

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

## Immunization Against Vasoactive Intestinal Peptide and its Effects on Prolactin, Steroid, Luteinizing Hormone and Egg Production During Mid Laying Cycle in Punjab Brown (PB3 ) Birds

I.J. Reddy, G. Ravi Kiran, S.K. Mondal and S. Anandan  
Animal Physiology Division, National Institute of Animal Nutrition and Physiology,  
Adugodi, Hosur Road, Bangalore -560 030, India

**Abstract:** Objective of the study is examine the effects of active immunization against chicken Vasoactive Intestinal Peptide (chVIP) on plasma prolactin (PRL) concentration, concentration of luteinizing hormone (LH) profile its interval and duration, Progesterone ( $P_4$ ), Estradiol ( $E_2\beta$ ), intersequence pause days and egg production in birds during the later stages of egg production from 48-72 weeks of egg lay in PB3 birds. Twenty-four PB3 birds of same age group were divided into 2 groups of 12 in each. Birds in control group were administered s/c with placebo. Equal volume of chVIP immunogenic protein was administered to treated group from 17<sup>th</sup> week of age to 36 weeks of age at an interval of 4 weeks. Hormonal profiles of immunized and control birds were quantified at weekly intervals from 48th to 72nd weeks of age in both the groups for prolactin, LH, estradiol, progesterone. Egg production and pause days were recorded in both the groups. At 59<sup>th</sup> weeks of age, blood samples from chVIP immunized and control birds were obtained every 4 h for 48 h to study the surges of LH. In immunized PB3 birds (against chVIP) plasma PRL concentration was lower ( $p < 0.01$ ) with high concentrations of  $E_2\beta$ ,  $P_4$ , LH and its 4 h LH surges in plasma. Significantly ( $p < 0.01$ ) higher egg production (13.62%) and less pause days were observed in chVIP immunized birds. It is mainly attributed due to low PRL concentration, associated with high concentrations of LH (with regular interval and duration of LH surges),  $E_2\beta$  and  $P_4$  concentration required for egg formation and subsequent ovulation. In conclusion, chVIP immunization advanced the LH surges, for release of matured oocyte, egg formation and egg lay enabled the birds to lay eggs at regular intervals due to active immunization against chVIP. Results indicate that control of chVIP may lead to more egg production with shorter duration of LH surges.

**Key words:** chVIP immunization, LH intervals, egg production

### INTRODUCTION

In domestic hen pause days and egg production are inversely related to each other and this problem is more pertinent in native fowls and backyard poultry. Of late it was observed that higher level of prolactin plays a negative role on egg production. Controlling prolactin levels through antiprolactin gave satisfactory results as observed by Reddy *et al.* (2001). Abnormal increase in prolactin concentration plays a role on egg lay in galliforms. Decrease in plasma PRL has been found before and during the preovulatory LH surge (Scanes *et al.*, 1977) but no experiment was conducted experimentally. This versatile hormone (PRL) is also involved in follicular growth in several species, but its role in native hen is very scanty. It is well known that turkeys are more susceptible to broodiness followed by other breeds of native birds may be a jungle female fowl too. Broodiness can be avoided by careful husbandry practices preventing the birds forming an attachment to a nest site, genetic selection etc., which consumes time and labor costs. In this context, it is essential to what is the exact physiological mechanism involved in taking pauses between the sequences of egg lay, broodiness, more pause days and low egg production. Besides

prolactin, LH surges also play a more role in taking pauses between the sequences of egg lay. In this context the present study is aimed to unravel profile of LH during the mid laying cycle and its relationship to steroid, LH surges and egg production. If the exact cause is known through the experiments, it possible to reduce/enhance the certain hormones during embryogenesis by using biotechnological approaches.

### MATERIALS AND METHODS

**Animals:** At the age of 17 weeks, PB3 birds housed in individual cages (one bird per cage) under two-tier battery system and were divided into 2 groups of 21 birds each. Feed was provided as per standard specifications. Same amount of feed was offered in both control and treated groups and feed intakes were not affected by the treatment. Clean water was made available round the clock through out the experimental period. All the birds were maintained under normal husbandry conditions. At 59<sup>st</sup> week of age, birds in chVIP immunized and control groups were bled at four hourly intervals. Daily egg production was recorded for each hen at the same time from 48<sup>st</sup> weeks of age to 72 week of age.

