ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 5 (7): 685-692, 2006
ISSN 1682-8356

Chemical Control of Prolactin Secretion and It's Effects on Pause Days, Egg Production and Steroid Hormone Concentration in Girirani Birds

I.J. Reddy*, C.G. David and S.S. Raju
National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore – 560 030, India

Abstract: The objective of this study was aimed to improve egg production by decreasing the intersequence pause days between the sequences of egg lay in girirani birds through chemical control of prolactin by using bromocriptine (an anti prolactin chemical) through feed. At 12 weeks of age, forty two girirani birds were randomly divided into control (n=21) and treated (n=21) groups. Birds in the treated group were fed bromocriptine through feed @ 350µg/bird/day from 17-36 weeks of age and controls were fed the same ration without bromocriptine. Birds fed with bromocriptine through feed significantly (P<0.01) reduced the prolactin concentration compared to control birds. Estradiol and progesterone were quantified in both the groups. Treated birds showed higher (P<0.01) concentration of estradiol and progesterone concentration from 19th to 72 weeks of age with intermittent fluctuations. Egg production, pause days, and hormonal parameters were correlated between the two groups. Prolactin is negatively correlated with egg production (r = -0.89), estradiol (r = -0.59) and progesterone (r = -0.37) hormones and positively correlated with inter sequence pause days with concomitant decrease in egg production. Birds fed bromocriptine through feed showed significantly (P<0.01) less pause days and more egg production. The results of this study showed that, controlling the prolactin secretion by chemical means resulted in 5.12% increase in egg production over control birds, further such an approach is more promising, practicable, economical without any traces of chemical in meat, egg and blood to improve egg production in backyard poultry farming. It is concluded that chemical control of prolactin plays a major role on pauses days , prolactin secretion and egg production in native breed of birds.

Key words: Chemical control of prolactin secretion; steroid hormones, egg production in girirani birds

Introduction

Egg laying pattern in domestic hen is characteristic to the breed of birds. Genetically superior birds' takes less pauses compared to native breed of birds developed for dual purpose, resistant to diseases and adverse climatic variables as a backyard poultry in rural areas. One such breed is a girirani bird developed for both egg and meat. Egg laying characteristics such as, age at first oviposition, sequence length, intersequence pause days, reproductive performance in terms of egg production and hormonal profiles in this new breed of birds is not reported and it is the first report available in this breed. Still it is not fully elucidated that, a change in the concentration of prolactin is responsible for timing of oviposition in domestic hen (Wentworth *et al*., 1983) or due to longer intervals of LH surges. Prolactin in orchestration with other hormones is responsible for follicular growth in several species (Sheep: Picazo *et al*., 2000; cow: Hoffmann *et al*., 1974), in addition to its role in the development of broodiness in poultry with reference to turkey and bantam hens (Sharp *et al*., 1988) nothing is reported in this breed. Earlier studies revealed that (Reddy *et al*., 2001) that prolactin inhibits gonadotrophin stimulated ovulation and estrogen production at ovarian level in chicken and a decrease in prolactin is found before and during the preovulatory LH surge (Zadworny *et al*., 1985). Several studies were conducted to reduce the secretion of prolactin through active and / or passive immunization in bantam hens to prevent development of broodiness (Sharp, *et al*., 1989) for improving the reproductive senescence. These studies were targeted through dopamine system since dopamine inhibits prolactin secretion via hypothalamus. In mammals, the biological effect of inhibition of prolactin is well established using a dopamine agonist bromocriptine but its application in avian species is limited compared to active immunization against prolactin and its secreting hormone, i.e., vasoactive intestinal peptide (VIP). However, not much attention has been given to the chemical control of prolactin in laying hen through simple and practicable ways. Hence, this study is conducted to control the prolactin concentration chemically by using bromocriptine through feed at micro quantities (which is practicable through feed) and studying its effects on pause days, egg production, and interrelation of steroid and prolactin hormones in this newly developed breed of girirani birds. More recently, we found that subcutaneous administration of bromocriptine from 17 to 36 weeks of age in white leghorn birds was able to suppress prolactin secretion and increase egg production thorough out one reproductive cycle up to 72 weeks of age (Reddy *et al*.,

