

Relationships between broodiness expression laying persistency and concentrations of hormones during the first productive period in turkey hens (*Meleagris gallopavo*)*

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Summary — The changes in egg production, in broodiness index and in plasma concentrations of LH, prolactin, oestradiol and progesterone were monitored throughout the first period of laying in turkey hens. The hens were subsequently classified according to their ability to express broodiness (33%) and their laying persistency; 25% were out-of-lay by the end of the experiment. A high percentage (67%) of the hens that went out-of-lay had previously been identified as broody. Altogether, a significant ($p < 0.05$) physiological stage effect was found when comparing prolactin, oestradiol and progesterone data obtained from short and long laying persistency hens and this stage effect was also significant for oestradiol and progesterone data obtained from broody and non-broody hens. Otherwise, plasma LH concentrations decreased slightly but significantly throughout the laying year in all hens but no significant differences between physiological states were observed, although the decrease was more pronounced in the hens that went out-of-lay. Plasma progesterone concentrations remained stable throughout in laying hens but decreased significantly in broody and/or out-of-lay hens. Plasma prolactin concentrations were maximal between the 5th and 12th week of egg production and the levels observed in laying hens that did not become broody or had a long laying persistency were twice those measured in broody and/or out-of-lay hens. In the meantime, plasma oestradiol concentrations were lower and stable in laying hens, whereas they were higher during the first half of the productive period in broody and short laying persistency hens. These results suggest that, under our experimental conditions, the hormonal profiles of prolactin and oestradiol for a given hen during the first 10 weeks of the laying cycle may provide predictive information for future changes in its physiological status. The inverse relationship that was observed here between high early plasma concentrations of oestradiol and laying persistency is original. In addition, the relationship between the ability to express broodiness and high and low early plasma concentrations of oestradiol and prolactin, respectively, in hens submitted to preventive broody treatment has not been reported previously. Furthermore, it seems clear that high initial concentrations in prolactin, far from exerting any deleterious effects on egg production are closely associated with a longer persistency of egg laying.

turkey hens / LH / prolactin / ovarian steroids / laying persistency / broodiness expression

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Résumé — Variations hormonales associées à l'expression du comportement d'incubation et la persistance de ponte durant un cycle de production chez la dinde (*Meleagris gallopavo*). Les évolutions de la production d'œufs, de l'expression du comportement d'incubation et des concentrations plasmatiques de LH, prolactine, œstradiol et progestérone sont observées au cours d'une première période de production chez la dinde. Respectivement 33 et 25% des dindes ont pu être classifiées a posteriori selon qu'elles aient exprimé le comportement de couvaision ou interrompu la ponte. Un pourcentage important (67%) des femelles, qui ont interrompu la ponte, ont antérieurement exprimé la couvaision. Globalement, un effet significatif ($p < 0,05$) du facteur «état physiologique» est mis en évidence lorsque les taux de prolactine, de progestérone et d'œstradiol mesurés chez les dindes ayant ou non une bonne persistance de ponte sont comparés, alors qu'il est significatif pour les taux de progestérone et d'œstradiol mesurés chez les dindes couveuses ou non-couveuses. Par ailleurs, les concentrations plasmatiques de LH diminuent graduellement pendant la période de production et cette décroissance est plus accentuée chez les dindes qui ont interrompu la ponte toutefois, aucune différence significative n'est mise en évidence. Les concentrations plasmatiques de progestérone restent stables chez les dindes pondeuses mais diminuent significativement chez les dindes couveuses et/ou en arrêt de ponte. Les concentrations plasmatiques de prolactine sont maximales depuis la 5^e jusqu'à la 12^e semaine de production et les taux mesurés durant cette période sont plus élevés chez les dindes pondeuses qui n'ont pas exprimé la couvaision et qui ont eu une bonne persistance de ponte. Parallèlement, les concentrations plasmatiques d'œstradiol sont plus faibles mais stables chez les dindes pondeuses, alors qu'elles sont plus élevées durant la première moitié de la période expérimentale chez les dindes qui arrêtent prématurément leur production. Ces résultats suggèrent que, dans nos conditions expérimentales, l'observation des profils plasmatiques de prolactine et d'œstradiol au cours des 10 premières semaines de production pourrait être un prédicteur de variations potentielles ultérieures de l'état physiologique, chez la dinde. La corrélation mise en évidence entre l'évolution des taux d'œstradiol et la persistance de ponte, de même que l'évolution inverse des taux circulants d'œstradiol et de prolactine n'ont jamais été décrites auparavant. Par ailleurs, il apparaît que, dans nos conditions expérimentales, des taux élevés de prolactine, loin d'avoir des effets inhibiteurs sur la production d'œufs, sont associés à une meilleure persistance de l'activité de reproduction.

