Relationship between Intersequence Pauses, Laying Persistency and Concentration of Prolactin during the Productive Period in White Leghorn Hens

I. J. Reddy*, C. G. David and Khub Singh

National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore - 560 030, India

ABSTRACT : Prolactin is considered to influence the taking of pauses in between ovulatory sequences in White Leghorn hens. Therefore modulating concentrations of prolactin using bromocriptine - a dopamine agonist during early life (17 to 36 weeks of age) could overcome the inhibitory effects of high concentration of prolactin on ovarian activity. The effect of modulation of prolactin concentration on egg production, sequence length and inter sequence pauses were studied by analyzing the oviposition records from 19 to 72 weeks were studied and compared with untreated controls. Bromocriptine administered subcutaneously (100 μg kg^{-1} body weight or orally through feed (640 μg day^{-1} $bird^{-1}$) resulted in a steady and sustained decrease in prolactin levels ($p<0.01$) during and after the withdrawal of treatment up to one reproductive cycle (72 weeks of age). The treated birds had comparatively longer sequences ($p<0.01$) and fewer pauses ($p<0.01$). Egg production increased ($p<0.01$) by fourteen per cent through subcutaneous administration and eleven per cent through oral feeding, over the control birds. It is concluded that the physiological pauses that occur during ovulatory sequences can be disrupted effectively using bromocriptine. Prolactin levels are modulated which may interfere with the follicular recruitment and subsequent oviposition thereby improve egg laying potential of the bird. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 5 : 686-691)***

Key Words : Bromocriptine, Inter Sequence Pauses, Ovulatory Sequences, Oral Feeding, Prolactin, White Leghorn

INTRODUCTION

Oviposition in domestic chicken is characteristic in a way that the poor layers have frequent pause days and therefore short sequences whereas the best hens have the ability to lay one egg at roughly the same time everyday. Thus by examining egg-laying characteristics such as, age at first oviposition, sequence length and intersequence pause lengths we can assess the reproductive fitness in single comb White Leghorns. Fluctuation in the concentration of prolactin is responsible for timing of oviposition in the domestic hen (Wentworth et al., 1983; Reddy et al., 2002; David et al., 2003). Prolactin in orchestration with other hormones is responsible for follicular growth in Sheep (Picazo et al., 2000), cow (Hoffmann, et al., 1974), sow (Horth and Farmer, 2000), Mares (Bennett-Wimbush et al., 1998.), in addition to its role in the development of broodiness in turkey and bantam hens (Sharp et al., 1988). Nevertheless it has been shown that prolactin inhibits gonadotrophin-stimulated ovulation and oestrogen production at the ovarian level in chicken and a decrease in prolactin is found before and during the preovulatory LH surge (Tanaka et al., 1971; Scanes et al., 1977; Zadworny et al., 1985).

Several measures have been taken to reduce the secretion of prolactin through active and/or passive immunization especially in turkey (El Halawani et al., 1995) and bantam hens (Sharp et al., 1989) to prevent development of broodiness. All these methods were targeted through the dopamine system since dopamine inhibits prolactin secretion via hypothalamus. In mammals the biological effect of inhibition of prolactin is determined using a dopamine agonist - bromocriptine (Horth and Farmer, 2000) but its application in avian species is limited compared to active immunization against the vasoactive intestinal peptide to combat broodiness. However, not much attention has been given to the role of prolactin in the laying hen. This may be due to the fact that commercial layers are quite refractory to the development of broodiness due to selection against broodiness. More recently, we found that subcutaneous administration of bromocriptine from 17 to 36 weeks of age was able to suppress prolactin secretion and increase egg production thorough out one reproductive cycle up to 72 weeks of age (Reddy et al., 2001). However, residual effect of bromocriptine has not been observed in egg. In commercial poultry industry even a slight increase in egg production would bring about an appreciable increase in profit to the farmer by decreasing the pauses between sequences. In this regard, suppression of prolactin secretion within physiological limits could improve egg production. This study was designed to study the pattern of oviposition in terms of ovulatory sequences and inter sequence pauses by oral feeding of bromocriptine and compared with those birds treated subcutaneously as well as untreated control birds.

