INTRODUCTION

Reproductive fitness in Single Comb White Leghorns can be assessed by examining the egg-laying characteristics such as, age at first oviposition, sequence length and intersequence pause lengths. PRL may be involved in follicular growth in other species (McNeill et al., 1982). PRL receptors have been localized on the granulosa cells late in follicular development (Hughes et al., 1988) and PRL in minimal levels has shown to increase steroidogenesis. The role of PRL in laying chicken has not received much attention. This is in contrast to the situation in turkeys and bantam hens that develop broodiness, which is dependent upon PRL (Wentworth et al., 1990). Albeit, commercial laying chicken are quite refractory to development of broodiness, PRL has been shown to inhibit gonadotrophin stimulated ovulation in chicken (Tanaka et al., 1971; Camper and Burke, 1977; Lea et al., 1981; Sharp et al., 1988) and a decrease in plasma PRL has been found before and during the preovulatory LH surge (Scanes et al., 1977). Zadworny et al., (1985), reported that PRL inhibits oestrogen production at the ovarian level.

PRL secretion is controlled by the tonic inhibition via hypothalamus. Most evidence indicates that dopamine is the inhibitory factor (Weiner and Ganong, 1978; Birge et al., 1979; Sharp, 1999). Bromocriptine is a dopamine agonist, often used in endocrinological studies in order to determine the biological effects of inhibition of PRL (Taverne et al., 1982; Farmer et al., 1998). Harvey et al. (1982) reported that bromocriptine decreases PRL release from fowl pituitaries in vitro. Although bromocriptine has been widely used in other species (Sheep: Niswender 1974; Picazo et al., 2000; cow: Hoffmann et al., 1974; sow: Horth and Farmer 2000; Mares: Bennett-Wimbush et al., 1998.) its application in avian species is limited (turkey: Bedrak et al., 1983). Instead, active immunization of vasoactive intestinal polypeptide (VIP) has been widely used in poultry to overcome broodiness (turkey: El Halawani et al., 1990, 1995; chicken: Sharp et al., 1989).

The hypothesis in the present study was that successful treatment of white leghorn hens with 2-bromo-α-ergocryptine would modulate the effect of dopamine consequently decreasing PRL and increasing egg production by decreasing the inter sequence pauses length, overcoming the inhibitory effects of high concentration of PRL on ovarian activity.

METHODS

Experimental animals

The experiment was carried out in one hundred white leghorn birds, housed in individual cages from 12 to 72 weeks of age. The birds were divided into two equal groups viz. control and treatment groups. The birds in the treatment group were administered at weekly intervals with 2-bromo-α-ergocryptine (100 µg kg⁻¹ s.c. Sigma, USA.) from 17 to 36 weeks of age. The control groups were administered...
Post treatment PRL concentration was higher in both the groups before the start of the treatment at 17 weeks of age. Thereafter the concentration in treated birds decreased significantly (p < 0.01) and remained at a lower level during different weeks of lay compared to the control birds. Values are Mean±SE; n=48.

with placebo containing only the vehicle in place of bromocriptine. The birds were kept under constant light to eliminate all diurnal variations from the pattern of ovipositions. 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, 1981).

Collection of samples
The birds were bled by brachial venipuncture at weekly intervals from 13 to 72 weeks of age. Serum was separated by centrifugation at 2,500 rpm for 20 minutes and stored at -20°C for the estimation of PRL using Radioimmunoassay (RIA).

Recording of egg production
Egg production was recorded for each hen at the same time each day for a continuous 343 days period. The incidences of soft shelled and shell less eggs were recorded. 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 pauses in each hen’s oviposition determined the number of sequences; the number of eggs laid on successive days by a particular hen determined the length of each sequence.