dinde / LH / prolactine / stéroïdes ovariens / persistance de ponte / incubation

INTRODUCTION

High plasma prolactin and low plasma LH concentrations are associated with the expression of broodiness in galliforms (Cogger *et al*, 1979; Sharp *et al*, 1979; Burke and Dennison, 1980; El Halawani *et al* 1980; Proudman and Opel, 1981; Harvey and Bedrak, 1984; Zadworny *et al* 1985) whereas an increase in prolactin level only occurs at the end of the incubation period and remains high during the rearing of the young in columbiforms (Goldsmith *et al*, 1981). Results from an early study suggested that prolactin induced broodiness in birds (Riddle *et al*, 1935). However, more recent studies using high doses of heterologous hormones (oPRL) brought controversial results (Opel and Proudman, 1980; El Halawani *et al*,

1986; Sharp *et al*, 1988; Youngren *et al*, 1991). Otherwise, a pharmacologically induced decrease in prolactin levels affects the expression of incubation behaviour (El Halawani *et al*, 1983; Guémené and Etches, 1989; Sharp *et al*, 1989) whereas the readiness to incubate after nest-deprivation does not appear to depend on high plasma prolactin concentrations (El Halawani *et al*, 1980; Goldsmith *et al*, 1984; Richard-Yris *et al*, 1993). On the other hand, ovine prolactin has been reported to induce ovarian regression (Opel, 1971; Opel and Proudman, 1980) and to inhibit LH-stimulated steroidogenesis (Camper and Burke, 1977; Zadworny *et al*, 1989). Depending on the period of treatment, it may also delay the photoinduced rise in LH (El Halawani *et al*, 1991) and decrease the rise in LH induced by nest-deprivation in incubating hens (Sharp *et al*,

1988). In addition, the injection of anti-prolactin serum increased plasma LH concentrations of incubating bantam hens (Lea *et al*, 1981) while high levels of prolactin blocked the post-castration increase in circulating LH in incubating hens (Zadworny and Etches, 1987). All these data strongly suggest therefore that prolactin exerts direct and/or indirect antigonadotrophic actions, while a causal relationship between an increase in plasma prolactin concentrations and the expression of broody behaviour has not yet been fully established.

The stimulatory effect of long photoperiods on LH secretion in birds including turkeys is well established (Follet and Davis, 1975; Godden and Scanes, 1977; Scanes *et al*, 1977). In the meantime, a large increase in plasma prolactin levels is associated with the onset of egg-production and the concurrent high plasma LH concentrations in hens of a high egg-producing strain (Etches and Cheng, 1982), but not in hens of a low egg-producing strain (Burke and Dennison, 1980). The physiological significance of this increase in prolactin is not known. It does not seem to be related to the expression of broodiness since it is observed in both broody and non-broody hens (Etches and Cheng, 1982) and no differences in basal plasma prolactin concentrations were observed between broody and non-broody hens (Bacon *et al*, 1983). The photoinduced increase in LH induces a rise in oestrogens (Bajpayee and Brown, 1972; Wineland and Wentworth, 1975) and in progesterone (Mashaly and Wentworth, 1974; Godden and Scanes, 1977). In addition, changes in the circulatory level of these steroids have been reported to be associated with the changes in physiological states: laying, incubating and out-of-lay (Guémené and Williams, 1992).