MATERIALS AND METHODS

Experimental birds

The study was conducted with 50 White Leghorn birds

* Corresponding Author: I. J. Reddy. Tel: +91-080-5711304, Fax: +91-080-5711420, E-mail: ijreddy123@rediffmail.com

Received September 22, 2004; Accepted December 27, 2004

Table 1. Egg production, egg sequence and pause days (mean±SE) for White Leghorn hens subject to bromocriptine treatment

	Control group	Subcutaneous treatment	Oral feeding
No. of birds	20	10	20
Age at first oviposition	127.00±0.00	127.30±0.15^{NS}	127.25±0.10^{NS}
No. of laying days	284.70±5.66^{a}	339.10±4.35^{b}	328.50±3.28^{b}
Total no. of sequences	63.05±2.65^{a}	32.50±4.28^{b}	39.50±2.15^{b}
Mean sequence length (days)	4.73±0.29^{a}	7.68±0.37^{b}	8.90±0.50^{b}
Maximum sequence length (days)	25.25±1.83^{a}	72.50±9.94^{b}	57.10±5. 57^{b}
Total pause days	93.30±5.64^{a}	38.90±4.35^{b}	49.25±3.17^{b}

^{a,b} Means having at least one common superscript do not differ at 1% level ($p<0.01$).
^{NS} Non Significant.

of strain were housed in individual cages (1'×1'×1') from 12 to 72 weeks of age under two-tier battery system. The birds were kept under constant light (2 lux at the level of the eye) to eliminate all diurnal variations from the pattern of oviposition. All hens were fed on the same layer ration (16 per cent CP and 11.72 MJ ME kg^{-1}) as per the standard recommendations (Ranjhan. 1993) and water provided ad libitum.

When the birds attained 17 weeks of age they were divided into control group (n = 20) and treatment groups 1 (n = 10) and 2 (n = 20). subjecting to completely randomized design. The birds in the treatment group 1 were administered with bromocriptine subcutaneously at weekly intervals in the wing web region (100 μg kg^{-1} body weight). where as those in treatment group 2 received 640 μg day^{-1} $bird^{-1}$ bromocriptine through feed up to 36 weeks of age. The control birds were left untreated during the same period of time. The effect of feeding of bromocriptine through feed was studied to increase egg production in the commercial poultry industry.

Recording of egg production

Egg production was recorded for each hen at the same time each day (1100 h) for a continuous 378 days period. The incidence of broken eggs and soft-shelled eggs were identified and recorded. All the birds in the two groups started to lay eggs around day 127. therefore oviposition records were calculated from day 127 to 504 for all analyses. Egg sequence length and the number of egg sequences were determined from oviposition records following the procedure reported by Blake and Ringer (1987). The number of eggs laid on successive days by a particular hen determined the length of each sequence and the number of pauses in each hen's oviposition determined the number of sequences. For each hen the length of laying sequence was determined on the day the last egg of the current clutch was laid. To calculate inter sequence pauses the oviposition records from days 127 to 504. were subdivided into 27. fourteen day periods to determine the inter sequence pauses for each hen. If a hen did not experience a pause during that period no value was recorded or else the actual number of pauses observed during that period was recorded.

Blood sampling:

Weekly blood samples were collected by brachial venipuncture. serum separated and stored at -20°C for analysis of prolactin using RIA.

Radioimmunoassay of prolactin

Plasma samples were analyzed for PRL using the method previously described by Scanes et al. (1976) for domestic fowl. Chicken PRL hormone (AFP-44448B) and antisera (AFP-151040789) were generously provided by Dr. Parlow (NIADDK. USA). The antiserum was used at a final dilution of 1:400,000. PRL standards ranged between 50 to 1.000 ng/ml (50. 100. 250. 500. 1.000). The bound and free fractions were separated using anti rabbit γ-globulin raised in goat at a final dilution of 1:100. The Intra and inter assay coefficients of variation for PRL were 7.22% and 9.50%. respectively.