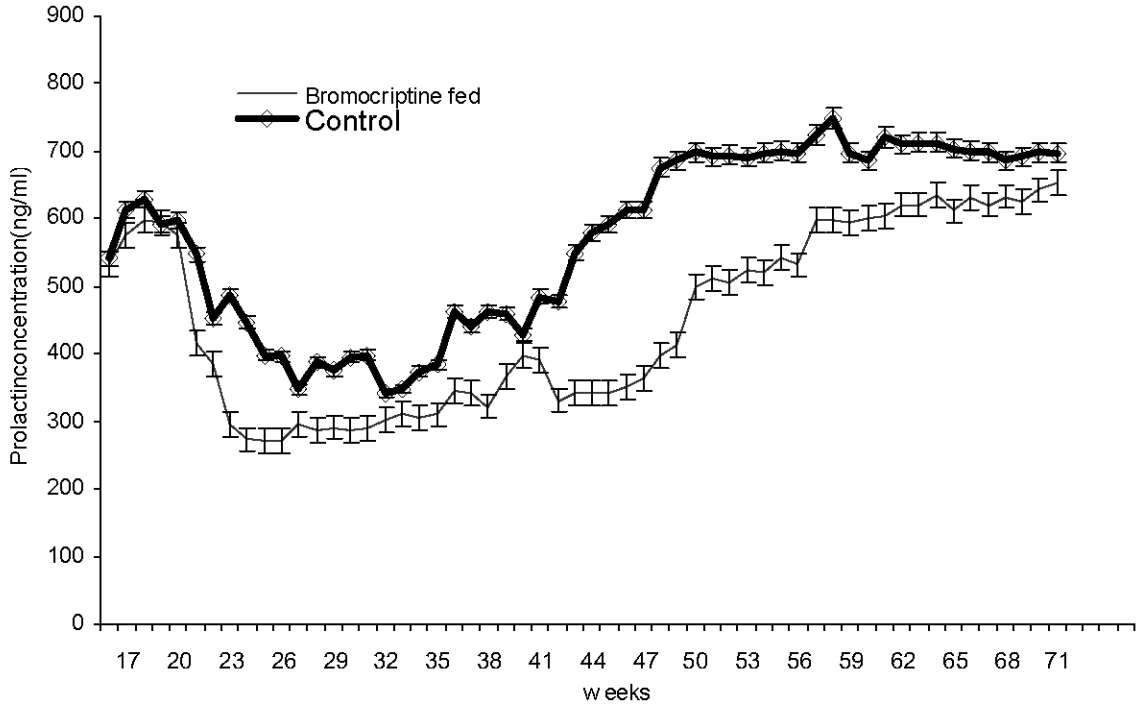

Fig. 1: Plasma prolactin concentration (ng/ml) between control & treated birds. Bromocriptine treated birds showed significantly lower ($P<0.01$) concentration over controls from 17 week to 72 weeks of age

2001). However, the experiment mainly focuses on feeding of bromocriptine through feed in girirani birds and its effects on egg production. Even a slight increase in egg production would bring about a tremendous increase in egg production with available resources under normal managemental conditions which will have an impact of proliferating of egg production especially to the poor and marginal farmers whose lively hood depends on livestock and poultry by adopting these techniques. Hence keeping the prolactin hormone under check (prolactin secretion within physiological limits) can improve egg production. The overall goal is to improve reproductive efficiency of native birds in terms of egg production. The objective of this study will focus on improving reproductive performance of native birds through nutritional physiological approaches to improve egg production in birds. Research advances and new biotechnologies will be developed to reduce losses due to reproductive problems in local breeds of birds to maximize per capita consumption of the of high quality products among poor and marginal farmers in the form of meat and eggs.

Materials and Methods

Experimental birds: The study was conducted in forty two girirani birds housed in individual cages from 12 to 72 weeks of age. All the birds were maintained under normal husbandry conditions with 16 hours light and 8 hours darkness. All the birds were fed on the same grower and layer rations as per the standard Specifications. When the birds attained 17 weeks of age they were divided into control (n=21) and treatment groups (21) birds respectively. The birds in the treatment group received bromocriptin 350µg day^{-1} $bird^{-1}$ bromocriptine through feed up to 36 weeks of age. The control birds were provided with the same ration without bromocriptine.

Recording of egg production: Egg production was recorded for each hen at the same time each day for a continuous 362 days period. Birds in the treated group started to lay eggs around day 140, therefore oviposition records were calculated from day 140 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 intersequence pause days. 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 140 to 504, were subdivided 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.