The objects of the present study were, therefore, to further increase knowledge about changes in the concentrations of plasma LH, prolactin, progesterone and

oestradiol throughout the first laying period of turkey hens submitted to a preventive broody treatment and to further study the relationships with the occurrence of broodiness expression and with the persistency of egg laying under specific experimental conditions.

MATERIALS AND METHODS

Animals

Thirty-six medium white turkey hens (Betina, 56250 Saint Nolf, Elven, France) aged 29 weeks, were randomly allocated to 3 pens with wood shaving on the floor ($n = 12$ per pen). They were raised under a 6 h photoperiod and thereafter exposed to a stimulatory photoperiod of 14 h until week 16 (lights on from 3.00 to 17.00 h) and of 15 h thereafter (lights on from 2.00 h). The minimum light intensity was 50 lux. They were fed a commercial diet provided *ad libitum* and had free access to water. Each pen was equipped with 4 nests to which access was controlled automatically. The hens were managed according to commercial practice and were not therefore allowed to incubate eggs. Furthermore, broodiness was partially prevented by frequent egg collections and the use, after the peak of lay (4th week of egg production), of regular nest ejections with a maximum of 14 ejections per day as described previously (Guémené, 1990a).

Experimental approach

Egg production and broodiness records were obtained daily for a 27-week production period from the onset of lay of the flock. The physiological stage (laying; broody; out-of-lay) was determined as follows. Broody hens were identified by twice daily inspections of the nest (9.00 and 16.00 h) as described previously (Guémené and Etches, 1990). Hens that were present on the nest at both tests and had no egg present in the oviduct at the second test were classified as broody. Identification of out-of-lay hens was carried out by oviducal palpation and measurement of the pelvic bone gap on weeks 2, 8, 12, 17 and 26.

Blood samples (5 ml) were taken weekly from a wing vein into an heparinized syringe. Plasma was separated from blood by centrifugation at 2 000 *g* for 10 min at 4°C and kept at -20°C until required for radioimmunoassay for LH, prolactin, progesterone and oestradiol. The first sample was taken 12 d after photostimulation just prior to egg production, which started to occur at 31 weeks of age, and weekly thereafter. Sampling was carried out between 3 and 1 h before the lights went off in order to minimise the possibility of sampling hens during the preovulatory peaks.

Radioimmunoassays

LH

Plasma were assayed in triplicate 100 or 50 µl samples according to Sharp *et al* (1987) using fraction PRC-AE1-s-1 as labelled hormone and standard, and the anti LH 3-3 as antiserum. The validation of this assay for measuring turkey LH has been reported previously (Guémené and Williams, 1992). The mean intraassay variation was 12.6% and the interassay variation for the 2 assays needed for this study was 10.8%.

Prolactin

Plasmas were assayed in triplicate 100 µl samples following the method of Etches and Cheng (1982), but using tPRL provided by Proudman and Opel (1981) as labelled hormone and standard and the anti-tPRL-2-B3 provided by Etches and Cheng (1982) as antiserum. The mean intraassay variation was 7.6% and the interassay variation for the 2 assays needed for this study was 9.4%.

Progesterone

Progesterone was assayed using the method described by Duplaix *et al* (1981). The mean intraassay variation and the interassay variation for the 5 assays needed for this study were 3.5% and 15.2%, respectively.

Œstradiol

Plasma concentrations of oestradiol were assayed in duplicate 0.6 ml plasma samples according to

Etches *et al* (1984). The mean intraassay variation and the interassay variation for the 5 assays needed for this study were 3.7% and 17.8%, respectively.