Statistical analyses

All quantitative data were subjected to one-way analysis of variance. Test for independent means was used to test the significance in the different patterns of egg sequences and pause length between the groups (Snedecor & Cochran. 1994). Statistical significance was set at $p<0.01$. Group data are presented as mean±SE.

RESULTS

All the hens in the two groups started to lay eggs by 19th week of age. The mean age at first egg was around 127 days in all the groups. Even though bromocriptine treatment did not change the age at first egg. an increase ($p<0.01$) in the number of laying days in subcutaneously treated (339.10± 4.35) and orally fed (328.50±3.28) birds was recorded compared to the control birds (284.70±5.66). with relatively less number of egg sequences (Table 1). The control group had significantly more numbers of egg sequences with <10 eggs. In contrast. birds of the treatment groups had significantly more ($p<0.01$) numbers of sequences with >11 eggs. Egg sequences with >100 eggs were also encountered in birds treated with bromocriptine. The mean sequence length was higher in the bromocriptine treated birds

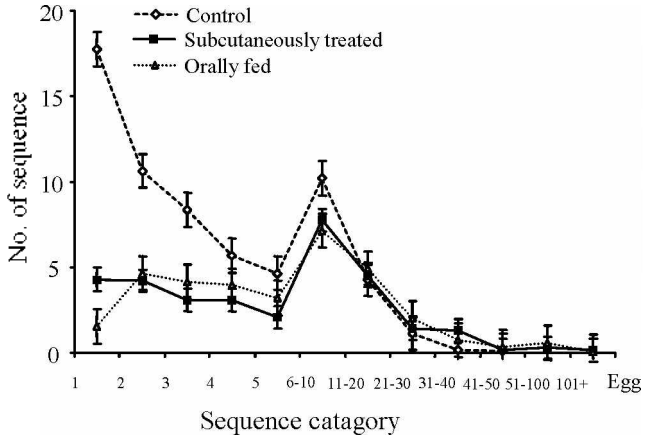

Figure 1. Changes in the sequence of egg production between the bromocriptine treated white leghorn hens and untreated control hens (means±SEM).

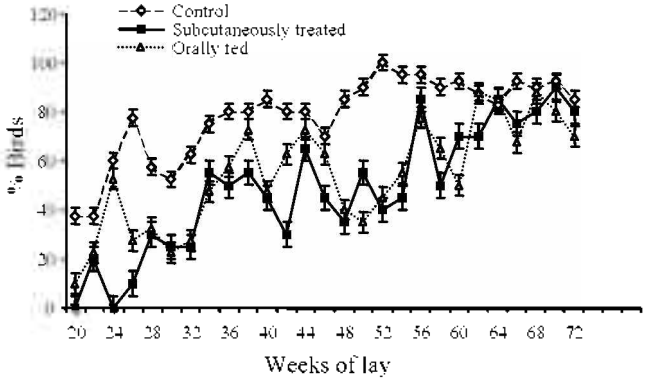

Figure 2. Percentage of hens experiencing pauses through out the productive period in white leghorn hens which were treated with bromocriptine subcutaneously (Group 1), orally (Group 2) or remained untreated (Control).

compared to the control birds (Figure 2B). Treatment with bromocriptine, while resulting in increase in egg production, reduced the number of laying pauses between sequences (Figure 2A, 2C) compared to the control group. Subcutaneous treatment of bromocriptine increased the number of laying days and reduced pauses compared to oral feeding.

Treatment with bromocriptine (from 17 to 36 weeks) resulted in a steady significant decrease ($p<0.01$) in the peripheral concentration of PRL (Figure 1). This decrease was sustained during and after the withdrawal of bromocriptine treatment. On the other hand, PRL levels were elevated in control birds throughout the 72-week period. However, during peak egg production the circulatory levels of PRL automatically fell in all the groups. The fall was steep in the treatment groups due to bromocriptine.