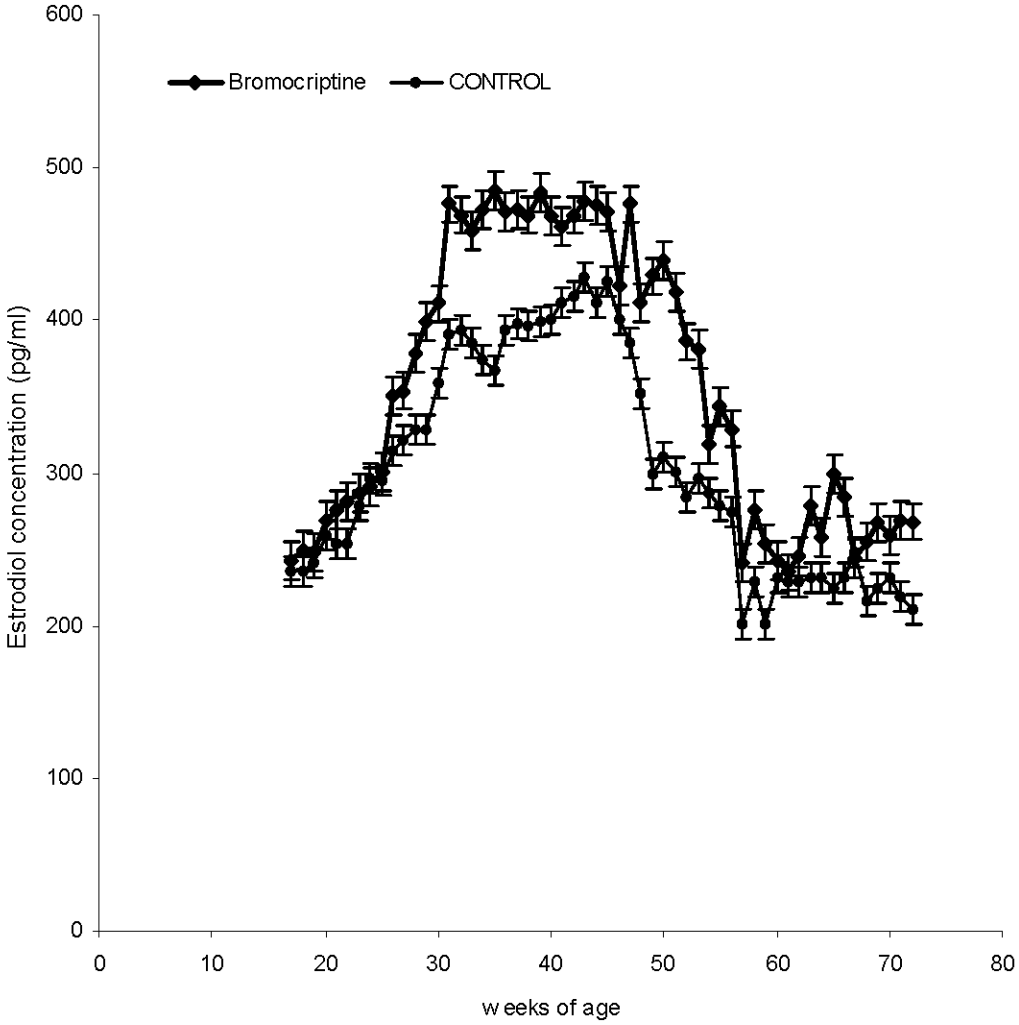

Fig 2: Plasma estrodiol concentration between control & treated birds. Treated birds showed significantly higher (P<0.01) concentration of hormone over control birds

Blood sampling: Blood samples were collected at weekly intervals from 17th to 72nd week in both the groups by brachial venipuncture. Plasma was separated and stored at -20°C for analysis of prolactin (Scanes *et al.*, 1976), estradiol and progesterone (Hall and Sufi 1981) by RIA method. Chicken prolactin antisera, pure hormone were procured from Dr. A.F. Parlow, USA, estradiol and progesterone antisera were provided as a gift from Dr. G.D. Niswender, Colorado., USA. The labeled hormone for estradiol and progesterone were purchased from M/s. Amersham, U.K.

Statistical analyses: All measurements were given as mean ± SE and F test was applied to analyze the significance of differences between means. To study the influence of the hormones on egg production and prolactin, estradiol and progesterone were subjected to correlation coefficient analysis. Differences were considered significant at a value of p<0.01.

