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PARTICULARITIES OF THE THYROID CONTROL OF LAYING CYCLE IN EGG-LAYING CHICKEN HYBRIDS DIFFERENTLY SELECTED FOR EGG PRODUCTION

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Abstract

The present work investigates the changes in thyroid control of the laying cycle in Lohmann and Leghorn egg-laying selected hybrids versus unselected Sussex hens. Blood thyroxine (T4) concentration showed an upward trend from 20 to 32 weeks of age (woa), then T4 concentration decreased until 100 wk. The blood T4 evolution curve in Sussex hens was consistently below hybrid chickens (P<0.05). Blood triiodothyronine (T3) also showed an upward trend from 20 to 32 woa, followed by a plateau until 36 woa, after which, the T3 showed a slow downward trend until 100 woa. The increase in T4 and T3 levels during the period of laying was related to the increase in photoperiod. Both hybrid and Sussex hens showed a decrease of T4 to T3 conversion after 32 woa. The conversion capacity decrease was higher in hybrids versus Sussex. Blood T3 evolution levels correlated negatively and weakly with egg-laying intensity (r=-0.81 in Sussex, r=+0.05 in Lohmann and r=+0.17 in Leghorn) but positively with oviduct weight/body weight ratio (r=0.81 in Sussex, r=0.66 in Leghorn and r=0.49 in Lohmann hens).

***Key words**: tetraiodothyronine, triiodothyronine, egg-laying cycle control, egg-laying intensity, egg-laying chicken hybrid.*

INTRODUCTION

In birds, as in mammals, there are two forms of iodinated thyroid hormones (ITH) circulating in the blood: the prohormone T4 and the active hormone T3. T3 is the biologically active form because it binds to thyroid hormone receptors with higher affinity than T4. In birds, peripheral tissues can convert T4 to T3 by the action of 5'-deiodinase (Harr, 2002; Hudelson and Hudelson, 2009; Kaneko, 1997, cited by Sönmez, 2021). However, the conversion of T4 to rT3 (reverse T3) is faster than its conversion to T3. Therefore, low levels of T4 may be present in the serum of birds when increasing amounts of rT3 are produced (Decuypere et al., 2005). Iodinated thyroid hormones get involved directly, through systemic actions, or indirectly, through metabolic actions, in the control of reproductive function in the hen. It is well known, for example, that in hypothyroidism, ovulation is inhibited, leading to infertility. Treatment with ITH restores normal ovarian activity. Sechman (2013) reproted that hyper-thyroid state evoked by administration of T3 for a few days diminishes LH, estrogen and progesterone levels, reduces the weight of the ovary, induces atresia of preovulatory follicles and eventually causes stoppage of egg laying. ITH are also involved in moulting of the birds (Dascălu et al., 2000). Cole (1965) described hereditary hypothyroidism in White Leghorn fowls with obesity, delayed maturity, poor laying rate, reduced egg size, decreased fertility and hatchability. Kowalik and Sechman (2017) published a study on the role of ITH in birds underlaying the genomic and non-genomic actions, and their effects on egg laying intensity. Sechman (2013) demonstrated that the ovary expresses mRNAs for membrane receptors for ITH in laying hens, revealing the direct intervention of the thyroid in the steroidogenesis processes of this organ. The process of genetic selection of inbred strains of egg-laying hybrids, however, led to changes in the response of the reproductive system to the control exerted by the pituitary-thyroid axis (Hanlon et al., 2021). Our study summarizes the extent to which the implications of the hypothalamic-pituitary-thyroid axis in the regulation of reproductive function differ in Lohmann and Leghorn hybrid birds compared to Sussex hens.

