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CHANGES OF THE PITUITARY-OVARIAN AXIS IN THE CONTROL OF THE EGG-LAYING CYCLE IN CHICKEN HYBRIDS DEFERENTIALLY SELECTED FOR EGG PRODUCTION

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Abstract

The selection process led to changes in the pituitary-ovarian axis control in egg-laying chicken hybrids. This paper investigates the particularities of the control exercised by the pituitary-ovarian axis on reproductive function in Lohmann and Leghorn egg-laying selected hybrids versus the Sussex breed. The blood level of follicle-stimulating hormone (FSH) of the hybrids was higher than that of Sussex hens in the first part of the laying cycle, falling below that of Sussex hens in the second part of the cycle. The concentration of LH showed a peak in all categories of hens, the values in hybrid hens being consistently higher than those of Sussex hens (P<0.05). The analysed hybrids showed particularities of the ovarian endo-secretor response, characterized by a higher oestrogen secretory peak at the beginning of laying and a higher secretory level in hybrids compared to Sussex hens (P<0.05), throughout the laying cycle. Both FSH and LH concentrations were positively correlated with laying frequency, the correlative values being higher for LH. Correlations of laying frequency and ovary weight/body weight ratio were also identified.

***Key words**: genetic selection, chicken egg-laying hybrid, pituitary control, egg-laying intensity.*

INTRODUCTION

Genetic selection, combined with advances in microclimate and nutritional conditions, has played a key role in maximizing the reproductive capacity of laying hens. With the development and implementation of clearly expressed phenotypic traits, breeding programs for laying hens have led to significant improvements in egg production, particularly by accelerating sexual maturity, increasing peak egg production, and reducing the ovulation interval (Wolc et al., 2014). The decrease in age at first egg laying (AFE) and the increase in total number of eggs have been of particular interest to specialists because they are traits with moderate to high heritability (h2) (Hanlon et al., 2021). Once the biological limit of laying onset and the interval between ovulations have been reached, the efforts of specialists in the field of selection have been focused on extending the duration of the laying cycle to 100 weeks of age (woa) (van Sambeek, 2010). Unfortunately, scientists placed limited emphasis on studying the physiological processes that underlie the achievement of these characteristics.

Selection for the traits presented above led to hens entering lay before photostimulation, maintaining a high level of egg production until the end of the laying cycle (Baxter and Bédécarrats, 2019). Since these birds have been pushed to their biological limits by genetic selection work, the physiological implications of this intensive, even abusive, selection must be considered by physiologists in their research topics. Hanlon et al. (2021) hypothesized that decades of genetic selection of purebred egg-laying hens have altered the activation and function of the HPG axis in supporting increased production. Therefore, our present study aimed to characterize and compare the reproductive parameters of three differentially selected laying hen strains (Lohmann brown, White Leghorn and Sussex), with a particular focus on the period around sexual maturation, and on egg persistence up to 100 woa. This will provide useful data to specialists on the (genetic) differences that have occurred between the three analysed hen lines/breeds regarding their productive and reproductive traits.

MATERIALS AND METHODS

Animals and experimental design

To achieve the objective of the present experiment, one-day-old chicks from each established line/breed Lohmann, Leghorn, Sussex were used. At 1-day age, the chicks were randomly distributed into 12 incubation cages: five cages for Lohmann chicks (n = 120 chicks; 24 chicks /cage), five cages for Longhorn chicks (n = 100 chicks; 25 chicks/ cage), and three cages for Sussex chicks (n = 78 chicks; 26 chicks/cage). At 18 weeks of age (woa), the chicks of each group were transferred to industrial adult hen houses at a density of 5.7 chicks/m^2, ensuring microclimate and care conditions according to the technology requirements. Birds were watered and fed *ad libitum*. All diets were obtained from Agromar and were formulated to meet or exceed NRC (National Research Council, 1994) requirement. Egg number of each group was registered daily and laying frequency was calculated as number of eggs/week/cap., from 18 to 100 woa. Blood was sampled at 5-week intervals for hormone assay. At 22, 32, 61, 65 and 100 woa five birds form each group were slaughtered to determine the ovary weight. The experiments were carried out in compliance with European Directive 2010/63/EU on the protection of animals used for scientific purposes.

Hormone assay

FSH, LH and oestrogen were assayed using dedicated ELISA FSH, LH and oestrogen kits. For each hormone, serial dilutions of the standard were made according to the kit supplier's instructions and standard absorption curves were drawn. Optical density (OD) was read at 450 nm (figures 1, 2 and 3).