Results

Birds in the two groups started to lay eggs by 19th week of age. The mean age at first egg was around 140 days in the two groups. Bromocriptine treatment did not change the age at first egg but an increase in the number of laying days in treated birds was observed compared to the control birds. (Table 1). Mean pause days were significantly (P<0.01) higher in control birds (9.48 days) compared to treated birds (8.47 days). Egg sequences of more than 46 egg were recorded in treated birds as against 28 eggs in control birds. The mean sequence length was 4.37 ± 0.30, 2.14 ± 2.14 days in treated and control group respectively with a maximum sequence length of 46.25 ± 1.32, and 28.42 ± 1.02 days in treated and control respectively (Table 1). Supplementing the bromocriptine in minute doses through feed (in treated group) resulted in significant (P<0.01) increase in percentage of egg production by 5.12% with concomitant decrease in intersequence

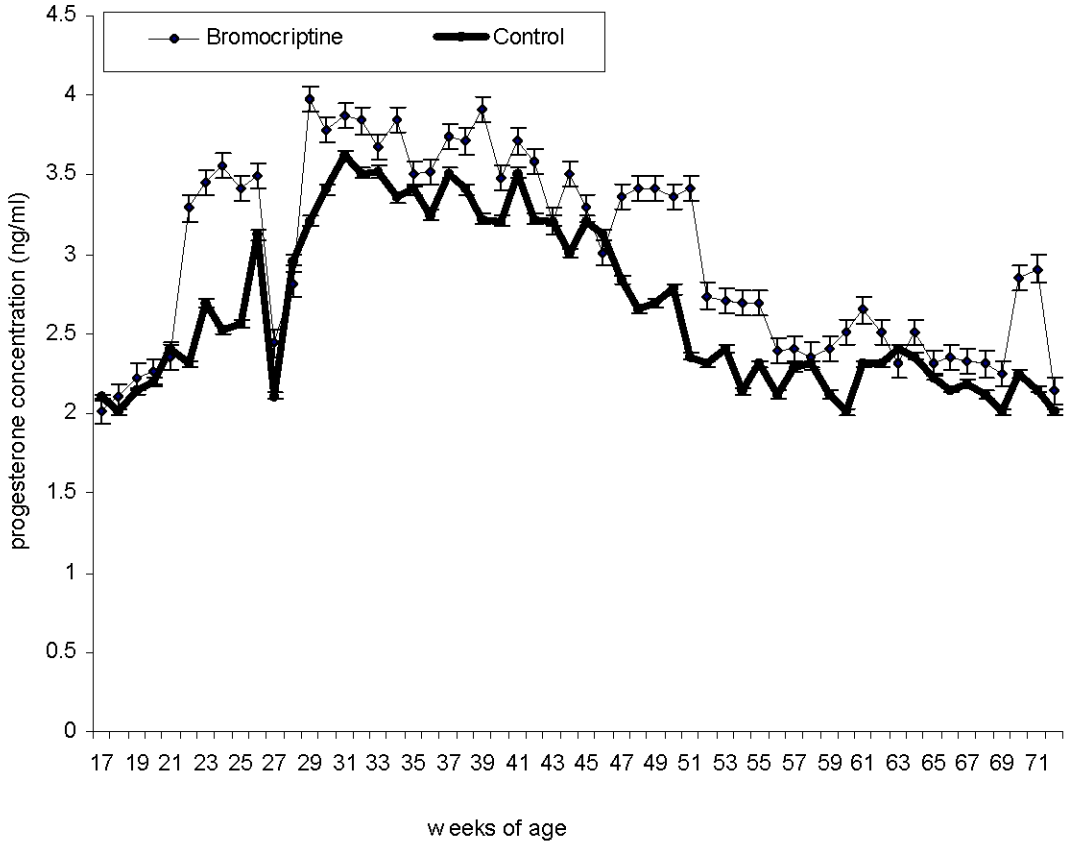

Fig. 3: Plasma Progesterone concentration in control & treated birds. Treated birds showed significantly (P<0.01) higher concentration over control birds

pause days in treated birds over the control birds (Fig. 4, 5). Bromocriptine treatment (from 17 to 36 weeks) resulted in significant decrease in the plasma prolactin concentration (Fig. 1) and this decrease in prolactin concentration is persistent during and even after withdrawal of bromocriptin through feed. Prolactin levels were significantly higher in control birds compared to treated birds. During peak egg production, the circulatory levels of PRL were lower in both the groups. Plasma estradiol level in control group of girirani birds of varied between 250 pg / ml to 350 pg / ml during 17th to 72 weeks week of age (Fig. 2) as against treatment group from 265 pg /ml to 380 pg/ml. The progesterone concentration in the two groups followed a similar pattern as estradiol (Fig. 3) However, intermittent hormonal fluctuations were observed in both control and treated groups. Egg production, was positively correlated with estradiol (r = 0.89) and progesterone (r = 0.87) where as prolactin level is negatively correlated with egg production (r = -0.89) estradiol (r = -0.59) and progesterone (r = -0.37).