MATERIALS AND METHODS

The research was carried out on three groups of hens: two groups of egg-laying hybrids (Lohmann brown classic and White Leghorn hybrid) compared to a group of Sussex hens, as a control, raised in industrial system. The birds were monitored from the age of one day. Feeding and watering of the birds were done *ad libitum*. The determinations began at the age of 18 weeks of age (woa) on a number of 48 capita in each group. Also, at the age of 18 woa, photostimulation began with the progressive increase of the light period from 8 hours, by 1:30 hour per week, until a duration of 14 h of light was reached (at 22 woa). This photoperiod was maintained until 61 woa, when an additional photostimulation of another 1 h was added and maintained until the end of the experiment (100 woa). The number of eggs laid was recorded daily. Blood samples were taken at different set time intervals, up to 100 woa, to determine serum T4 and T3 concentrations in all the three groups. Body weight was monitored at the same time intervals. At the ages of 22, 32, 61, 65 and 100 woa, 5 animals from each group were slaughtered to determine the weight of the oviduct. Serum T4 and T3 concentrations were determined by ELISA using dedicated kits provided by DBC (Diagnostics Biochem Canada Inc) for "research only" purposes. A standard curve was drawn for T4 determination, relating the colour intensity to the T4 concentration (Figure 1).

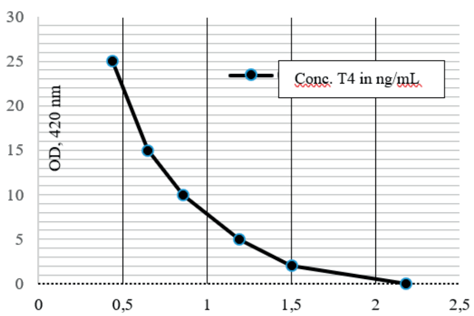

Figure 1. Standard curve for T4 concentration

Absorbance (OD) was read at 420 nm. T3 concentration (in ng/mL) was determined similarly using dedicated reagents.
The data were statistically processed. SAS v9.4 Software (SAS Institute Inc. 2015. SAS/STAT® 14.1 User's Guide. Cary, NC: SAS Institute Inc) was used for the statistical analysis and interpretation of the data. The data regarding the production and growth para-meters were analysed with ANOVA (PROC MIXED). Pearson correlation coefficient (r) was also calculated and expressed as strong, moderate, weak or none. Differences between groups were considered significant for the probability of the null hypothesis $P < 0.05$.

RESULTS AND DISCUSSIONS

Particularities of ITH secretion during the laying cycle
The overall analysis of the T4 evolution graph reveals a general ascending path for all three groups (Figure 2). The slope became more pronounced after the 32nd week of life of the hens, which suggests a forcing of the negative feedback mechanism regulating hypothalamic-pituitary T4 secretion after this age, decreasing in turn its degree of conversion to T3, the active hormone, as shown in Figure 4. The T4 concentration presents higher average values in hybrids compared to Sussex ($P = 0.029$). It is also noted that the two hybrid groups showed higher T3 levels than the Sussex hens, with the differences over the entire period from 18 to 100 woa being 2.31 ng/mL in the Lohmann group and 2.85 ng/mL in the Leghorn group ($P = 0.011$). The standard deviation values were also higher in the hybrid groups. The differences between the Sussex hens and the hybrid groups were significant ($P < 0.05$) at the ages at which they were calculated: 21, 32, 61 and, respectively, 100 woa for T3.
The T3 evolution curves of the hybrid groups show wider variations from one determination to another, generating a "sawtooth" appearance, compared to Sussex hens (Figure 3). We can speculate that hybrid hens are more sensitive to stress, reacting more intensely to the action of different factors: environmental, nutritional or other, compared to Sussex hens. After reaching the peak of laying, the overall trend of the T3 evolution curves was a downward one in all groups ($P < 0.01$), which may be associated with a decrease in oxygen consumption and the decrease in thyroid activity or a decrease in the intensity of metabolic processes as they age.