All the birds in the two groups started to lay eggs around day 168, therefore oviposition records were calculated from day 162 to 504 for all analyses. During the period of study two birds in each group died and oviposition records of the surviving 48 hens in each group was taken into consideration for all analytical purpose. For each hen the length of laying sequence was determined on the day the last egg of the current clutch was laid. From days 162 to 504, the oviposition records were subdivided into 18.5, fourteen day periods to determine the inter sequence pause 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 during that period observed was recorded.

Radioimmunoassay of prolactin
Plasma samples were analyzed for PRL using the method previously described by Kaprowski, and Tucker (1971). Chicken PRL hormone (AFP-44448B) and antisera (AFP-151040789) were 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 and 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 and Cochran, 1994). Statistical significance was set at p < 0.01. The relationship between the length of the largest sequence, PRL concentration and egg production were examined by calculation of Pearson correlation coefficient. Group data are presented as Mean±SE.

RESULTS
Effect of 2-bromo-α-ergocriptine on circulating concentration of prolactin
The circulating concentration of PRL in control and treatment group was estimated using RIA. The concentrations were found to increase with age in both the groups from 13th week of age onwards (Figure 1). Treatment with 2-bromo-α-ergocriptine (from 17 to 36 weeks) resulted in a steady significant decrease in the peripheral concentration of PRL. This decrease was found sustainable during and after withdrawal of 2-bromo-α-ergocriptine treatments. On the other hand, PRL levels were found elevated in birds of the control group throughout the 72-week period. However, during peak egg production the circulatory levels of PRL automatically fell in both the groups, albeit the fall was steep in the treatment group due to 2-bromo-α-ergocriptine (p≤0.01).

Effect of modulation of PRL on egg production, sequence and intersequence pause
All the hens in the two groups started to lay eggs by 24th week of age. The mean age at first egg was 152.08±0.89 and 151.85±0.98 in the control and treatment group.

![Figure 1. Concentration of prolactin in the plasma of control and 2-bromo-α-ergocriptine treated white leghorn hens.](image-url)
**TABLE 1. Egg production, sequence length and intersequence pause data (Mean±SE.) for white leghorn breed hens sorted into control and 2-bromo-α-ergocriptine treated birds.**

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Treated group</th>
</tr>
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<tbody>
<tr>
<td>No. of hens</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Age at first oviposition (day)</td>
<td>152.08±0.89</td>
<td>151.85±0.98 NS</td>
</tr>
<tr>
<td>No. of laying days</td>
<td>300.13±0.35</td>
<td>313.13±0.52 **</td>
</tr>
<tr>
<td>No. of Sequences</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34.58±1.70</td>
<td>25.67±1.15 b</td>
</tr>
<tr>
<td>1 egg</td>
<td>4.52±0.47**</td>
<td>2.28±0.27</td>
</tr>
<tr>
<td>2 eggs</td>
<td>4.26±0.44**</td>
<td>2.08±0.17</td>
</tr>
<tr>
<td>3 eggs</td>
<td>3.80±0.44**</td>
<td>2.40±0.29</td>
</tr>
<tr>
<td>4 eggs</td>
<td>3.29±0.34**</td>
<td>2.40±0.23</td>
</tr>
<tr>
<td>5 eggs</td>
<td>3.55±0.40**</td>
<td>2.18±0.14</td>
</tr>
<tr>
<td>6-10 eggs</td>
<td>8.73±0.62**</td>
<td>7.74±0.55</td>
</tr>
<tr>
<td>11-20 eggs</td>
<td>4.98±0.35</td>
<td>5.54±0.32**</td>
</tr>
<tr>
<td>21-30 eggs</td>
<td>4.98±0.35</td>
<td>5.54±0.32**</td>
</tr>
<tr>
<td>31-40 eggs</td>
<td>1.30±0.13</td>
<td>1.48±0.15**</td>
</tr>
<tr>
<td>41-50 eggs</td>
<td>1.09±0.09</td>
<td>1.18±0.13**</td>
</tr>
<tr>
<td>51-100 eggs</td>
<td>1.42±0.14**</td>
<td>1.23±0.08</td>
</tr>
<tr>
<td>100+eggs</td>
<td>0</td>
<td>2.00±0.00**</td>
</tr>
<tr>
<td>No. of pauses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18.94±0.58</td>
<td>16.29±0.52 b</td>
</tr>
<tr>
<td>1 day</td>
<td>7.13±0.35</td>
<td>9.08±0.37**</td>
</tr>
<tr>
<td>2 days</td>
<td>5.43±0.37</td>
<td>4.96±0.33 NS</td>
</tr>
<tr>
<td>3-10 days</td>
<td>6.44±0.55**</td>
<td>2.73±0.24</td>
</tr>
<tr>
<td>11+days</td>
<td>2.00±0.00</td>
<td>2.00±0.00 NS</td>
</tr>
</tbody>
</table>