Discussion

Egg production in birds declines with increasing concentration of prolactin. Further, elevated levels of prolactin in birds plays a negative role on reproductive performance and decreases the egg sequence lengths (clutch length) by increasing the intersequence pauses between the sequences of egg lay in native birds. This is particularly pronounced in native breed of birds and control of prolactin secretion during the early age of productive period, decreases prolactin concentration later in the productive period (Reddy *et al.*, 2002) with concomitant increase in egg production by decreasing the intersequence pause days. However, the physiological mechanism involved in inter sequence pauses (taking pauses between the sequences of egg lay) is not fully explained. Present study provides new information to establish whether declining in egg production (due to more pauses) is correlated to plasma concentrations of prolactin, estradiol and progesterone or due to genetic composition of girirani birds, is not known but appears that, it is partly related due to genetic potential and physiological constraints. Feeding of bromocriptin through feed to girirani birds from 17th to 36th week of age during early in the productive period associated with an increase egg production (Table 1), estradiol (Fig. 2) and progesterone (Fig. 3) with concomitant decrease in plasma PRL concentration (Fig. 1) indicates the major role physiological factors

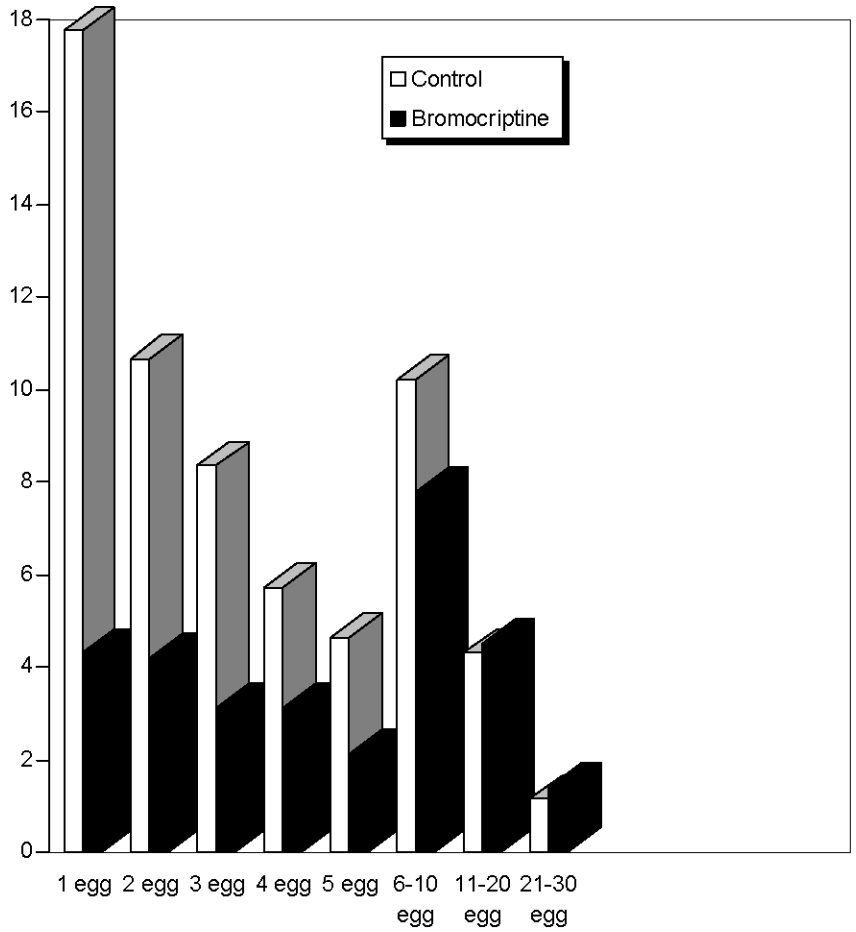

Fig. 4: Fluctuations in the sequence of egg production between the bromocriptine treated girirani breed of hens and untreated control hens. (Means ± SEM).