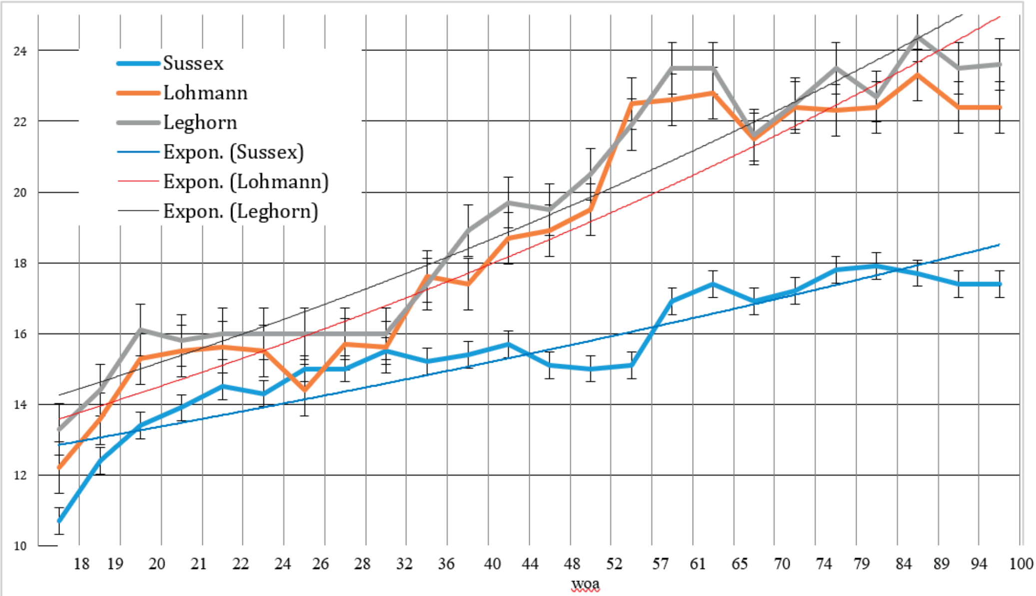

Figure 2. Evolution of the tetraiodothyronine concentration (as ng/mL) in blood serum of the three breed/hybrids of egg-laying hens from 18 to 100 weeks of age. From 61st week of age, the photoperiod was supplemented by 1 hour of light

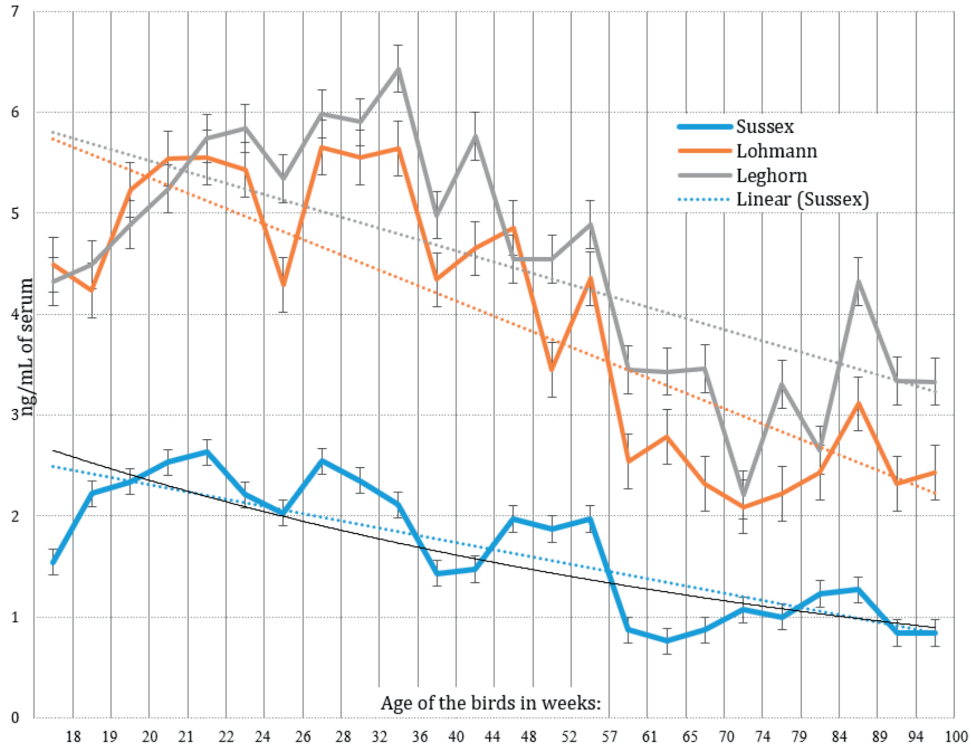

Figure 3. Evolution of triiodothyronine concentration (as ng/mL) in blood serum of the three breed/hybrids of egg-laying hens from 18 to 100 weeks of age. From 61st week of age, the photoperiod was supplemented by 1 hour of light