* Means having at least one common superscript do not differ at 1% level (p<0.01). ** p<0.01, NS Non Significant.

respectively (Table 1). However, between the two groups the age at first egg was not significantly altered by modulation of PRL levels due to 2-bromo-α-ergocriptine treatment, whereas there was a significant increase (p≤0.01) in the number of laying days in the treated birds (313.10±0.52 days) as against (300.13±0.35 days) in the control group, with a significant decrease (p≤0.01) in the intersequence pause length (Figure 2A, 2C).

The total number of sequences was significantly higher in control group compared to treatment group (34.58±1.7 and 25.67±1.15). The birds of the control group had significantly more numbers of egg sequences with <10 eggs. On the contrary, birds of the treatment group had significantly more numbers of sequences with >11 eggs. Egg sequences with >100 eggs were also encountered in birds treated with 2-bromo-α-ergocriptine. The mean sequence length was 9.92±0.63 and 14.04±1.12 days in control and treatment group respectively with the maximum sequence length of 46.04±3.09 days in the control group and 59.33±4.44 days in the treatment group (Figure 2B).

**Correlation coefficients**

On the flock basis, there was a strong correlation between the prime sequence length and total egg output in the control (r=0.53; p≤0.01) and treated (r=0.64; p≤0.01) groups. Plasma PRL levels were negatively correlated with egg production in the control (-0.47; p≤0.01) and treated (r= -0.41; p≤0.01) groups.

**DISCUSSION**

**Effect of 2-bromo-α-ergocriptine on prolactin**

The administration of 2-bromo-α-ergocriptine from 17 to 36 weeks of age in layer birds resulted in a sustained decrease in the circulatory levels of prolactin. The inhibitory effects of dopamine on PRL secretion are mediated by D2 receptors that act via G\(_\text{i}\), the inhibitory GTP protein in the membrane to inhibit intra cellular generation of cAMP (Ganong, 1989), hence 2-bromo-α-ergocriptine
the long acting dopamine agonist is a potent inhibitor of PRL secretion. The D2 receptors are distributed in the lactotrophs present in the anterior pituitary gland. The action of 2-bromo-α-ergocriptine is therefore restricted to the inhibition of PRL secretion from the anterior pituitary and not from the hypothalamus (Youngren et al., 1998). The primary action of 2-bromo-α-ergocriptine on PRL secretion appears to be at the transcriptional level (Weinstein et al., 1981) thereby inhibiting secretion of newly synthesized but not pre existing prolactin. Johansen et al. (1986), demonstrated in rat pituitary adenoma cells (GH3) that bromocriptine completely blocks only the release of PRL stimulated by TRH or elevated K+ concentration and basal PRL levels were moderately affected, where it inhibits Ca2+ efflux from the preloaded cells. The physiological significance of the inhibitory effects of dopamine in the control of PRL has not been established in birds. Alternatively, it has been suggested that the inhibitory effect of dopamine on PRL release at the level of anterior pituitary gland may modulate the secretion of PRL releasing hormone and/or VIP induced changes in PRL secretion with reproductive status (Hall and Chadwick 1984; Youngren et al., 1998). However, Harvey et al. (1982), found in vitro that several dopaminergic agonists inhibited both the basal release of immunoassable PRL and the release of PRL stimulated by complete hypothalami or hypothalamic extracts from chicken pituitary gland.