DISCUSSION

Our results support the notion that circulating prolactin levels play a role in timing the oviposition in domestic hen. An increase in the level of prolactin in pullets may be correlated to growth since prolactin promotes growth in birds Scanes et al., 1976, even though it will not maintain high rates of growth. This ambiguity concludes that increase in prolactin levels should be responsible for increased folliculogenesis and preovulatory LH surge resulting in the first oviposition Harvey et al. (1979).

Age at first egg

The age at first egg was not much altered between the two groups by modulation of prolactin levels through bromocriptine treatment. The age at first oviposition is influenced by external stimuli in addition to the genetic constitution of individual birds. The influence of supplemental lighting on age at first egg is not very well understood (Lupicki, 1994; Robinson et al., 1996; Robinson et al., 2001). In our study continuous lighting decreased the age at first egg but no treatment differences were observed. Our observations were in contrast to Robinson et al. (1993) where early age at first oviposition was found to impair egg production. It is likely that there is a greater allocation of energy for the ovary at a young age, which may impair ovarian control as observed in broiler breeds (Bedecarrates, et al., 1997). In addition, the limited body reserves would have been depleted by the time of peak egg production (Robinson et al., 1990). We conclude from our observations that high egg production was primarily a function of higher rates of lay throughout the laying period of 72 weeks rather than the age at sexual maturity.

Egg sequence

Bromocriptine treatment increased the number of laying days in treated birds compared to the control birds with relatively fewer egg sequences (Table 1). Convincing evidence has been presented implicating increased prolactin secretion as the cause of reduced circulating gonadotrophins, ovarian regression and the shift from egg laying to the incubation phase of reproductive cycle in the hen (Crisostome et al., 1998). This is further fortified by the findings of Ogawa et al. (1977) that intravenous injection of mammalian prolactin in hens 6-7 h before the expected second ovulation, blocks the second ovulation but not when given 5 or 8-14 h before the second ovulation. In our study we have observed reduced laying pauses and longer sequences in birds treated with bromocriptine. On the contrary Bedrack et al. (1983) reported that bromocriptine treatment impaired the resumption of lay in broody turkey hens compared to clomiphen citrate an anti oestrogen drug, which might be due to the difference in dosage of bromocriptine as well as the treatment regimen employed.

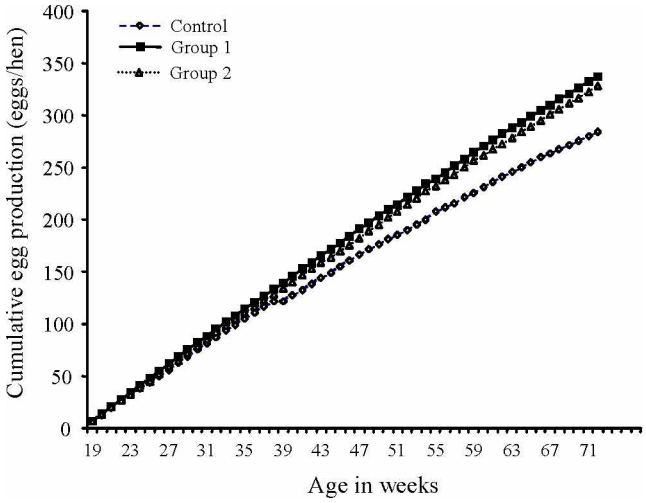

Figure 3. Cumulative egg production of White Leghorn hens, which were injected subcutaneously with bromocriptine or fed orally compared to untreated control.