Statistic processing of the data

The obtained data were statistically processed by determining the mean, standard error of the mean and standard deviation. The difference between groups was considered significant when the probability of the null hypothesis $P < 0.05$. The correlation of blood levels of FSH, LH and oestrogen with laying frequency and ovary weight were calculated using the Pearson correlation coefficient.

RESULTS AND DISCUSSION

At 18 woa, FSH values were quite close (in ng/mL): 5 ng/mL in Sussex and Leghorn and 6.4 ng/mL in Lohmann (Figure 4). Two weeks later, the differences between the groups were already statistically significant ($P = 0.010$), being higher in hybrid hens. From 21 to 28 woa, FSH values increased in all groups, the differences becoming statistically insignificant ($P > 0.05$). A peak in FSH levels was not observed in any of the groups. FSH levels continued to increase slowly until 61 weeks. After 61 woa, blood FSH levels in hybrid hens showed only a slight and slow upward trend. The blood FSH levels of Sussex hens were consistently above those of Lohmann and Leghorn hens, which is attributed to a more pronounced hypothalamus feedback stimulation exerted by the decrease in oestradiol secretion.

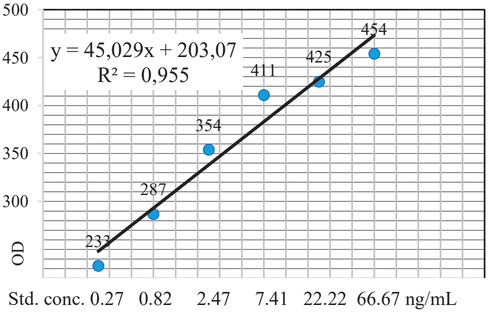

Figure 1. Plloting of the FSH determintion standard curve (as ng/mL)

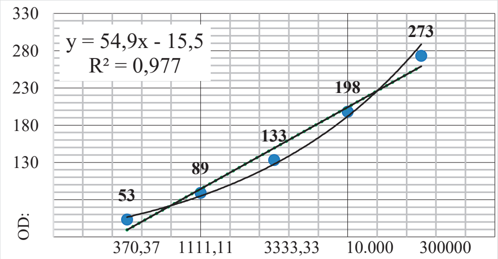

Figure 2. Standard curve for the calculation of the LH concentration (as pg/mL)

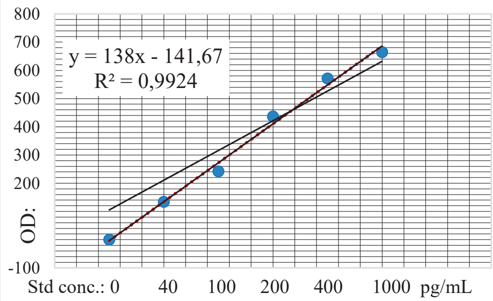

Figure 3. Standard curve for the calculation of the estradiol concentration

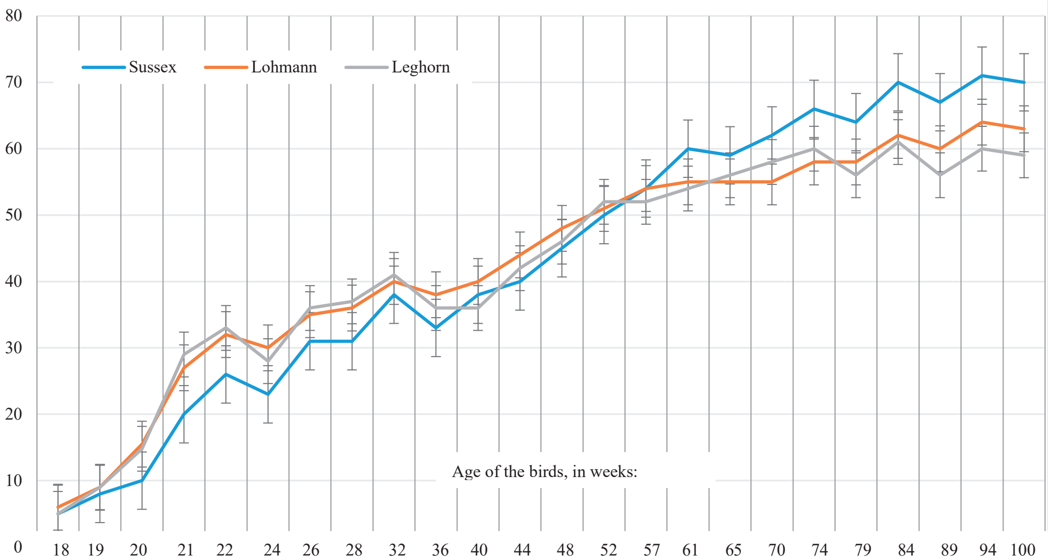

Figure 4. Graphic representation of the evolution of FSH values in the blood serum of hens from 18 to 100 weeks of age