**Collection of blood samples:** Blood samples were collected from each bird by superficial venepuncture of the brachial vein starting from 20th weeks of age onwards at weekly intervals and continued until the end of experimental period at 72 weeks of age. At 48th weeks of age, blood samples from chVIP immunized and control birds were obtained every 4 h for 48 h starting at 6.00 h to study the surges of LH. The sampling took about 1 h and the birds were always sampled in the same order to ensure a period of 4 h between each sample. Lights were off from 10pm to 6am. Plasma was separated and stored at -20°C for hormone assay.

**Analysis of hormones:** Chicken PRL anti serum, chicken PRL iodination grade and pure chicken PRL hormone were obtained from NIADDK., USA. Plasma PRL levels were estimated by radioimmunoassay assay (Kaprowski and Tucker, 1971) using highly specific antiserum to chicken PRL. Intra and inter assay coefficient of variation for PRL were 6.47 and 8.95%, respectively and the sensitivity of the method was 5ng/tube. The antiserum had a specificity of 100% for chicken PRL and less than 1% for chicken growth hormone. Highly purified chicken PRL one ampoule, approximately 100 micrograms was provided and stored in 20-30 micrograms of aliquots. Chicken LH was obtained from John. A. Proudman, USDA as a gift from USA. The intra and inter coefficient variation for chLH was 6.00 and 9.02%, respectively with sensitivity of the hormone 0.021 ng/mL per tube as per the method described by (Sharp *et al.*, 1987). E<sub>2</sub>β and P<sub>4</sub> hormones and chemicals used for RIA of hormones. E<sub>2</sub>β antisera and progesterone antisera in lyophilized form were procured from Prof. G.D. Niswender, Colorado, 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, England, UK. Plasma progesterone and E<sub>2</sub>β were estimated using RIA following the standard method (Hall and Sufi, 1981). Intra and inter assay coefficient of variation for E<sub>2</sub>β were 5.23 and 7.14%, respectively and 5.32 and 9.98%, respectively for P<sub>4</sub> and the sensitivity of the method was 1 pg/ mL for E<sub>2</sub>β and 14 pg/mL for progesterone.

**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 prolactin, E<sub>2</sub>β and P<sub>4</sub> were subjected to correlation coefficient analysis to study the influence of the hormones on egg production. Differences were considered significant at a value of p<0.01. The statistical analyses were carried out following the standard method (Snedecor and Cochran, 1994).

**RESULTS**

Egg production in ch VIP immunized group from 48th to 72nd weeks of age was significantly (p<0.01) higher than the controls (Fig. 1). During this period the average number of eggs laid per bird was 59.97±1.69, 46.35±1.91 in chVIP immunized and control hens respectively. The difference in egg production was significant between 2 groups from 48th weeks of age to 72 weeks of age. Egg production in chVIP immunized group between 48th and 72nd weeks of age, improved by 13.62% over control group. The plasma PRL level in the control group varied between 651.09±0.15ng/mL and in chVIP immunized group to 497.76 ± 2.21 ng/mL during 48 to 72 week of age (Fig. 1). Significantly increased (p<0.01) plasma LH concentration was observed in immunized group over the controls (ranging from 3.12±0.11 ng/mL to 6.47±0.13 ng/mL during 48th to 72 week of age). In control group LH levels were fluctuated at around 3.5-4.0 ng/mL to 5.10 ng/mL. In chVIP immunized group, the increase in LH level was of greater magnitude in chVIP immunized birds 48-72 weeks of age (Fig. 4). Four hourly preovulatory surges of LH in chVIP immunized birds were occurred mostly between the 8 am, 2.00 pm with an highest concentration where as these surges were occurred around 4-6 pm in the controls with an highest concentration of 6.99 ng/mL (Fig. 4). The plasma E<sub>2</sub>β level in birds of control group varied between 243.97±0.99 pg/mL to 231.09±0.45 pg/mL during 48-72 weeks week of age (Fig. 2). In chVIP immunized group plasma E<sub>2</sub>β increased during 48-72th weeks of age. Similar pattern was observed in progesterone concentration between the two groups (Fig. 3). However, intermittent hormonal fluctuations were observed in both control and treated groups. Egg production, was positively correlated with E<sub>2</sub>β (r = 0.69), P<sub>4</sub> (r = 0.6892) and LH (r = 0.6454), P<sub>4</sub> (r = -0.6513) and LH (r = -0.6988).