Statistical analysis

The endocrinological data were analysed using a 2-way ANOVA (time and physiological stage effects (broody vs non-broody and out-of-lay vs laying at week 26) and Fisher PLSD (protected least significant difference) *post hoc* test if appropriate ($P \leq 0.05$, ANOVA). The standard error of the mean was used to express data variability.

RESULTS

Husbandry and behavioural observations

The onset of egg production occurred 2 weeks after exposure to the stimulatory photoperiod and during the week following the first blood sampling. The peak of production occurred 2 weeks after the first egg was laid (fig 1). Egg production declined gradually thereafter throughout the experiment, but a larger decrease was observed between the 12th and the 15th week of production.

Nearly 33% of the hens (10 out of 36) were identified as broody at least once during the reproductive cycle and from 3 to 12% of the hens were identified as broody each week from the 4th week until the end of the production period (fig 1). All hens studied in this experiment were laying on weeks 2, 8 and 12 whereas 14% (5 out of 36) and 25% (9 out of 36) were out-of-lay in weeks 17 and 26, respectively. None of the hens that were out-of-lay on week 17 resumed egg production afterwards. A high percentage (67%) of the hens that became out-of-lay had previously been identified as broody.

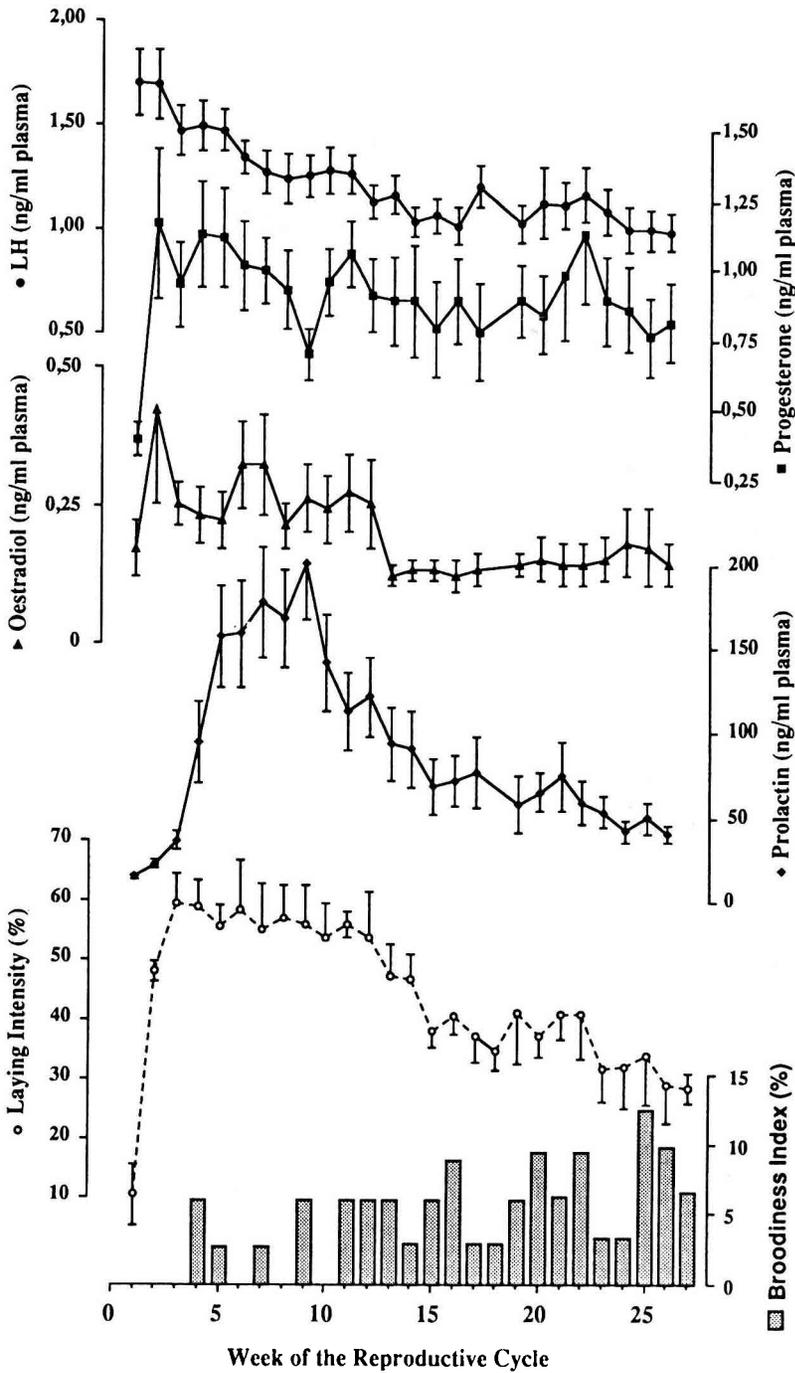


Fig 1. Plasma concentrations (ng/ml) of LH (—●—), progesterone (—■—), oestradiol (—▲—) and prolactin (—◆—), egg production (---○---) and broodiness index (▒) in turkey hens during the first laying period (means ± SEM).

Endocrinological data