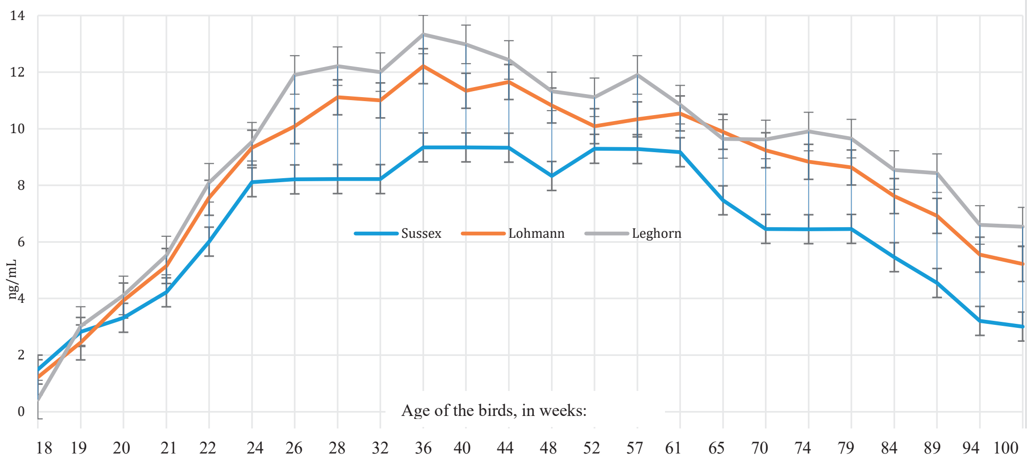

Figure 5. Graphic representation of the evolution of LH values in the blood serum of hens from 18 to 100 weeks of age

LH values at 18 woa were (in ng/mL, Figure 5): 1.49+0.21 in the Sussex, 1.22+0.03 in Lohmann and 0.43+0.21 in White Leghorn ($P > 0.05$). In the following weeks, LH values increased in all groups ($P < 0.05$). At the peak laying, the largest differences in LH levels were recorded between Sussex hens and hybrid hens, with values 2.79 ng/mL in Lohmann and 3.67 ng/mL in Leghorn. Subsequently, the three groups showed a slow downward trend, as physiological ageing process, the evolutionary position of the three curves being maintained throughout this period ($P = 0.016$ at 100 woa).

Oestradiol showed a secretory peak at the start of laying period, with levels generally lower in Sussex throughout the laying cycle (Figure 6). Young hybrid hens showed higher oestradiol levels at 18 woa versus Sussex (P < 0.05). A secretory peak of oestradiol in hybrid hens was reached after 2 weeks of photostimulation. The differences between Sussex and hybrid hens became more pronounced at 20 woa ($P = 0.009$).