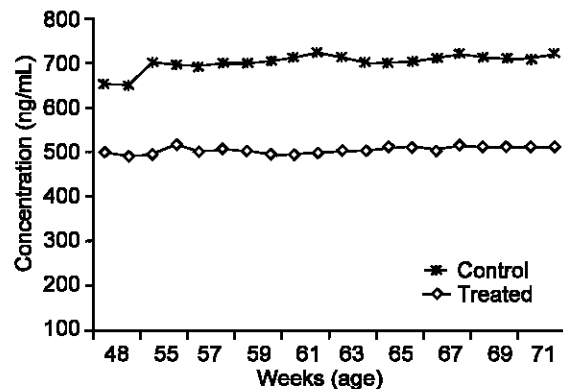


Fig. 1: Plasma prolactin concentration in control and treated (chVIP immunized) birds. Immunized showed lowered (p<0.01) PRL concentration over controls

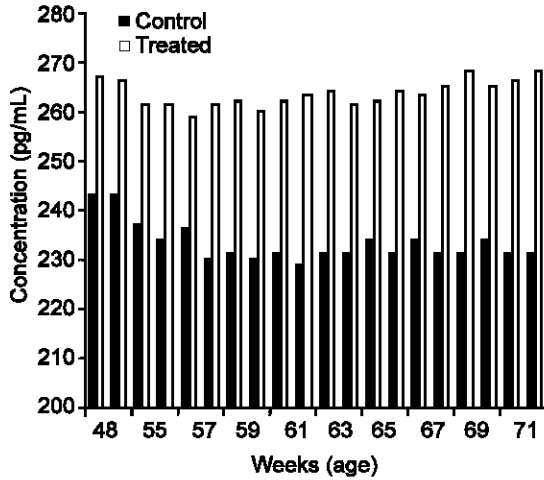


Fig. 2: Plasma estradiol-17b concentration in control and ch immunized birds. Immunized birds showed higher concentration of estradiol over

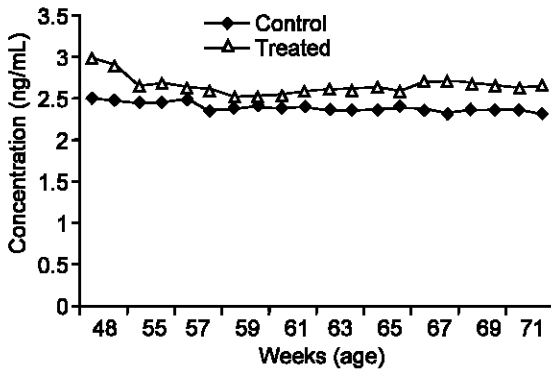


Fig. 3: Plasma progesterone (ng/mL) concentration in control and treated (chVIP immunized). Immunized birds showed significantly ( $p < 0.01$ ) higher concentration over control bird

### DISCUSSION

Egg production is more in chVIP immunized birds compared to controls (Fig. 5). Mechanism in taking pauses between the sequences of egg lay is explained to certain extent (Reddy *et al.*, 2007). This experiment provides new information to establish whether declining in egg production with reproductive status in birds is correlated to plasma concentrations of PRL, LH,  $E_2\beta$  and  $P_4$ . Birds immunized against VIP showed an increase egg production with significant ( $p < 0.01$ ) increase in  $P_4$ ,  $E_2\beta$  and LH concentration and significantly ( $p < 0.01$ ) plasma PRL concentration. It has been reported that, the secretion of PRL from the anterior pituitary gland is regulated (Youngren *et al.*, 1998). Active immunization of chicken vasoactive intestinal peptide (VIP), a PRL releasing hormone decreased the PRL secretion (El-Halawani *et al.*, 1990). Productive system of