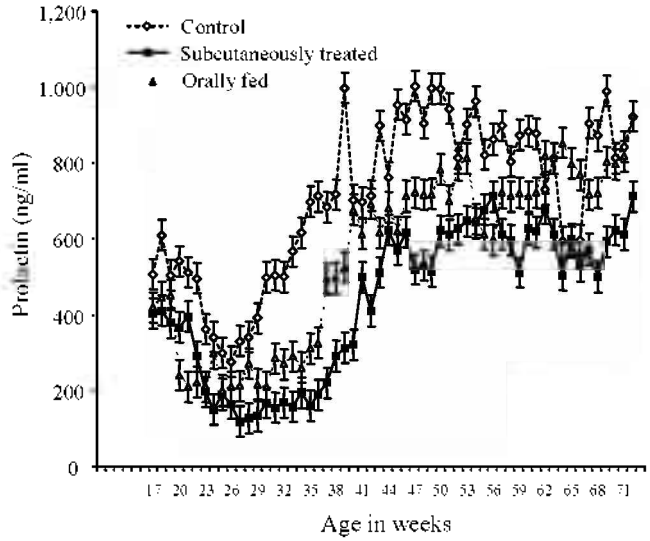

Figure 4. Changes in prolactin concentration (ng/ml plasma) in White Leghorn hens, which were injected with bromocriptine orally (640 μg day^{-1} bird^{-1}) compared with subcutaneously treated (100 μg kg^{-1} body weight) and untreated controls (means±SEM).

The increase in egg production is also due to the rate at which follicles enter their final phase of rapid growth, which is also under the influence of prolactin. At high concentration, prolactin interferes with follicular steroidogenesis in avian species (Emmerson et al., 1991; Porter et al., 1991; Dajee et al., 1998) and only minimal amounts are required for normal growth. This fact is also emphasized in studies with human granulosa cells that failed to grow and secrete progesterone *in vitro* in the absence of prolactin even in the presence of adequate amounts of gonadotrophins (McNatty et al., 1975). In our earlier study we observed a negative correlation between prolactin with progesterone and estradiol 17β (Reddy et al., 2002) which also support the earlier reports.

Laying pauses

Even though the intersequence pause length increases with age the occurrence of pauses of 2 days duration may be the consequence of reduced rate of follicular maturation and its subsequent recruitment into the hierarchy following ovulation which is partly regulated by FSH (Etches and Cheng, 1981). Prolactin at high levels suppresses the FSH induced estradiol production through the aromatase enzyme system (Wang et al., 1980) resulting in reduced steroidogenic potential within the follicles. This reduced steroidogenic potential is not able to produce progesterone sufficient to elicit a positive feedback of LH required for ovulation (Dorrington and Gore-Langton, 1981). We also observed an increase in the concentration of estradiol 17β and progesterone in plasma of birds treated with bromocriptine compared to those of the control birds (Reddy et al., 2002). In support of our statement that modulation of prolactin using bromocriptine overcomes the inhibitory effect of prolactin on follicular development and subsequent oviposition, we observed at necropsy that ovaries of bromocriptine treated birds had greater number of yellow yolk follicles compared to the control group. This may explain the cause for longer sequences and reduced laying pauses in the treated birds. However, the occurrence of more than 11 days of laying pauses in birds of both groups may be due to the genetic constitution of individual birds.

The mechanism responsible for ovulation and its failure, which lead to skipped days has been much studied but not clarified. Even though the role of prolactin in occurrence of broodiness in turkey and bantam hens is well known it was not extended to laying chicken, particularly in relation to laying pauses in between clutches, which has been emphasized in this study. In the present study the treatment with bromocriptine either subcutaneously or through oral feeding, during the initial weeks of laying was able to control egg production throughout one reproductive cycle up to 72 weeks of age in White Leghorn hens. This is supported by the observations of Gumene and Williams (1994) that low initial concentrations of prolactin (far from exerting any deleterious effects on egg production) is closely associated with longer persistency of egg laying and that the hormonal profiles for a given hen during the first 10 weeks of the laying cycle may provide productive information for future changes in the physiological status. We conclude that the physiological pauses that occur during ovulatory sequences can be disrupted effectively using bromocriptine Prolactin levels, are modulated which may interfere with the follicular recruitment and subsequent oviposition thereby improving egg production and improve egg laying potential in White Leghorn hens.

ACKNOWLEDGEMENTS

We acknowledge Dr. A. F. Parlow, (Director, Pituitary Hormones and Antisera Center, Harbour - UCLA Medical Center, Torrance, CA), for donating chicken prolactin hormone and anti chicken prolactin antibody, and Mr. K.S. Guru Murthy for the technical assistance.

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