like, (endogenously secreted hormones) compared to genetic composition of birds. This is further concurred that, dopamine a neurotransmitter stimulates the prolactin secretion from anterior pituitary gland (Youngren *et al.*, 1998). Prolactin concentration is mainly due to the stimulatory action of Vasoactive intestinal peptide [a PRL releasing hormone through (D_2) receptors], at hypothalamic level. Bromocriptine acts as a dopamine agonist, inhibits the prolactin secretion. Due to feeding of bromocriptine, the reproductive system of girirani hen changed from low functional sate to a high functional state during 17 to 72 weeks of age. Within 3-4 weeks of weeks, estradiol and progesterone increased significantly following the treatment. Action of bromocriptine is attributed to lowering prolactin concentration and low levels of prolactin increases progesterone levels by inhibiting 20α-hydroxysteroid dehydrogenase enzymes, which catabolizes progesterone to 20α - dihydroprogesterone (Veldhius *et al.*, 1981; Wong *et al.*, 1979). Stimulating precursors, enzymes and receptors required for estradiol synthesis at ovarian level elevate estradiol concentration in treated birds. These changes are due to low concentration of prolactin by the actions of bromocriptine at ovarian level which is further confirmed by other studies (McNeilly *et al.*, 1982) that, infusion of PRL into the ovarian arterial circulation decreased the steroid secretion. Estradiol is essential for initiation of the vitellogenic stage of ovarian follicular, oviduct development. Low concentration of steroid hormones in controls is due to interference of high prolactin concentration attributed to one of the reasons for lower egg production in control birds. Initiation of oviposition is associated with increase in estradiol, progesterone and low levels of prolactin, even native birds. It clearly indicates control of prolactin enhances egg production.

Age at first egg: In both the groups, the age at first egg production is more are less similar. First oviposition is mainly dependent upon external stimuli, the genetic constitution of individual birds in addition to lighting schedule. Role of lighting schedule on age at oviposition

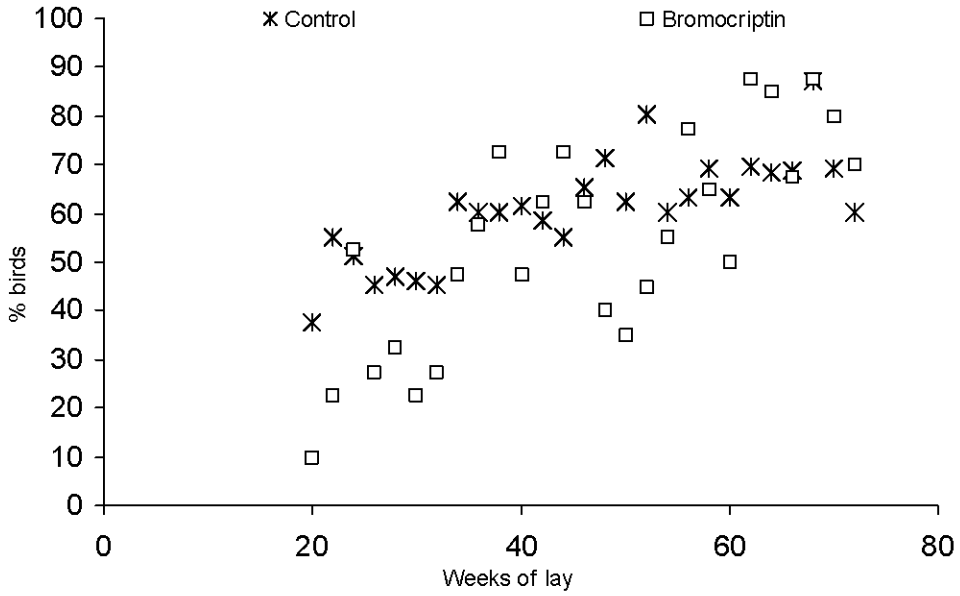

Fig. 5: Percentage of hens experiencing pauses through out the productive period in girirani hens, which were fed bromocriptine through feed and controls.

is not fully known (Robinson *et al*., 2001). In this study, birds were maintained under normal husbandry conditions and hence the light might not have played a major role on age at first egg. Hence, bromocriptine treatment through feed did not alter age at sexual maturity.

Egg sequence: Feeding of bromocriptine to birds resulted in increased number of egg laying days compared to control birds and the same has been proved in our earlier studies (Reddy *et al*., 2001; 2002) that, increased prolactin concentration above physiological ranges implicated in low secretion of circulating gonadotrophins, ovarian regression and the shift from egg laying to incubation phase of reproductive cycle in hen (Crisostome *et al*., 1998). This is further supported by the findings of Ogawa *et al*. (1977) that intravenous injection of mammalian prolactin to hens 6-7 hours before expected second ovulation blocks the second ovulation but not when given 5 or 8 –14 hours before second ovulation. In this study, we have observed that reduced laying pauses, and longer sequences in birds fed with bromocriptine were mainly due to decreased concentration of prolactin in treated birds over the control birds. 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 (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 this study and in our earlier studies we observed a negative correlation between prolactin with progesterone and estradiol (Reddy *et al*., 2002) which support the earlier reports.