The plasma concentrations of 4 hormones in the turkey hens throughout the first laying year are shown in figures 1 and 2 and results of the statistical analysis are reported in tables I and II. Plasma LH concentrations were maximal at the onset of egg production and declined progressively and significantly ($P < 0.01$) thereafter. No significant effect of the physiological stage ($P > 0.05$; broody *versus* non-broody or out-of-lay *versus* laying) was found, whereas a significant interaction ($P < 0.05$) between the time and physiological stage in the comparison of the out-of-lay *versus* laying hens was found ($p < 0.05$). This is due to the presence of higher levels of LH in the plasma during the first few weeks of egg production and lower

levels later (from week 20) for the out-of lay hens.

The plasma concentrations of prolactin changed significantly ($p < 0.01$) throughout the reproductive cycle. Initially, they were low but they then increased to reach a maximum 7–12 weeks after photostimulation and declined thereafter. However, there were clear differences from the 5th to the 12th week of the reproductive cycle between hens which became broody and those which did not exhibit any incubation behaviour, and between hens showing a long laying persistency and those which went out-of-lay before the end of the experiment. The plasma prolactin concentrations measured were lower in broody than in non-broody hens during this initial period and significantly lower ($p < 0.01$) throughout the repro-

Table I. Summary of p values obtained by ANOVA for time and physiological stage effects on 4 hormones of turkey hens with short or long laying persistency.

Variable	Factor		
	Time	Physiological stage	Interaction
LH	0.001	0.60	0.03
CEstradiol	0.10	0.003	0.41
Progesterone	0.33	0.001	0.87
Prolactin	0.003	0.001	0.59

Table II. Summary of p values obtained by ANOVA for time and physiological stage effects on 4 hormones of broody or non-broody hens.

Variable	Factor		
	Time	Physiological stage	Interaction
LH	0.001	0.95	0.97
CEstradiol	0.12	0.003	0.85
Progesterone	0.43	0.001	0.94
Prolactin	0.001	0.24	0.85