The decrease was more pronounced in Sussex compared to hybrid groups. The rate decrease conversion of T4 to T3 is revealed by the increase in the T4/T4 ratio values (Figure 4).

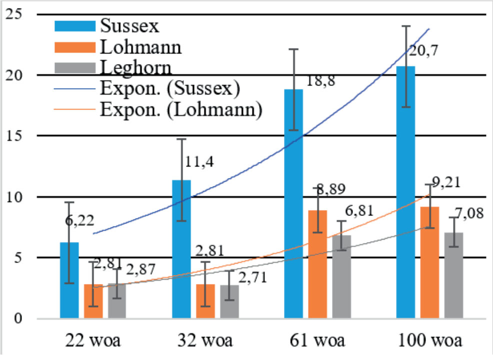

Figure 4. T4/T3 ratio in Sussex hens and laying hybrids, at four key times of the laying cycle: 21 woa (entry into laying), 32 woa (peak of laying), 61 woa (change in photoperiod) and 100 woa (end of technology exploitation period); woa = weeks of age

As a mechanism, the decrease trend in T3 levels as seen in Figure 3 may be the result of a conversion rate of T4 to T3 (Stojević et al., 2000).

Particularities of ITH secretion under the effect of photoperiod

In our research, the three groups of birds benefited from a directed light program. The effect of photoperiod on the blood concentration of thyroid hormones can be analyzed during the interval from 18 to 22 woa, when the birds of the three groups were applied a photostimulatory light program (14 hours lighting), and from the age of 61 woa, when a photostimulation hour was added (15 hours of lighting). According to data from the literature (Bédécarrats et al., 2009; Bunaciu et al., 2009; Zhao et al., 2023) the upward evolution of the T3 concentration from 18 to 22 woa in all three groups of birds has a double explanation: on one hand it can be attributed to the increase in photoperiod. On the other hand, it is considered that only the reaching of the maturity weight would be sufficient to determine these hormonal evolutions, the consequence of which being the onset of egg laying. The increase in the duration of the light period at the age of 61 woa did not generate significant changes in T3 or T4. However, the specialized literature has proven in recent years that, in birds, an adequate level of ITH is of paramount importance for the normal functioning of the female reproductive system.

ITH/egg-intensity ratio and ITH/oviduct weight ratio

Pearson correlation between the evolution of T3 and T4 concentration as independent variables and the laying frequency as dependent variable at the ages of 22, 32, 61, 65 and 100 woa allowed the observation that, in Sussex hens, the laying intensity correlates more strongly with the blood level of T4 and T3 than in hybrid hens (Table 1). In the Lohmann hybrid, the values of the correlation coefficient r were 0. This fact illustrates a higher sensitivity of Sussex hens to the action of ITH compared to Lohmann or Leghorn hens. In other words, in Lohmann and Leghorn hens, the laying frequency does not correlate with the secretion of ITH. On the other hand, according to Stojević et al. (2000) the variations in the intensity of production (of meat) largely follow the variations in the blood T3 concentration of young broiler hens. In contrast, the slight increase in T4 concentration during the experimental period is most likely related to the higher rate of iodinated thyroid hormone production.

It is worth noting that blood plasma in birds does not contain a specific protein for binding and transporting ITH as in mammals (Farer et al., 1961), which is why T3 and T4 bind to plasma albumin whose affinity and transport capacity are lower for T3 than for T4 (Sturkie, 1986).

