

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

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

Inter Sequence Pause Days, Egg Production, Steroid and Luteinizing Hormone in Domestic Hen (*Gallus domesticus*) Immunized Against cProlactin

I.J. Reddy, C.G. David and S.S. Raju

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

Abstract: Prolactin (PRL) is a versatile hormone synthesized and secreted by the specialized cells in vertebrate anterior pituitary and is one of the important hormone involved in regulating endocrine hormones and egg production in birds. The aim of this study was to lower the cPRL concentration in White leg horn birds (WLH) by active immunization against cPRL during the productive period (from 19-72 weeks of age), for studying and comparing the effects of low prolactin levels on the circulating concentrations of luteinizing hormone (LH), progesterone, estradiol, sequence length, intersequence pause days and egg production with the control birds of the same age group. WLH birds at 13 weeks of age were divided into two experimental groups consisting of 21 birds each in control and immunized birds. At 17th week of age, 21 birds were immunized with cPRL (conjugated with keyhole limpet haemocyanin KLH-cPRL), followed by three booster doses at four weekly intervals with a total of four immunizations. Controls were given placebo in place of immunogen. Active immunization of the birds against cPRL significantly ($P < 0.01$) decreased the circulating PRL concentration. cPRL antibody titers were checked at monthly intervals and found from 20th week of age to age to till 72 weeks of age in immunized birds. Estradiol, progesterone and LH concentrations were increased significantly ($P < 0.01$) during and after the withdrawal of immunizations. Egg production was positively correlated with estradiol-17 β ($r = 0.42$) progesterone ($r = 0.52$) and LH ($r = 0.75$), whereas cPRL level was negatively correlated with egg production ($r = -0.14$), estradiol-17 β ($r = -0.25$) progesterone ($r = -0.32$) and LH ($r = -0.15$). The total number of pause days during the production period decreased significantly ($p < 0.01$) in the immunized group resulting in a 5.40% increase in egg production. It is concluded that there is a consistent relationship between plasma prolactin in the physiological range, intersequence pauses and laying performance in domestic hen.

Key words: Active immunization against cPRL, intersequence pause days, steroid and LH concentration

Introduction

Hen lays an egg a day and continues to lay for certain period of time and takes a gap of one or few days (pause days). The exact physiological mechanism involved in taking pause between the sequences is not fully known. Of late it is attributed that increased concentration of cPRL plays a role in cessation of egg lay and broodiness during the active period of lay (Sharp *et al.*, 1998). cPRL probably acts at all levels of the hypothalamo-hypophysial-gonadal axis and inhibits reproductive function (You *et al.*, 1995). Elevated levels of serum cPRL has a negative effect on reproductive performance resulting in decreased egg production and broodiness in domestic hen and turkey (Sharp *et al.*, 1989). It has been suggested that an increase in the concentration of plasma cPRL during incubation period may depress LH secretion (Sharp *et al.*, 1998), inducing gonadal regression. The secretion of cPRL from the anterior pituitary gland is regulated by dopamine. Dopamine inhibits the stimulatory action of VIP through D₂ receptors, thereby bring about the release of cPRL (Youngren *et al.*, 1998). In the recent past active or passive Immunization against cPRL (Crisostomo *et al.*, 1998) or VIP (Reddy *et al.*, 2006) have been applied to

alter cPRL secretion in birds. But physiological mechanism that is responsible for taking pause days between the sequences of egg lay, intersequence pause length, clutch length in hen is very scanty. Even if a slight increase in clutch length is achieved, it will result into increase in egg production with the available resources and similar managerial practices. This study was carried out to examine the influence of active immunization against cPRL, to extend the clutch length and decrease pause days, to enhance egg production in domestic hen and also to correlate the profiles of steroid, LH hormones with egg production and plasma cPRL levels in birds. This approach is of great practical interest, although their use needs to be carefully evaluated under commercial conditions.

Materials and Methods

Experimental Birds: The study was conducted with 42 White Leghorn birds of strain were housed in individual cages (1'x 1' x 1') from 13 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 NRC recommendations and water was made available throughout the day.

Immunization: Chicken PRL was conjugated by the glutaraldehyde method to Key hole limpet haemocyanin (KLH ; Sigma , USA) by standard method. The first dose of the immunogen containing 125µg cPRL was given as KLH-cPRL conjugate in 1 ml of Freund's adjuvant made up to 2 ml with distilled water. The mixture was emulsified and intradermally injected (@ 2 ml/bird) into the lateral thoracic wall under the wings. Subsequent boosters were given with KLH - cPRL conjugate containing 25µg cPRL in Freund's incomplete adjuvant. Control hens were injected placebo in place of immunogen. Treatment was repeated at four weeks interval with a total of four immunizations (@ 2 ml/bird) spanning between 17th to 32nd weeks of age.