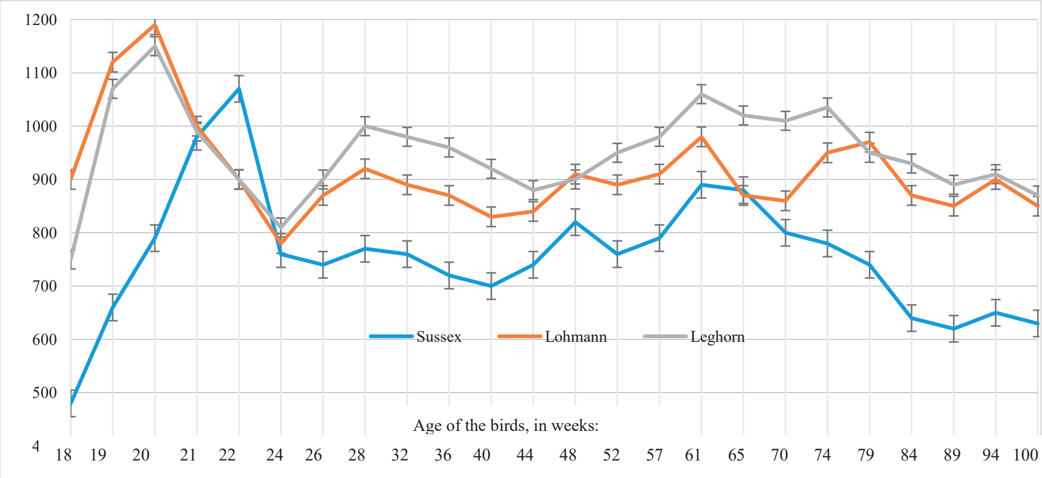

Figure 6. Graphic representation of the estradiol concentration in the blood serum of hens from 18 to 100 weeks of age

Sussex hens reached a peak of oestradiol later than hybrid hens, being 80 pg/mL lower than Leghorn and 120 pg/mL lower than Lohmann. At peak laying, the differences between hybrid and Sussex were amplified ($P = 0.003$). After a relative plateau up to 65 wk, oestradiol showed a downward trend, the differences being maintained up to 100 wk ($P = 0.002$), the decrease being more pronounced in Sussex.

The egg-laying frequency evolution curves primarily reveal the similarity of the two hybrid groups, selected for intensive laying (Figure 7). Both groups reached the same egg-laying peak, at the same age. However, the subsequent evolution reveals the Leghorn egg-laying curve below that of the Lohmann hens, the differences progressively increasing up to 100 woa. The Sussex hens laid their first egg about two weeks later, reached a less high and more delayed egg-laying frequency peak, and their evolutionary curve was located well below that of the two hybrid groups.

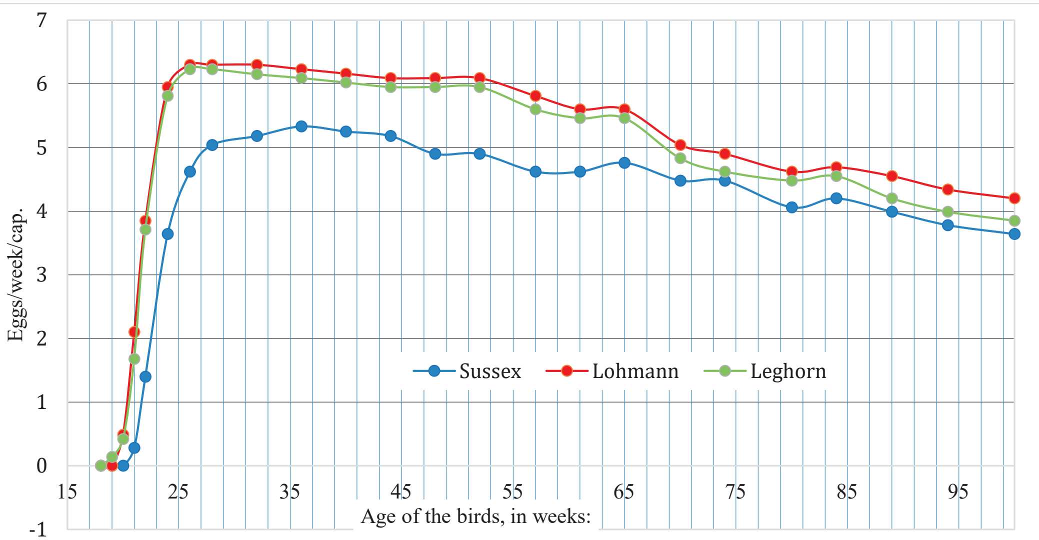

Figure 7. Evolution of the egg-laying frequency in hen groups from 18 to 100 weeks of age

The ovary weight/body weight ratio reveals that hens with a smaller body size (Leghorn and Lohmann, in particular) have a larger ovary than hens with a larger body weight (Sussex) (Figure 8). The analysis of the evolutionary dynamics of this value reveals a downward trend in the three groups, on the one hand due to the increase in weight during the laying cycle and on the other hand due to the decrease in ovary weight. The slope of weight loss went down around the age of 61 woa, especially in Lohmann. The decrease in the values of this ratio in the range of 65-100 woa is more pronounced in Sussex than in hybrid hens, which can be interpreted as a particularity of hybrids, generated by the selection process to which they were subjected.