Laying pauses: Intersequence pause length advances as the bird ages and this is mainly due to longer duration of LH surges in older birds. Longer intervals of LH duration and higher levels of prolactin are the factors that play a role in longer intervals of pause days in birds. In this study, also, as bird advances with age, the occurrence of pauses of 2 days duration might be the consequence of reduced rate of follicular maturation and its subsequent recruitment into the hierarchy following ovulation that is partly regulated by FSH (Etches and Cheng, 1981). Prolactin at high levels suppresses the FSH induced estradiol production through 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 and progesterone in plasma of birds treated with bromocriptine compared to those of the control birds (Reddy *et al*., 2002). Further in support of our statement that modulation of prolactin using bromocriptine through feed at minute quantities to over come the inhibitory effect of prolactin on follicular development and subsequent oviposition effectively. We

Table 1: (Mean±S.E.) Egg production, egg sequence and pause days between control and bromocriptine fed birds from 19th to 72nd weeks of age in giriranibreed of birds a,b Means having at least one common superscript do not differ at 1% level (P<0.01)

	Birds fed bromocriptine through feed	Control birds
No. of birds	21	21
Age at first oviposition	139.90^{a}±2.24	142.02^{b}±1.29
No. of days	362	362
Number of laying days	184.08^{a}±1.32	162.85^{b}±1.38
Total number of sequences	31.59^{a}±1.89	61.76^{b}±1.95
Maximum sequence length (days)	31.08^{a}±3.98	29.12^{b}±0.95
Mean sequence length (days)	4.37^{a}±0.32	2.14^{b}±0.95
Mean pause length	8.47^{a}±2.28	9.48^{b}±4.91
Total Pause days	178.01^{a}±2.05	199.15^{a}±0.192
Percentage of egg production	50.10^{a}±0.36	44.98^{b}±0.01
Difference in percentage of egg production	5.12	

NS Non Significant.

observed at necropsy that ovaries of bromocriptine treated birds had more number of yellow yolk follicles compared those of the control group. This explains the cause for longer sequences and reduced laying pauses in the treated birds. Yet the occurrence of more than 8 days of laying pauses in birds of both groups may be due to the genetic constitution of individual birds.

In recent past, the mechanism responsible for ovulation and its failure, which lead to skipped days has been much studied but little clarified even in genetically superior birds. Still the role of prolactin in occurrence of broodiness in turkey and bantam hens was well clarified and it was not extended to laying chicken in relation to the laying pauses in between clutches, that has been emphasized in this study. In the present study, the feeding of bromocriptine through feed 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 by Guemene 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 first 10 weeks of the laying cycle may provide productive information for future changes in the physiological status. It is conclude that the physiological pauses that occur during ovulatory sequences can be disrupted effectively using bromocriptine, which modulates prolactin levels, that may interfere with the follicular recruitment and subsequent oviposition thereby improving egg production in girirani birds thereby improve egg laying potential of the bird.

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 Dr. G.D. Niswender for providing etradiol and progesterone antisera.

Reference

Blake, A.G. and R.K. Ringer, 1987. Changes in ring-necked pheasants (*Phasianus colenicus*) egg formation time oviposition lag time and egg sequence length due to adhemeral light- dark cycles. Poult. Sci., 66: 231-236.

Crisostome, S., D. Gumene, G.M. Mills, C. Morvan and D. Zadworny, 1998. Prevention of incubation behaviour expression in turkey hens by active immunization against prolactin. Theriogen, 50: 675-690.

Dajee, M., G.H. Fely and J.S. Richards, 1998. Stat 5b and the orphan nuclear receptors regulate expression of the alpha 2-macroglobulin (alpha 2M) gene in rat ovarian granulosa cells. Mole. Endocrinol., 12: 1393-1409.

Dorrington, J. and R.E. Gore-Langton, 1981. Prolactin inhibits oestrogen synthesis in the ovary. Nature (Lond.), 290: 600-602.

Etches, R.J. and K.W. Cheng, 1981. Changes in the plasma concentrations of luteinizing hormone, progesterone, oestradiol and testosterone and in the binding of follicle stimulating hormone to the theca of the follicles during the ovulation cycle of the hen (*Gallus domesticus*). J. Endocrinol., 91: 11-22.