Sampling of blood: The birds were bled on weekly intervals from 13th to 72nd week of age as well as immediately before and 15 days after each immunization. Brachial venous blood (~2 cc) was collected from each bird in heparinized tubes. The blood was centrifuged at 2500 rpm for 15 minutes and plasma was harvested and stored at -20°C until assayed for cPRL, tested for the presence of antibodies for cPRL. Hormone analysis is carried at 16th week of age to 72 weeks of age.

Egg production: Egg production was recorded for each hen at the same time each day for a continuous 378 days period. Egg sequence length and the number of egg sequences were determined from oviposition records following the procedure described 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. 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.

Radioimmunoassay of hormones: The Chicken PRL hormone and antisera used in the assay were procured from NIH, USA. The hormone PRL was iodinated following the procedure of Sharp *et al.* (1989). The assay was carried out following the detailed procedure of Koprowski and Tucker (1971). The antiserum was used at a final dilution of 1:4,00,000. PRL standards ranged between 50 to 1000 ng/ml. 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 6.54% and 8.43%, respectively. Chicken LH was obtained from John. A.

Proudman, USDA as a gift from USA. The intra and inter coefficient variation for cLH was 5.77% and 9.34% respectively with sensitivity of the hormone 0.051 ng/ml per tube as per the method described by (Sharp *et al.*, 1987). Plasma progesterone and estradiol were estimated using RIA following the method described by Hall and Sufi (1981). Intra and inter assay coefficient of variation for oestradiol-17β were 4.76% and 6.22%, respectively and 6.5% and 9.63% respectively for progesterone. Unless specified, reagents were purchased from Sigma -Aldrich Co., USA. Radiochemicals viz. (2,4,6,7-3H) estradiol, 85.0 Ci /mmol and (1,2,6,7 -³H) Progesterone , 93.0 Ci /mmol were purchased from Amersham Life Science, Nycomed Amersham plc ,England, UK.

Titer evaluation: cPRL binding study was carried out as per the method described by Mauro *et al.* (1992). Antibody titers were checked periodically using 125 I- Monoiodinated cPRL. The titres were checked periodically using 125- Monoiodinated cPRL till 72 weeks of age in immunized birds, which gave more than 50% of the binding.

Statistical analysis: Measurements were given as mean ± SE. The significance of differences between means was analyzed by F test. The data on egg production and cPRL, oestradiol-17β progesterone and LH were subjected to correlation coefficient analysis to study the influence of the hormones on egg production. The statistical analyses were carried out following using Microsoft statistical package.

Results

Immunization against cPRL and its effect on cPRL levels: The immune response, measured by the percentage binding of monoiodinated cPRL to plasma at a dilution of 1:1000, with the maximum binding of 16.82 ± 1.63% after the administration of final booster dose at 29th week of age. The plasma samples collected from control birds contained no detectable antibody to cPRL. cPRL concentration in control and immunized birds were estimated using RIA. The concentration of PRL from 16th week of age was increased in both the groups (Fig. 1). Following active immunization against cPRL at 17th week of age, profiles of PRL significantly decreased during and after the immunization till 72 weeks of age, where as PRL levels were higher in the control group throughout the 72-week period. During peak egg production (beginning from 28th weeks of age to 30th weeks) the circulatory levels of PRL were lower in both the groups.

Active immunization against cPRL and its effects on egg production, sequence length and intersequence pauses: Birds in two groups started to lay eggs by 19th

Table 1: (Mean \pm S.E.) Egg production, egg sequence and pause days between control and cPRL immunized birds from 19th to 72nd weeks of age in White Leghorn birds

	cPRL Immunized birds	Control birds
No. of birds	21	21
Age at first oviposition	131.90a \pm 0.24	135.02b \pm 0.19
No. of days	378a	378 a
Number of laying days	284.69a \pm 1.99	264.28b \pm 1.99
Total number of sequences	31.59a \pm 1.89	61.76b \pm 1.95
Maximum sequence length (days)	62.88 a \pm 3.98	21.36 b \pm 5.79
Mean sequence length (days)	7.42a \pm 0.98	3.72b \pm 1.12
Mean pause length	4.44a \pm 0.18	5.40b \pm 0.91
Total Pause days	93.31b \pm 1.98	113.72a \pm 1.92
Percentage of egg production	75.31a \pm 1.89	69.91b \pm 1.77
Difference in percentage of egg production	5.40	