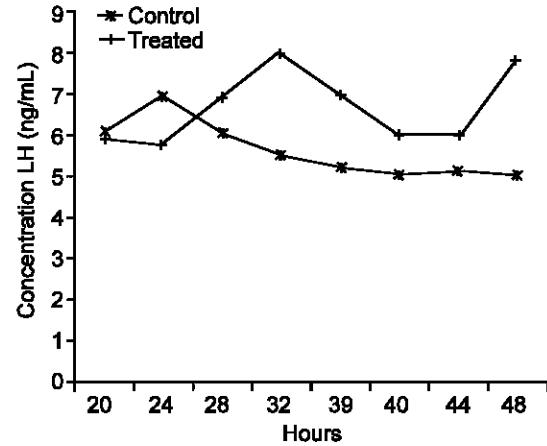


Fig. 4: Hourly (at 4 h intervals) Plasma luteinizing hormone concentration between control and treated birds. Treated birds showed 2 peaks of LH while control had one LH peak

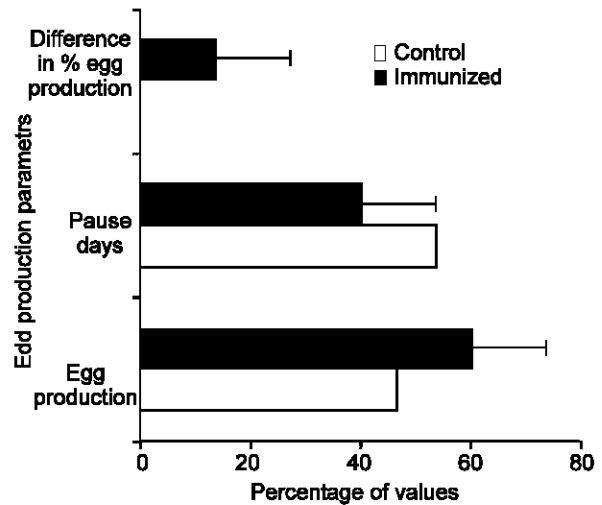


Fig. 5: Egg production parameters between control and Immunized birds showed significant ( $p < 0.01$ ) increase control birds

domestic hen remained same to a high functional state during 19-36th weeks of age following chVIP immunization. Elevated levels of plasma concentrations of  $P_4$ ,  $E_2\beta$  and LH were observed by inhibiting steroid dehydrogenate enzymes catabolizing  $P_4$  to  $20\alpha$ -dihydroprogesterone (Vildhius *et al.*, 1981) and  $E_2\beta$  levels by stimulating precursors, enzymes and receptors required for  $E_2\beta$  synthesis at ovarian level (Fortune *et al.*, 1986). This is further supported by other studies (McNeilly *et al.*, 1982) that, infusion of PRL into the ovarian arterial circulation decreased the steroid secretion. Further,  $E_2\beta$  is essential for initiation of the vitellogenic stage of ovarian follicular and oviduct

development, while  $P_4$  is essential for albumen secretion and egg formation (Chapman *et al.*, 1994). The possible effects of the concentration of LH on egg production remains unknown, but high concentration of LH has previously been shown to be associated with initiation of egg production (Liu *et al.*, 2002). Plasma concentrations of LH declined during laying period of hens (controls) coincident with advancing age, decreasing egg production and increasing intersequence pause days in controls. The decline in egg production was strongly related to more pause days, an increase in plasma PRL concentrations and decreases in LH and steroid hormone concentrations.