Table 1. Pearson correlation coefficient (r) of serum T4 and T3 and the laying frequency of different-age hen groups during egg-laying cycle

	Age (in weeks):	22	32	61	65	100	r values
Sussex	Laying fr*	1.4	5.18	4.62	4.76	3.64	-
	T4	13.9	15.5	16.4	17.4	17	0.68
	T3	2.53	1.35	0.87	0.76	0.84	-0.81
Lohmann	Egg fr.	3.85	6.3	5.6	5.6	4.2	-
	T4	15.5	15.6	22.6	22.8	22	0.00
	T3	5.54	5.55	2.54	2.78	2.43	0.00
Leghorn	Egg fr	3.71	6.15	5.46	5.46	3.85	-
	T4	15.8	16	23.5	23.5	23	-0.04
	T3	5.24	5.9	3.45	3.43	3.33	0.17

*Laying fr = laying frequency, expressed as number of egg/week/bird.

To eliminate possible errors determined by the influence of breed size, the analysis of the correlation between the evolution of ITH blood level and oviduct weight was performed using the oviduct weight/body weight ratio values. The results of this monitoring are presented in Table 2.

Table 2. Pearson correlation coefficient (r) of serum T4 and T3 (as ng/mL) and the oviduct weight (ovw)/body weight (bw) ratio in different-age hen groups during egg-laying cycle

Age in weeks:		22	32	61	65	100	r values
Sussex	T4	13.4	15.5	16.4	17.4	17.4	-0.84
	T3	2.53	1.35	0.87	0.76	0.84	0.81
	Ovw/bw	2.78	2.54	2.53	2.56	2.33	-
Lohmann	T4	15.3	15.6	22.6	22.8	22.4	-0.60
	T3	5.54	5.55	2.54	2.78	2.43	0.65
	Ovw/bw	3.41	3.13	3.14	3.12	2.73	-
Leghorn	T4	15.8	16	23.5	23.5	23.6	-0.54
	T3	5.54	5.9	3.45	3.43	3.33	0.49
	Ovw/bw	4.34	3.83	3.96	3.92	3.43	-

From the analysis of the data presented in Table 2, it is found that the Sussex breed presented the highest correlation values, both for T4 and T3. In all of hens used in the experiment, T4 correlated negatively with the age of the birds, while T3 correlated positively with the age of the birds, and this asynchronicity is difficult to explain. Analysis of the period from 61 woa (the time of application of the one-hour photoperiod supplement) to 65 woa (five weeks of photostimulation) illustrates slight decreases in the oviduct weight/body weight ratio ($P > 0.05$) in hybrids, with a slight increase being present in Sussex, which could be interpreted as a higher sensitivity of the Sussex breed to supplemented photoperiod. Data in the literature regarding the influence of ITH on oviduct weight in laying hens are scarce. Mimura (1937) reports a weight value of 45.5 g for the oviduct in adult Leghorn hens, with a correlation coefficient (r) value with body weight calculated at r = +0.59, while Mahajan & Joshi (2020) report a weight of 57 g for the same structure in 25-year-old Leghorn. According to Dawson (2001), treatment of birds with thyroxine can mimic the effects of long photoperiods, which confirms our results where an increase in photoperiod from 8 hours to 14 hours in 18-wk-old chicken was accompanied by an increase in ITH concentration.

CONCLUSIONS

The research of particularities of thyroid control of the laying cycle in hens selected for egg-laying (Lohmann and Leghorn) allowed to identify some particularities of ITH secretion compared to an unselected breed of hens (Sussex). Over an evolution period from 18 to 100 woa, the curve of blood T4 evolution in Sussex chickens was constantly below that of hybrid chickens. The increase in iodinated thyroid hormones, both T4 and T3, during the period of entry into laying was related to the increase in photoperiod. Supplementation of photoperiod in the middle of the laying cycle did not significantly modify the evolution of the concentration of the two ITHs in either hybrids or Sussex. The evolution values of T4/T3 ratio were constantly higher in Sussex versus hybrid chickens. The blood concentration of T4 and T3 does not correlate with either the frequency of laying or the weight of the oviduct in hybrids versus Sussex chickens.

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