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

week of age. The mean age at first egg was 131.90 ± 0.24 days and 135.02 ± 0.19 days in the immunized and control group respectively (Table 1). Decrease in the age at first egg was observed between the immunized (131.90 ± 0.24) and control birds (135.02 ± 0.19), with a significant increase ($P < 0.01$) in the number of laying days in the immunized birds (284.69 ± 1.99 days) as against control group (264.28 ± 1.99 days). The total number of sequences (31.59 ± 1.89) in immunized birds were significantly lower than the controls (61.76 ± 1.95), with a maximum sequence length of 62.88 ± 3.98 days in immunized birds (continuous laying without pauses) compared to the controls with 21.46 ± 5.79 . Mean sequence length of 7.42 ± 0.98 to 3.72 ± 1.12 days in immunized and control birds respectively. The total numbers of sequences were significantly higher in control group (61.76 ± 1.95) compared to immunized birds (31.59 ± 1.89). Mean inter sequence pause length (skipped days/days without an egg) over a period of 54 weeks was lower in immunized birds (4.44 ± 0.18 days) as against controls (5.40 ± 0.92 days). Significantly ($P < 0.01$) higher percentage of egg production was observed in immunized birds $75.31 \pm 1.89\%$ compared to controls $69.91 \pm 1.77\%$. There is a significant increase in egg production of about 5.40% in immunized birds over controls.

Discussion

The results of the present study show that active immunization of white Leghorn hens with KLH – cPRL was able to prevent the rise in circulating PRL during age at first egg up to peak egg production and increase the egg production potential of the birds till 72 weeks of age, compared to control hens. The effect of immunization against cPRL on decreasing circulatory concentration of PRL is consistent with the endogenous neutralization of excessive cPRL due to presence of antibodies to cPRL supported by other indirect studies (Chen *et al.*, 1997) with a concomitant reduction in plasma PRL and PRL mRNA content after active immunization with cVIP antibodies *in vitro* (which is a potent releaser of cPRL). Direct measurement of cPRL

in the hypophysial portal blood in the turkey demonstrates that the amount present is directly related to concentration of PRL in the peripheral circulation (Youngren *et al.*, 1996). Immunomodulation of circulating PRL release in incubating bird's blocks photo induced PRL secretion and thus improves egg production in turkey hens and in 56-week-old Taihe hens (Chen *et al.*, 1997). This study indirectly supports that active immunization against cPRL resulted in significant ($p < 0.01$) reduction in PRL concentration in the peripheral circulation that sustained throughout the experimental period (Fig. 1.), which supports the earlier findings of El - Halawani *et al.* (1995) in turkey hens that, when turkeys immunized with cVIP conjugated to keyhole limpet haemocyanin showed lower PRL levels compared to control birds.

Age at first egg: There was a significant decrease in the age at first egg between immunized and control birds. It is observed that external stimuli such as supplemental lighting (Robinson *et al.*, 2001) influence the age at first oviposition in addition to the genetic constitution of individual birds. The early age at first oviposition observed in the present study might be due to the synchronized effect of continuous lighting and immunization with KLH-cPRL. This is in conformity with the reports of El- Halawani *et al.* (1995) in turkey hens, where immunization with cVIP and photo stimulation resulted in increased egg production. 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 length: Immunization of birds against cPRL increased the number of laying days in immunized birds compared to the control birds with significantly fewer egg sequences (Table 1 and Fig. 5). Convincing evidence indicates that increased PRL secretion may be the cause of reduced circulating gonadotrophins, ovarian regression and the shift from egg laying to the incubation phase of reproductive cycle in the hen