Additionally, the duration of one ovulation cycle was prolonged due to aging of the birds and this may explain the increase of pause days. Blocking of prolactin with chVIP immunization enhanced reproductive performance in laying hens by reducing the number of pause days and shortening of the ovulation cycle, which was reflected in hormonal parameters. The longer intervals and more pause days suggest that the synchronization between follicular development and LH peaks during the ovulation cycle is not 'optimal' in control birds, but that this can be improved by blocking PRL in this study in chVIP immunized birds. 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 after chVIP immunization. These observations are based on weekly blood samples taken during the active immunization of birds against chVIP. However, 4 h intervals of blood sampling (for LH surge intervals and duration, for 48 h) during middle of the treatment, showed variation in LH surge intervals and frequency of LH surges to within 24 h (but not more than 30 h with more number of incidences of LH surges) in treated hens as against >38 in control birds. Further, longer interval of LH surges of >30 h are associated with more intersequence pause days in controls over treated hens. Active immunization of hens against chVIP had a decrease in LH surge intervals (24 h), with more number of incidences of LH surges and over all increase in LH concentration (coupled with priming of estradiol and  $P_4$  for eliciting LH responses) resulting in increase egg production by 13.62% (Fig. 5). Thus, keeping the PRL concentration under check increases egg production by decreasing the inter sequence pause days in birds. Longer intervals between preovulatory surges of LH during the egg production are associated with decline in egg production in controls. Our results clearly indicate the importance of active immunization against chVIP on PRL concentration, on preovulatory LH surges, its duration, steroidogenesis, incidence of intersequence pause days and egg production in domestic hen during the reproductive period. In conclusion, several factors may contribute to low egg production during the active period

of egg lay in hen. These include, decrease in LH surges, longer intervals, resulting in longer intervals between ovipositions, an increase in the incidence of intersequence intervals of oviposition >34 h between clutches of eggs, resulting in a decrease in clutch size, an increase in follicular atresia, resulting in a lower rate of follicular maturation and number of follicles available for ovulation, a loss of synchronization of ovulation and oviposition resulting in an increase in the number of defective eggs late in the reproductive period and abnormal secretion of progesterone, associated with a blockage of LH surge secretion and ovipositions. Decreasing the excessive PRL concentration in birds irrespective of the laying period could ameliorate all these problems for sustainable egg production with available resources under similar managerial conditions with more profits to poultry industry.

#### ACKNOWLEDGEMENTS

The authors are thankful to Director, NIAMP, Bangalore, for providing necessary facilities to carryout the work. We are thankful to Dr. A.F. Parlow, Director, NIADDK, California (USA), providing the chicken prolactin hormone and antisera and Prof. G.D.Niswender, Colorado-USA, for providing the antisera for estradiol and progesterone as a gift for the above study and John. A. Proudman, USDA, USA, for providing chLH hormone and antisera as a gift.

#### REFERENCES

- Chapman, D.P., W.L. Bacon, D.W. Long, K. Kurima and W.H. Burke, 1994. Photo stimulation changes the pattern of luteinizing hormone secretion in turkey hens. *Gen. Comp. Endocrinol.*, 96: 63-74.
- El Halawani, M.E., J.L. Silsby and Mauro, 1990. Enhanced vasoactive intestinal polypeptide induced prolactin secretion from anterior pituitary cells of incubating turkeys (*Meleagris gallapavo*). *Gen. Comp. Endocrinol.*, 80:138-145.
- 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.
- 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.
- Kaprowski, J.A. and H.A. Tucker, 1971. Radioimmunoassay of Prolactin. *J. Dairy Sci.*, 54: 1675-1680.
- Liu, H.K., D.W. Longand and W.L. Bacon, 2002. Interval between preovulatory surges of luteinizing hormone increases late in the reproductive period in turkey hens. *Biol. Reprod.*, 66: 1068-1075.
- 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.

- Reddy, I.J., C.G. David and S.S. Raju, 2007. Effect of suppression of plasma prolactin on luteinizing hormone concentration, intersequence pause days and egg production in domestic hen. *Domestic Anim. Endocrinol.*, 33: 167-175.
- Reddy, I.J., C.G. David, P.V. Sarma and Khub Singh, 2001. Prolactin hormone and intersequence pause days in domestic chicken. *Vet. Rec.*, 149: 590-592.
- Scanes, C.G., P.J. Sharp and A. Chadwick, 1977. Changes in plasma prolactin concentration during the ovulatory cycle of the hen. *J. Endocrinol.*, 72: 401-402.
- 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.
- Snedecor, G.W. and W.G. Cochran, 1994. *Statistical Methods*. 8th Edn. Iowa state Press.
- Veldhuis, J.D., P. Klase and J.M. Hammond, 1981. Divergent effects of prolactin upon steroidogenesis by porcine granulosa cells *in vitro*: influence of cytodifferentiation. *Endocrinology*, 107: 42-46.
- 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.