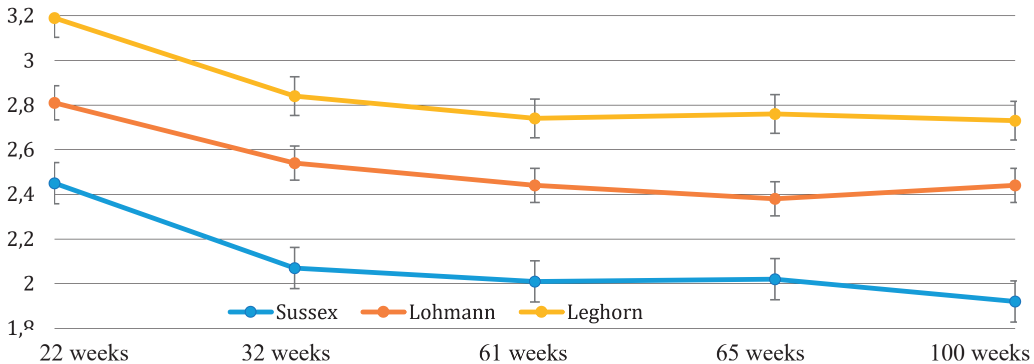

Figure 8. Evolution according to age of the ovary weight/body weight ratio in hybrids and Sussex

Correlation between the level evolution of the analysed hormones, FSH, LH and oestrogen as independent variables and the laying frequency, as dependent variable expressed as number of eggs/head/weeks revealed that both FSH and LH correlate with laying frequency (high positive values), the correlative values being higher for LH.
However, no differences were noted between hybrid and Sussex hens. In contrast, the serum oestrogen concentration does not correlate with laying frequency in any breed or hybrid analysed (low negative values).

Table 1. Pearson correlation coefficient (r values) of laying frequency with FSH, LH and oestrogen

Hormone	Sussex	Lohmann	Leghorn
FSH	0.62	0.61	0.58
LH	0.78	0.93	0.95
Oestrogen	-0.04	-0.68	-0.13

Note:
Values have been calculated based on the data in figures 4, 5 and 6 as independent variables and data in figure 7 as dependent variable.

The secretion of gonadotropic hormones in the hen is influenced by age, species, photoperiod and, probably, other factors (Bunaciu et al., 2009; Bunaciu & Dojană, 1982; Hanlon et al., 2021). New factors were added to this group: the endocrine ones, based on feed-back mechanisms that include ovarian oestrogens, progesterone and inhibin.
In the case of the ultra-specialized hybrids for egg production investigated in this study, the effect of genetic selection is recognized, materialized in higher blood serum values (in the case of LH), secretory oscillations with wider variations in the case of FSH, suggesting an improved pituitary sensitivity to stimulator input, or wider ovarian oestrogen secretory responses in the case of oestrogen.
The effects of the two pituitary gonadotropin hormones on the ovary were materialized in a larger ovarian volume and a slower weight evolution during the egg-laying cycle. Among the works that addressed the subject of changes induced by the selection process on the response of the hypothalamic-pituitary-ovarian axis, or chicken hybrid particularities Yang et al. 1997, Robinson et al., 1988, Robinson et al., 2003 and Hanlon et al. (2021) could be reminded.
The decline in LH and oestrogen secretion observed in our study was also described by Maddineni et al. (2008) in broiler chicken lines.
In their study on changes in the hypothalamic-pituitary-gonadal axis in the control of the egg

cycle in differently selected chicken lines, namely Lohmann, Shaver and Smoky Joe, the authors concluded that the key elements that changed throughout the selection process were the level of secretion of LH and oestrogens, on one hand. On the other hand, they found an increase in the sensitivity of target tissues to the action of these hormones, respectively, the increase in the density of specific receptors of ovarian follicular granulosa stem cells for FSH and of specific receptors of oviductal epithelial cells for ovary oestrogen.

CONCLUSIONS

The research on the changes of the pituitary-ovarian axis in the control of the egg-laying cycle in chicken hybrids selected for egg production allowed the identification of secretory particularities of pituitary and ovary glands compared to hens not subjected to the egg-selection process.

Higher evolutionary serum values of LH, larger secretory oscillations and wider variations of FSH were found during egg-laying cycle. These suggest an improved pituitary sensitivity to stimulator factors. Wider ovarian oestrogen secretory responses were also found in hybrid chickens.

Plasma levels of FSH and LH correlate with egg-laying frequency. One consequence of these changes of the pituitary control in hybrids is a higher ovary weight/body weight ratio, and egg-laying intensity curve superior to that of un-selected hens, and the possibility of extending their technological exploitation to 100 woa.

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