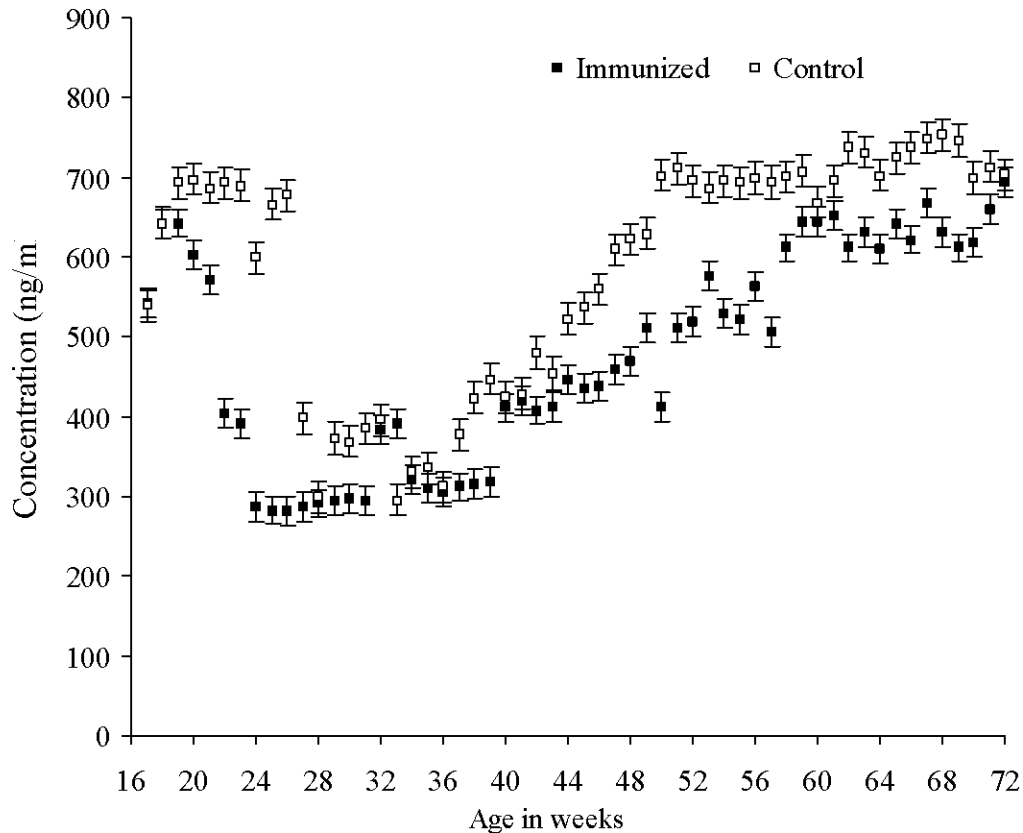
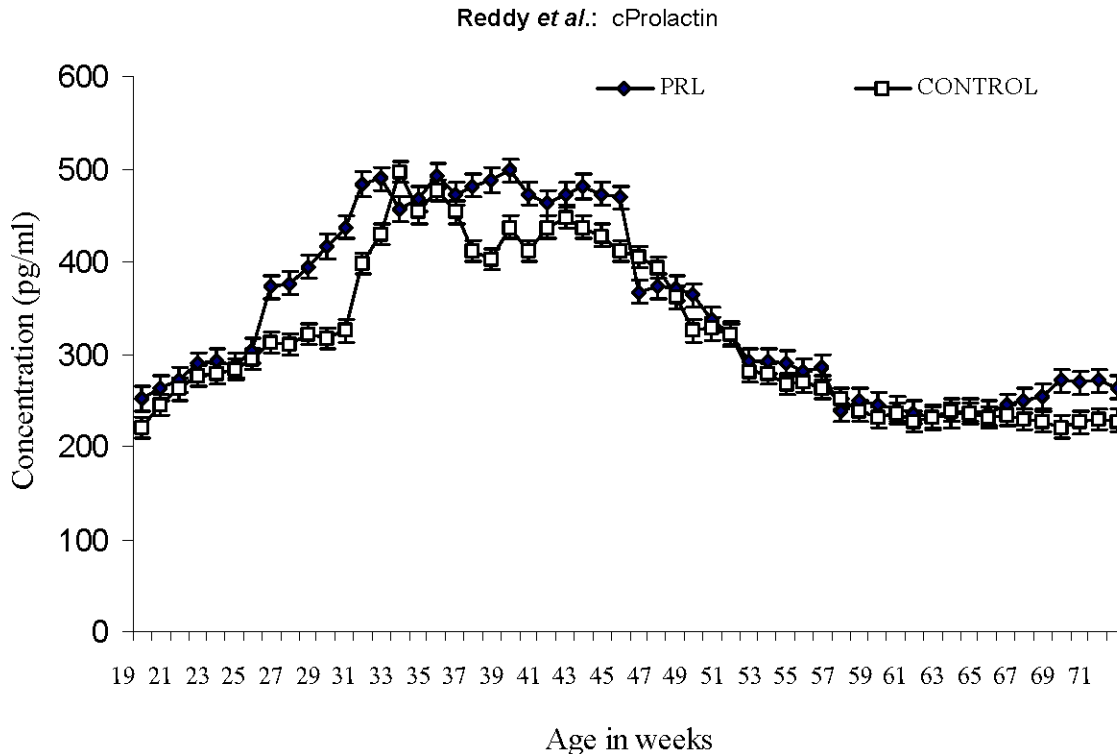


Fig. 1: Plasma PRL concentration (ng/ml) in control (21) and immunized birds against cPRL (n=21). Controls are having significantly ($P < 0.01$) higher levels of PRL over treated birds.