Guemene, D. and J.B. Williams, 1994. Relationships between broodiness expression, laying persistency and concentrations of hormones during the first productive period in turkey hens. Reprod. Nutr. Devt., 34: 371-381.

Hall, P.E. and S.B. Sufi, 1981. Programme for the provision of matched assay reagents for the RIA of hormones. In reproductive physiological method manual, Geneva, Switzerland.

Hoffmann, B., D. Schalm, R. Bopp, M.L. Ender, T. Gimenez and H. Karg, 1974. Luteotrophic factors in the cow, Evidence for luteinizing hormone rather than prolactin. J. Reprod. Fertil., 40: 77-85.

McNatty, K.P., J.G. Bennie, W.M. Hunter and A.S. McNeilly, 1975. Antibodies to gonadotrophins and the subsequent rate of progesterone secretion by human granulosa cells *in vitro*. Clin. Exp. Immunoreprod., 3: 41-66.

McNeilly, A.S, G.Anna, J. Julie P.W. Howie, 1982. Evidence for direct inhibition of ovarian function by prolactin. J Reprod Fertil; 65: 559-569.

Ogawa, K., S. Matsuo and H. Tojo, 1977. Inhibitory effects of prolactin on ovulation and eggshell formation in the hen. Jap. J . Zootechnol. Sci., 48: 341-346.

Picazo, R.A., A. Gonzalez de Bulnes, A. Gomez Brunet, A. del Campo, B. Granados, J. Tresguerres and A. Lopez Sebastian, 2000. Effects of bromocriptine administration during the follicular phase of the oestrous cycle on prolactin and gonadotrophin secretion and follicular dynamics in merino monovular ewes. J. Reprod. Fertil., 120: 177-186.

Reddy, I.J., C.G., David, P.V. Sarma and Khub Singh, 2001. Prolactin hormone and inter sequence pause days in domestic chicken. Vet. Rec., 149: 590-592.

Reddy, I.J., C.G. David, P.V. Sarma and Khub Singh, 2002. The possible role of prolactin on laying performance and steroid hormone secretion in domestic hen (*Gallus domesticus*). Gen. Comp. Endocrinol., 127: 549-557.

Robinson, F.E., R.A. Renema, H.H. Oosterhoff, M.J. Zuidhof and J.L. Wilson, 2001. Carcass traits, ovarian morphology and egg laying characteristics in early versus late maturing strains of commercial egg type hens. Poult. Sci., 80: 37-46.

Scanes, C.G., A. Chadwick and W.J. Bolton, 1976. Radioimmunoassay of prolactin in plasma of domestic fowls. Gen. Comp. Encrinol., 30: 12-20.

Sharp, P.J., M.C. Macnamee, R.J. Sterling, R.W. Lea and H.C. Pedersen, 1988. Relationship between prolactin, luteinizing homone and broody behaviour in bantam hens. J. Endocrinol., 118: 279-286.

Sharp, P.J., R.J. Sterling, R.T. Talbot and N.S. Huskisson, 1989. The role of hypothalamic vasoactive intestinal polypeptide in the maintenance of prolactin secretion in incubating bantam hens. Observations using passive immunization, Radioimmunoassay and immunohistochemistry. J. Endocrinol., 122: 5-13.

Veldhius, J.D., P. Klase and J.M. Hammond, 1981. Divergent effects of prolactin upon steriodogenesis by porcine granulose cells *in vitro*: influence of cytodifferentiation. Endocrinol., 107: 42-46.

Wang, C., A.J.W. Hsuch and G. Erichron, 1980. Prolactin inhibition of estrogen production by cultured rat granulosa cells Molecular and Cellular Endocrinol., 20: 135-144.

Wong, C., A.J.W. Hsuch and G.F. Erickson, 1979. Induction of functional prolactin receptors by follicle stimulating hormone in rat granulose cells *in vivo* and is *in vitro*. J. Biol. Chem., 257: 11330-11336.

Wentworth, B.C., J.A. Proudman, H. Opel, M.J. Wineland, N.G. Zimmermann and A. Lapp, 1983. Endocrine changes in the incubating and brooding turkey hen. Biol. Reprod., 29: 87-92.

Youngren, O.M., Y. Chaischa and M.E. El-Halawani, 1998. Serotogenic stimulation of avian prolactin secretion requires an intact dopaminergic system. Gen. Comp. Endocrinol., 112: 63-68.

Zadworny, D., J.S. Walton and R.J. Etches, 1985. The relationship between plasma concentration of prolactin and consumption of feed and water during the reproductive cycle of the domestic turkey. Poult. Sci., 64: 401-410.