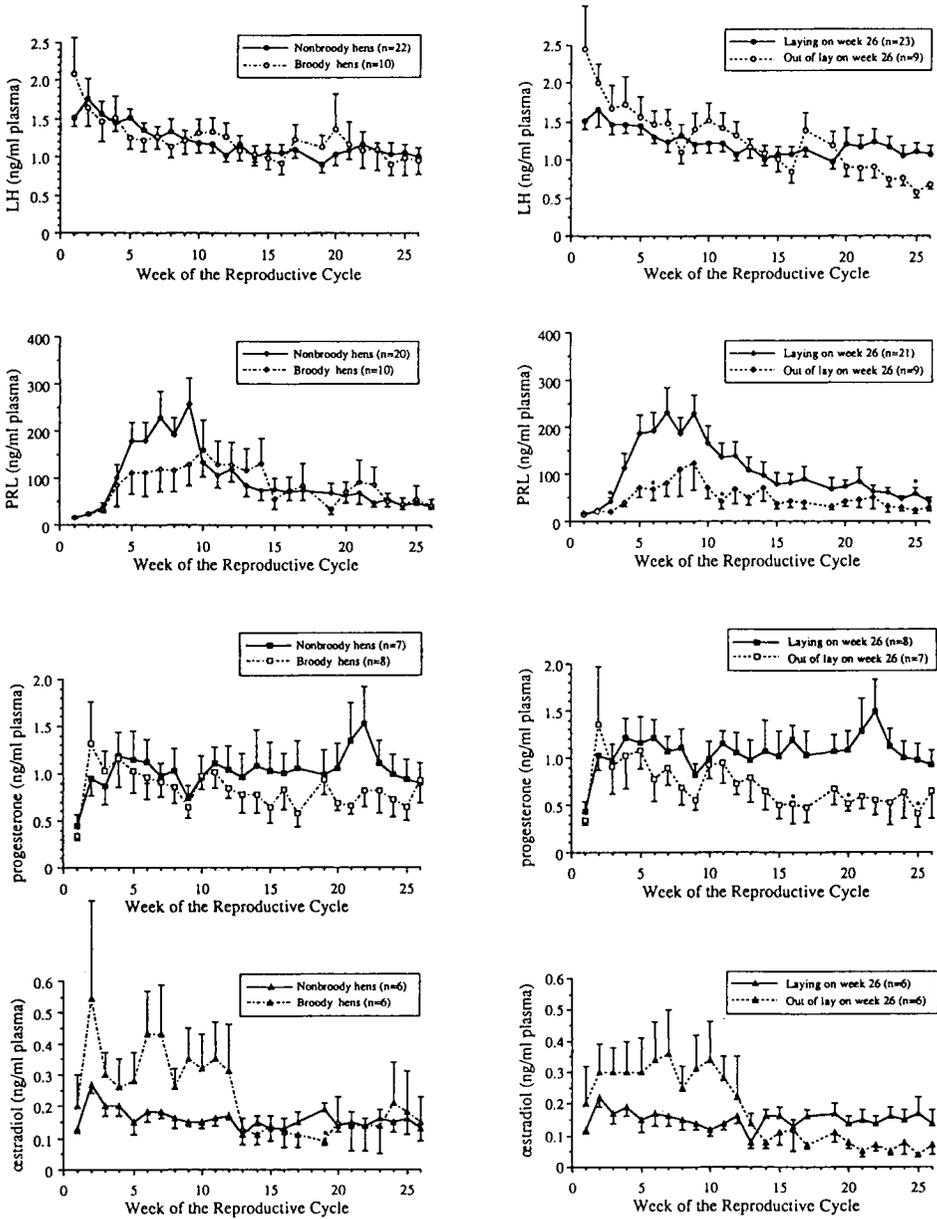


Fig 2. Plasma concentrations (ng/ml) of LH, prolactin, progesterone and oestradiol in turkey hens which have been classified broody at least once (open symbols and dashed line) or not (closed symbols and solid line) and of turkey hens with short (open symbols and dashed line) or long laying persistency (closed symbols and solid line) during the first laying period (means \pm SEM). * $P < 0.05$ for comparison at a specific time (protected least significant difference procedure).

ductive cycle for the hens which went out-of-lay compared with hens which displayed good persistency of lay.

Plasma oestradiol concentrations reached a maximum at the 2nd week of lay and remained higher until week 12. Afterwards, these concentrations were much lower than those measured during the first part of the laying year. A significant difference in the changes in oestradiol concentrations in relation to the physiological status ($p < 0.01$) was observed in both comparisons (broody *versus* non-broody and out-of-lay *versus* laying). The plasma concentrations of oestradiol were quite stable in non-broody or in hens which showed good persistency of laying. On the other hand, the broody or the out-of-lay hens displayed high oestradiol concentrations in plasma during the first half of the experiment. These concentrations were lower in out-of-lay than in laying hens after the 17th week of egg production, while they were comparable in broody *versus* non-broody hens except on week 19.