(Crisostome *et al.*, 1998). This is further supported by the findings of Ogawa *et al.* (1977) that intravenous injection of mammalian PRL in hens 6-7 hours before the expected second ovulation, blocks the second ovulation but not when given 5 or 8-14 hours before the second ovulation. In our study we have observed reduced laying pauses and longer sequences in immunized birds, which may be due to the low concentration of PRL, as higher levels of PRL have negative effect on LH, estradiol and progesterone hormones. 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 PRL. At high concentration, PRL 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 PRL even in the presence of adequate amounts of gonadotrophins. In our earlier study using bromocriptine to decrease PRL levels, we observed a negative correlation between PRL with progesterone and estradiol (Reddy *et al.*, 2002) and in this study LH as well, indicates that higher levels of

PRL have negative effect on steroid and gonadotrophic hormones (LH), which are essential for egg yolk synthesis, calcification of egg (estradiol) albumen secretion (progesterone) ovulation (LH surges) and oviposition in domestic hen. Lower concentration of these hormones delays egg formation and oviposition in birds. This may be the reason for shorter sequences of egg lay in controls.

Pause days: Increase in intersequence pause length of more than 3-4 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). PRL 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). In our earlier studies we also observed an increase in the concentration of estradiol and progesterone in plasma of birds treated with anti PRL

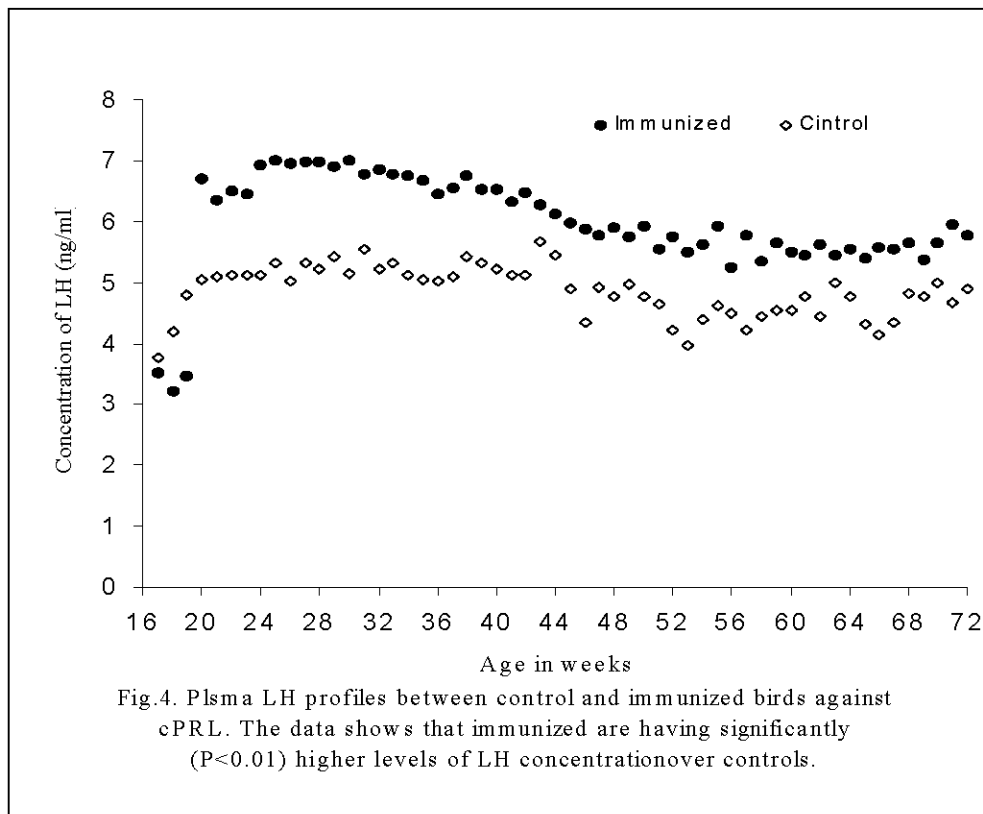
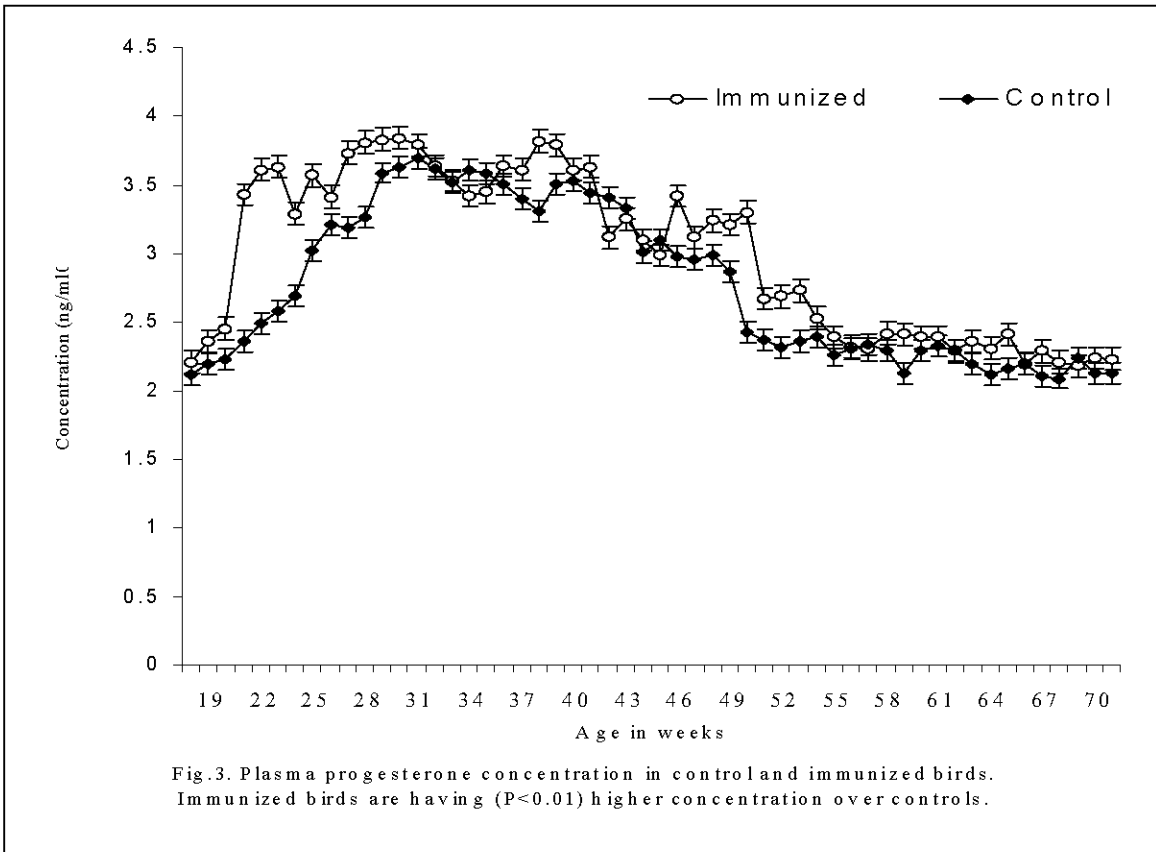


