine secretion. The thyreostatic substances (antithyroidal) such as: Thiouracil, Methylthiouracil, Thiourea, Thiocianate, Resorcine, Sulfaguanidina and Metimazol, lead to hypothyroidism conditions with an increase in the thyroid weight and volume but suppress thyroxine synthesis. Thiouracil administration to chickens may lead to a block of thyroxine synthesis between mono-iodine tyrosine and di-iodine tyrosine because the relation between the two hormones is bigger 48 hours after the Thiouracil administration than after 6 hours since its administration, which indicates mono-iodine tyrosine accumulation.

Low oxygen consumption and a drop in the base metabolism levels characterize a hypothyroidism condition by 20 – 30%. Chicken producers have been concerned with lowering the metabolism levels by use of thyreostatic substances in order to obtain weight gains and improve carcass quality by increasing the fat deposit. Observations made by Herbert and Brunson (quoted by Sturkie, 1965) following experiments conducted on broilers which were administered thyreostatic substances have shown an increase in fat deposits and a pleasant commercial aspect, yet, the protein quantity, humidity and ash in the carcass were low.

The hystopathological examination of the thyroid in chickens treated with Methylthiouracil has shown unequal thyroidal follicles out of which some were very large. The inter follicle walls appear to have thickened sometimes. In the convergent areas between neighboring follicles there is a discrete area of conjunctive tissue. At the outer area of the follicle the blood vessels are dilated and sometimes present hemorrhages. The thyroidal follicles are full of colloid and many of them have a clearly hemorrhagic aspect. Hematomas may be observed inside the follicles together with dilated follicles whose walls have degenerated in a cyst like manner, the epithelium is flattened and of a pavements type. The epithelium of the thyroidal follicles features cells with an isodiometrical tendency and a chromatin rich nucleus; at the same time one can observe a series of vacuolization in the cytoplasm at the perinuclear level.

The control batch chickens display thyroidal follicles of different size with the large ones prevailing. The epithelium consists of iso-diametrical cells, a nucleus relatively rich in chromatin, a compact appearance of the cytoplasm, the colloid fills the follicle cavity with occasional vacuolization and the discrete stroma features a moderate vasodilatation.

References

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