The plasma concentrations of progesterone measured in turkey hens throughout the laying year were lowest in the first experimental sample and higher thereafter. However, no significant time effect ($p > 0.05$) was found in this experiment. On the other hand, significant differences between the physiological states ($p < 0.01$; broody *versus* non-broody and out-of-lay *versus* laying) were found. This was due to the decrease in plasma progesterone concentrations with time observed in the broody and out-of-lay hens.

DISCUSSION

The findings of the present study confirm knowledge of the changes in plasma hormone levels associated with different physiological states in turkey hen. The passage from short to long photoperiods stimulates the secretion of LH (Godden and Scanes,

1977; Burke and Dennison 1980; Lea and Sharp, 1982; El Halawani *et al*, 1984), and in this experiment the onset of lay which followed photostimulation was associated with higher LH concentrations. A tendency for greater initial concentrations was observed in the hens that were the least persistent layers. Although we did not record individual egg production in this experiment, data from another study using hens of a similar strain (Guémené, 1990b) showed that short persistency hens were no more precocious than long persistency hens. The larger decrease in plasma LH concentrations which occurred during the last few weeks of this experiment in the short-persistency hens is consistent with egg-laying disruption. A decline in circulating LH throughout the laying year was also observed in long persistency laying turkey hens. Such a decrease has not been reported previously (Lea and Sharp, 1982; El Halawani *et al*, 1984). However, it did not result in a decrease in ovarian steroidogenic activity as measured by oestradiol and progesterone secretions.

The increased concentrations of plasma oestradiol and progesterone occurring at the onset of the laying year is attributable to the stimulatory effects of increasing plasma LH concentrations on the ovary (for review, Sharp, 1989). Progesterone secretion does not increase until the ovary is fully developed (Williams and Sharp, 1977) as the chief site of synthesis is the granulosa cell layer of the largest follicle of the hierarchy (Imai and Nalbandov, 1978; Culbert *et al* 1980; Etches and Duke, 1984). The observation that plasma progesterone concentrations reached a maximum at the peak of egg-laying is consistent with this idea. The decrease in plasma progesterone concentrations throughout the laying year in broody and out-of-lay hens is clearly related to their physiological states. Mashaly and Wentworth (1974), Mashaly *et al* (1976), and Scanes *et al* (1979) also reported a similar progressive decrease in plasma progesterone

terone concentrations during the first year of lay in hens which showed a low rate of egg production.

An increase in plasma prolactin concentrations associated with the peak of lay has been reported previously by Etches and Cheng (1982). However a such change was not observed previously by Burke and Denison (1980). The results of the present study therefore support the findings of Etches and Cheng (1982). Otherwise, we found a smaller increase in plasma prolactin concentrations in broody than in non-broody hens, and in short persistency than in long persistency hens over a 5-week period following the peak of lay. A high percentage of the hens that ceased egg laying earlier had been classified broody on a prior occasion and we cannot exclude the possibility that other hens may have expressed some symptoms which were not observed under the present experimental conditions. Lower plasma prolactin concentrations in hens that were to express broodiness later is indeed a puzzling observation. Furthermore in another experiment conducted in a different room with hens not submitted to broody treatment, broodiness expression was associated with high plasma prolactin levels (Bédécarrats *et al*, 1993). The present observation does not exclude however an increase in prolactin concurrently with broodiness expression. Similarly, Bacon *et al* (1983) did not find any difference in the base-line concentrations of plasma prolactin between hens that became broody and those which did not. We suggest that the hens that became broody were probably more inclined to stay longer in the nest and were therefore more often submitted to automatic nest ejections combined with frequent egg collection. We cannot exclude that certain hens are more sensitive to preventive treatments in terms of lowering their circulating concentrations of prolactin without preventing broodiness expression. It is clear from this study that low circulatory levels of

prolactin do not prevent these hens becoming broody. Likewise, icv perfusions of ovine prolactin in turkey hens induce a drop in circulating levels of prolactin while persistent nesting activity was displayed (Youngren *et al*, 1991). We cannot exclude therefore that under our experimental procedure these hens had low circulating prolactin levels but high central levels of prolactin.