Age in weeks

Fig.2. Plasma estradiol concentration between control and immunized birds against cPRL. Immunized birds showed higher levels of estradiol concentration ($P < 0.01$) over the control birds.

agent (bromocriptine) compared to control birds (Reddy *et al.*, 2002). In support of our statement that modulation of PRL either by using bromocriptine (Reddy *et al.*, 2001) or by active immunization against cPRL in turkeys as observed by El - Halawani *et al.*, 1998, supports the fundamental principle that the high PRL concentration has inhibitory effects on follicular development, subsequent oviposition with significantly higher number of pause days in control birds (Table 1), and control of higher levels of PRL by active immunization against cPRL improved sequence length, reduced the number of pause days, thereby increased the egg production. However, the occurrence of more than 10-15 days of 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 PRL in occurrence of broodiness in turkey and bantam hens is well known and it was not extended to laying chicken, particularly in relation to laying pauses in between clutches, which has been emphasized in this study. The increase in estradiol and progesterone level observed in immunized birds suggested that increased concentration of cPRL might interfere with steroidogenesis pathway. Presence of cPRL receptors in the granulosa cells and their absence from theca cells (Dunaif *et al.*, 1982) suggest that cPRL at high concentration may interfere with the synthesis of

androgens or their subsequent aromatization to estradiol and progesterone (Fortune *et al.*, 1986). This is supported by other studies, wherein infusion of cPRL into the ovarian arterial circulation decreased the progesterone and estradiol secretion, where as administration of anti cPRL agent increased the steroid hormones (McNeilly *et al.*, 1982). Sharp *et al.* (1992) reported that lower plasma concentrations of LH and high PRL levels reduces the pituitary responsiveness to chicken LH to LH releasing hormone (LHRH) in vivo thereby reducing ovulation which may be attributed to increasing the pause days in control hens. This suggests that initiation of oviposition is associated with increase in estradiol, progesterone and preovulatory surges of LH. Similarly, in our study, all these hormones increased in immunized birds after active immunization against cPRL (Fig. 2, 3 and 4) leading to improved egg production. It is logically concluded that, the increase in steroid and peptide is due to the active immunization against cPRL that in turn decreases the circulating concentration of cPRL. Thus, keeping cPRL levels under check can reduce the inter sequence pause days and broodiness in birds thereby increasing egg production. In conclusion active immunization against cPRL 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 Guemene and Williams (1994)