The control of PRL secretion involves the interaction of external stimuli with endocrine mechanisms. Photo induced gonadal recrudescence initiates the rise in circulating PRL (Burke and Dennison 1980). Another increase occurs during the onset of sexual maturity and is dependent upon ovarian steroids (El Halawani et al., 1983). These two PRL elevations were suppressed in 2-bromo-α-ergocriptine treated birds. These findings are interpreted to suggest that external stimuli and endocrinological mechanisms modulating PRL secretion and synthesis may in part act through dopaminergic neurons. Admittedly, the data on PRL release to photo stimulation are very limited (Burke et al., 1981), the findings in the present study appears to indicate that continuous light regimen induced rise in circulating PRL is diminished by 2-bromo-α-ergocriptine treatment. The prevailing steroid milieu in maintaining PRL secretion is important. The increase in PRL during egg laying is dependent on the presence of gonadal steroids. In the present study PRL remains low and stable during egg laying cycle in 2-bromo-α-ergocriptine treated birds (Figure 1). This is supported by the finding of El Halawani et al., (1986), who reported that ovarioctomy prior to photostimulation has no effect on post photostimulation increase in PRL but prevents that associated with laying, whereas hens ovarioctomized during egg laying exhibit a gradual decline in circulating prolactin. These findings support the hypothesis that ovarian steroids influence PRL secretion by modulating the action of dopamine. The steroid site of action is probably mediated at the pituitary level (Kansaku et al., 1994).

Modulation of PRL on egg production parameters

Each hen has a characteristic oviposition pattern (Lillipers and Wilhelmson, 1993) poor layers have many short sequences and frequent pause days whereas the best hens have the capacity to lay one egg at roughly the same time every day. Our data support the notion that fluctuation in circulating concentrations of PRL plays a role in timing of the oviposition in domestic hen.

The role of PRL in growing pullets remain obscure, even though it has been reported that PRL promotes growth in birds (Scanes et al., 1977) this hormone apparently is not able to maintain high rate of growth. Yet, the observed increase in circulating levels of PRL in growing pullets may be responsible for increased folliculogenesis and pre ovulatory LH surge that result in first oviposition (Harvey et al., 1979).

Convincing evidence has been presented implicating increased PRL secretion as a cause of reduced circulating gonadotropins, ovarian regression and the shift from egg laying to incubation phase of the reproductive cycle in chicken (Bedecarrates et al., 1997; Crisostome et al., 1998). Involvement of PRL in timing of preovulatory LH surge is suggested by the observation that intravenous injection of mammalian PRL blocks the second (C2) ovulation of a hen’s sequence when given 6-7 hours before expected (C2) ovulation but not when given 5 or 8-14 hours before C2 ovulation (Ogawa et al., 1977). In the present study 2-bromo-α-ergocriptine treated birds had a higher egg output, with reduced laying pauses and a significantly higher prime sequence. This observation is contradicting the findings of Bedrak et al. (1983), who reported that pharmacological disruption of broodiness using 2-bromo-α-ergocriptine treatment impaired the resumption of lay which improved only after administration of clomifencitract an anti oestrogen drug in turkeys. The increase in egg production might be due to the rate of which follicles enter their final phase of rapid growth. This recruitment of follicles is influenced by PRL since high levels of PRL interfere with follicular steroidogenesis in avian species (Robinson et al., 1990; Porter et al., 1991; Emmerson et al., 1991; Dajee et al., 1998) and only minimal amount of PRL is needed for normal growth. It does appear that some PRL is necessary for steroidogenesis because human granulosa cells failed to grow in vitro in the absence of PRL failed to secrete progesterone even in the presence of adequate amounts of gonadotropin (McNatty et al., 1975). Tanaka et al. (1971), reported that exogenous administration of PRL tends to inhibit LH induced premature ovulation in hen. These
findings support the results obtained in the present study wherein a negative correlation between PRL and egg production was obtained. This negative correlation observed in the present study clearly indicates that PRL secretion regulates egg production. Further, in this study even after withdrawal of treatment, egg production in treated birds was significantly higher than the control birds, which indicates that for a given hen the concentration of PRL during first ten weeks of the laying cycle may provide predictive information for future changes in its physiological status. Guemene and Williams (1994) reported that the low initial concentrations in prolactin are closely associated with longer persistency of egg laying, which support the results obtained in this study.