Alternatively, the observed difference in plasma prolactin concentrations between long and short laying persistency hens may perhaps clarify the differing results obtained with hens from a high producing strain (Etches and Cheng, 1982) and with hens from a low producing strain (Burke and Denison, 1980). Indeed, the present data suggest the existence of a proportional relationship between laying persistency and the presence of high plasma concentrations in prolactin between the 5th and 11th week of production. On the other hand, prolactin has been reported to inhibit LH secretion and to exert antigonadal effects in turkey hens (Opel and Proudman, 1980; Zadworny and Etches, 1987; El Halawani *et al*, 1991). However, one of the major problem remains the heterologous nature of the prolactin used in all these studies and it is likely that these preparations contain other pituitary contaminants and not impossible that heterologous prolactins have different affinities for the avian receptor and even elicit different responses.

Selection against broodiness and selection for increased egg production probably affect different endocrinological mechanisms. For the strain used in the present study, a decrease in the sensitivity to high peripheral concentrations of prolactin as a result of the selection process cannot be excluded since high levels were associated with egg laying persistency and not with broodiness expression. Furthermore, the differences found in prolactin concentrations did not correspond to any large differences in LH concentration. The present data

also confirm previous studies (Lea and Sharp, 1982; Bacon *et al*, 1983; Zadworny *et al*, 1986) showing that large increases in plasma prolactin concentrations do not anticipate the development of gonadal regression and/or photorefractoriness in turkey hens unlike some wild galliform species (Stokkan *et al*, 1988) or other wild species of birds (Dawson and Goldsmith, 1983).

As turkey hens come into lay, increasing concentrations of oestrogens enhance the release of prolactin (El Halawani *et al*, 1983; El Halawani *et al*, 1986; Saeed and El Halawani, 1986). It was observed in the present experiment that the changes in prolactin and oestradiol concentrations were inversely related. Moreover, the decrease in circulating prolactin in laying hens occurs independently of any change in oestradiol concentrations, therefore the facilitatory action of this steroid may become attenuated with time or counteracted by another mechanism. On the other hand, prolactin has been reported to inhibit the secretion of oestradiol from the small follicles (Zadworny *et al*, 1989) which are the major source of this steroid (Robinson and Etches, 1986). Therefore, the observed high concentrations of prolactin in long persistency hens may be the cause of their lower concentrations of plasma oestradiol. Alternatively, the high initial level of oestradiol may be the cause of early cessation of egg laying through a negative feed-back. These low persistency hens also displayed a decrease in progesterone concentrations with time and the largest ovarian follicle is the main source of plasma progesterone in birds (Imai and Nalbandov, 1978; Etches and Duke, 1984). We cannot exclude therefore that these hens were always poor layers having a full set of maturing yellow-yolky follicles producing oestradiol and less or no fully mature pre-ovulatory follicles producing progesterone.

We conclude that, under our experimental conditions, information on hormonal

changes such as prolactin and oestradiol secretion at the beginning of the laying year may provide predictive information on the laying persistency. The inverse relationship that was observed here between high early plasma concentrations of oestradiol and laying persistency has not been reported previously. Furthermore, it appears that the hens that had a longer egg-laying persistency had slightly lower levels of LH during the first 5 weeks of lay. In addition, the relationship between the ability to express broodiness and high and low early plasma concentrations of oestradiol and prolactin respectively in hens submitted to preventive broody treatment has not been reported previously either. Furthermore, it seems clear that high initial concentrations in prolactin, far from exerting any deleterious effects on egg production are closely associated with a longer persistency of egg laying.

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