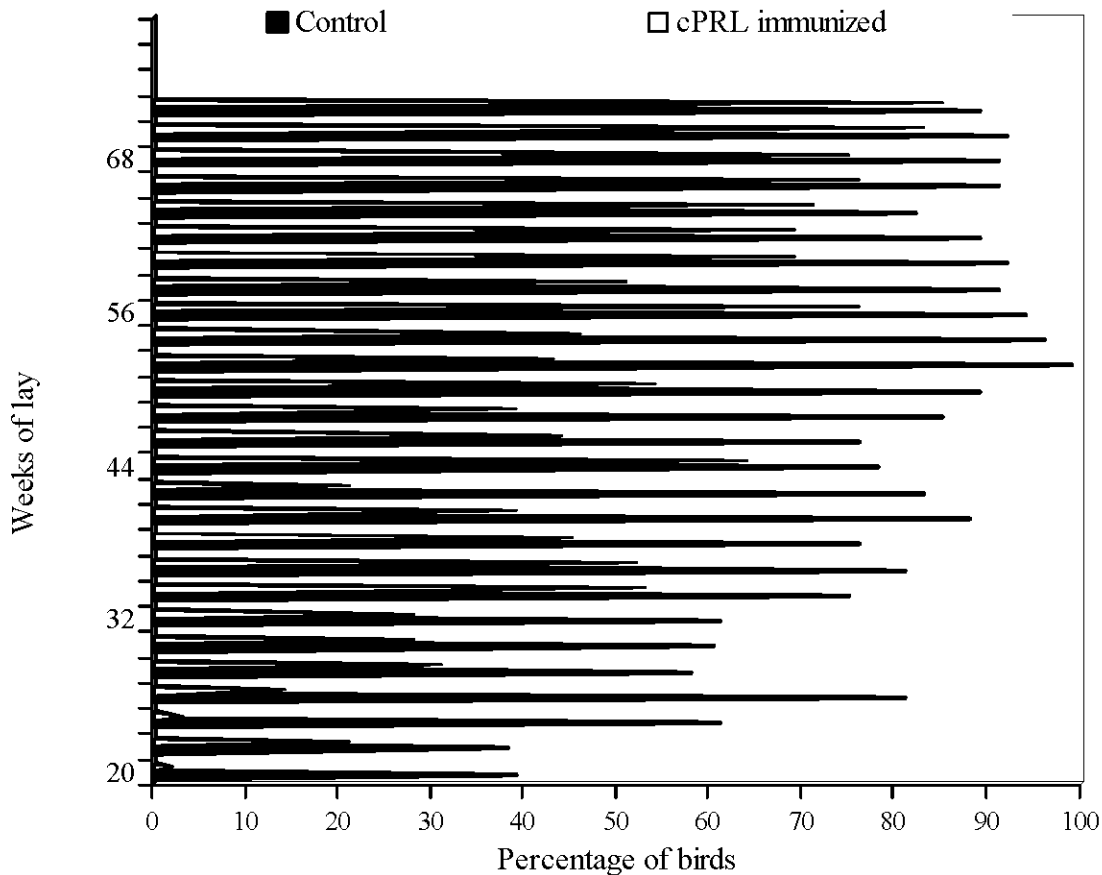


Fig.5. Percentage of birds showing inter sequence pauses in control and cPRL immunized birds.

that low initial concentrations of PRL (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 ten 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 active immunization against cPRL, eliminates the negative effects of PRL on hypothalamo-hypophysial- gonadal axis for higher follicular recruitment and subsequent oviposition thereby improving egg production and improve egg laying potential in White Leghorn hens.

Acknowledgements

We acknowledge the Director, NIANP, Bangalore, India for providing necessary facilities to carry out the work. We acknowledge, NIADDK, USA., for providing cPRL, cPRL antisera., Dr. John. A. Proudman, USDA, USA, and Dr. Prof. G. D. Niswender, Colorado, USA., for generously donating the estradiol and progesterone antisera .

References

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 light- dark cycles. *Poult. Sci.*, 66: 231-236.