The number of laying pauses is influenced by the genetic constitution of the birds, date of hatch and season (Venkatasubramaniam and Micheal 1979). Zakaria et al. (1984) reported that termination of the clutch in the hen might be the result of a failure of the follicles to transform during early stages of development or a failure of the hen to ovulate. Secondly, the ovary of mature hen contains a hierarchy of yolk filled follicles and the recruitment of a new follicle into the hierarchy as soon as the largest follicle ovulates is partly regulated by secretion of FSH (Etches and Cheng, 1981). Previous studies in other species have reported that high levels of PRL suppress the FSH induction (Cheng, 1981). The age at first oviposition is partly regulated by secretion of FSH (Etches and Cheng, 1981). Previous studies in other species have reported that levels of PRL suppress the FSH induction of oestradiol production through aromatase in this system (Wang et al., 1980; Dorrington and Gore Langton, 1981). This results in a decreased steroidogenic potential within the follicle (McNatty, 1979) thereby impaired oviposition. The modulation of PRL using 2-bromo-α-ergocriptine effectively over came the inhibitory effects of PRL on ovarian follicular development and subsequent oviposition. This might be the reason for the pattern of oviposition observed in this study with more 1 day pauses occurring in treated birds than in control birds. The occurrence of more than 11 days of laying pauses in both the groups may be due to the genetic constitution of the individual birds.

It has been postulated that age at first oviposition is influenced by external stimuli in addition to the genetic constitution of the birds. The age at first ovulation is influenced by supplemental light (Eitan and Sollan, 1991; Robinson et al., 1996) or not (Lupicki, 1994), in the present study maintaining birds at continuous light resulted in decrease in age at first oviposition. Although the decrease was lower than those reported by Lupicki, (1994) the decrease was not significantly altered due to 2-bromo-α-ergocriptine treatments. Robinson et al. (2001) reported that early age at first oviposition impairs egg production since greater allocation of energy is towards ovary in young age, which might have caused impairment in ovarian control (Robinson et al., 1993). Secondly, limited body reserves would have been depleted by the time of peak egg production as reported by Robinson et al. (1996). The results observed in the experiment indicate that high total egg production was primarily a function of higher rates of lay through out the laying period of 72 weeks, rather than age at sexual maturity. Since in the present study 2-bromo-α-ergocriptine treatments did not significantly alter the age at first oviposition the role of PRL on age at first oviposition needs further investigation.

To conclude, the mechanism responsible for ovulation and its failure, which lead to skipped days has been much studied but little clarified. Earlier studies have shown that secretion of PRL from anterior pituitary gland is controlled by dopamine and higher levels of PRL in circulation to be antgonadotrophic, presumably either blocking the secretion of gonadotrophins from the anterior pituitary gland or its action on the gonad, which leads to cessation of lay, and broodiness in poultry. The present study showed that the cessation of egg lay between ovulatory sequences in chicken is usually caused by increased PRL secretion from the anterior pituitary gland, therefore modulating the PRL levels in circulation with a dopamine agonist can result in longer ovulatory sequences by decreasing the inter sequence pause length which ultimately results in increased egg production.

REFERENCES


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