Chen, F., Z.D. Shi- Y.Z Bi- and Y.C. Cao, 1997. Effect of active immunization against cVIP on reproductive performance in Taihe hens. *Journal of South China Agricultural University* , 18 (Suppl.) 45-50.

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 PRL. *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- macro globulin (alpha 2M) gene in rat ovarian granulose cells. *Mol. Endocrinol.*, 9: 1393-1409.

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

- Dunaif, A.E., E.A. Zimmerman, H.G. Fresen and A. Frantz, 1982. Intracellular localization of prolactin receptor and Prolactin in the rat ovary by immunocytochemistry. *Endocrinol*, 110: 65-71.
- El Halawani, M.E., J.L. Silsby, I. Rozenboim and G.R. Pitts, 1995. Increased egg production by active immunization against vasoactive intestinal peptide in the turkey (*Meleagris gallopavo*). *Biol. Reprod.*, 52: 179-183.
- El Halawani, M.E., S. Fehrer, B.M. Hargis and T.E. Porter, 1998. Incubation behavior in the domestic turkey: Physiological correlates. *CRC Crit. Rev. Poult. Biol.*, 1: 285-314.
- 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.
- Fortune, J.E., R.N. Wisler and S.E. Vincent, 1986. Prolactin-steroidogenesis by rat granulosa cells II effects on estradiol. *Biol. Reprod.*, 35: 92-99.
- 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.
- Koprowski, J.A. and H.A. Tucker, 1971. Radioimmunoassay of PRL. *J. Dairy Sci.*, 54: 1675-1680.
- Mauro, I.J., O.M. Youngren, J.A. Proudman, R.E. Phillips and M.E. El-Halawani, 1992. Effects of reproductive status, ovariectomy and photoperiod on cVIP in the female turkey hypothalamus. *Gen. Comp. Endocrinol.*, 87: 481-493.
- McNeilly, A.S., G. Anna, J. Julie and 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 cPRL on ovulation and eggshell formation in the hen. *Jap. J. Zootec. Sci.*, 48: 341-346.
- Reddy, I.J., C.G. David, P.V. Sarma and Khub Singh, 2001. cPRL 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 cPRL on laying performance and steroid hormone secretion in domestic hen (*Gallus domesticus*). *Gen. Comp. Endocrinol.*, 127: 549-557.
- Reddy, I.J., C.G. David and S.S. Raju, 2006. Active Immunization Against cVIP and its Role on the Pattern of Sequence Length and Pause Days in Domestic Hen (*Gallus domesticus*). *International. J. Poult. Sci.*, 5: 156-161.
- 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.
- Sharp, P.J., A. Dawson and R.W. Lea, 1998. Control of Luteinizing hormone and prolactin secretion in birds. *Comp. Biochem. Physiol. part C.*, 119: 275-282.
- Sharp, P.J., I.C. Dunn and Cerolini, 1992. Neuroendocrine control of reduced persistence of egg laying in domestic hens: evidence for the development of photo refractoriness. *J. Reprod. Fertil.*, 94: 221-235.
- Sharp, P.J., R.J. Sterling and T.R. Talbot, 1989. The role of hypothalamic cVIP in the maintenance of PRL secretion in incubating bantam hens; observation using passive immunization, radioimmunoassay and immunohistochemistry. *J. Endocrinol.*, 122: 5-13.
- Sharp, P.J., I.C. Dunn and R.T. Talbot, 1987. Sex differences in the LH responses to Chicken LHRH-I and II in the domestic fowl. *J. Endocrinol.*, 115: 323-331.
- Wang, A.J.W. Hsueh and G.F. Erickson, 1980. Induction of functional PRL receptors by follicle stimulating hormone in rat granulosa cells *in vivo* and *in vitro*. *J. Biol. Chem.*, 254: 11330-11336.
- Youngren, O.Y., R. Chaiseha, E. Phillips and M.E. El-Halawani, 1996. cVIP concentration in turkey hypophyseal portal blood differs across the reproductive cycle. *Gen. Comp. Endocrinol.*, 104: 225-230.
- Youngren, O.Y., Y. Chaischa, M.E. and El-Halawani, 1998. Serotogenic stimulation of avian prolactin secretion requires an intact dopaminergic system. *Gen. Comp. Endocrinol.*, 112: 63-68.
- You, S., L.K., Foster, J.L. Silsby, M.E. El-Halawani and D.N. Foster, 1995. Sequence analysis of the turkey LH beta subunit and its regulation by gonadotrophin releasing hormone and prolactin in cultured pituitary cells. *J. Mol. Endocrinol.*, 14: 117-129.