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 6 (9): 637-641, 2007
ISSN 1682-8356

Prolactin, Luteinizing Hormone and Steroid Hormone Concentration in Punjab Brown (PB3) Birds Immunized Against Vasoactive Intestinal Peptide During the Early Stages of Egg Production

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: This study aims at 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_2ß), intersequence pause days and egg production in birds during the initial stages of egg production from 20-36 weeks of egg lay in PB3 birds. Twenty-four PB3 birds of same age group were divided into two groups of twelve in each. Birds in control group were administered s/c with placebo. Equal volume of chVIP immunogenic protein was administered to treated group from 17th week of age to 36 weeks of age at an interval of four weeks. Hormonal profiles of immunized and control birds were quantified at weekly intervals from 17th to 36th weeks of age in both the groups for prolactin, LH, estradiol, progesterone. Egg production and pause days were recorded in both the groups. At 31st weeks of age, blood samples from chVIP immunized and control birds were obtained every 4 h for 48 hours to study the surges of LH. In immunized PB3 birds (against ch VIP) plasma PRL concentration was lower ($p<0.01$) with high concentrations of E_2ß, P_4, LH and, its 4h LH surges, in plasma ($p<0.01$). Significantly higher egg production (9.71%), 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_2ß 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

The pituitary hormone prolactin (PRL) has long been associated with the expression of incubation behavior with more number of pause days between the sequences of egg lay (Reddy *et al*., 2007). Blood levels of PRL increase six-to 10-fold at the onset of reproductive behaviour. "Our studies have concentrated on understanding the roles of PRL and its effects in avian reproduction at the Steroid, LH hormone, egg production, pause days. "A greater understanding of the endocrinology may provide development of new methods for increasing egg production in dual purpose birds specifically designed for back yard poultry. Poultry is big business in south Asian countries, providing enumerable number of employment to skilled and semi skilled rural youth, associated with various aspects of the industry, contributing close to billions of dollars to the economy. It is the goal of the Avian Physiology researchers to protect the investment, particularly the laying chickens that eventually find their way into our stores and onto our tables. Our role in poultry research is aimed at improving the egg production in cross breed of poultry with least resistance to diseases at rural areas a factor that can contribute least rate of mortality in the birds besides women empowerment to provide the nutritious food to her family. Hence we explored and investigated the use of active immunization against chVIP to enhance egg production in hens meant for dual purpose. Further, the new research means all work should be done locally. At present all the tools are available to understand the biology of the chicken. But certain other conditions are not controlled, like the need to strike a balance between cost and return. Probably these new methodologies may be of more important to get returns more towards the positive side in poultry. Newly developed dual purpose birds, even though domesticated, retain some of their wild instincts, which include the nurturing/nesting instinct; in a rural setting this tends to slow down production. "our objectives are of interest in the biology of maternal instincts," "It is necessarily a priority to increase production, but a backyard bird will stop eating and drinking while incubating its eggs. Our research is to question how the behavior can be modified."Higher levels of prolactin more than six to ten fold are inversely proportional to egg production in birds (Reddy *et al*., 2007). It is mainly due to feedback mechanisms of endogenous hormones. PRL and its secretory hormone VIP are the major

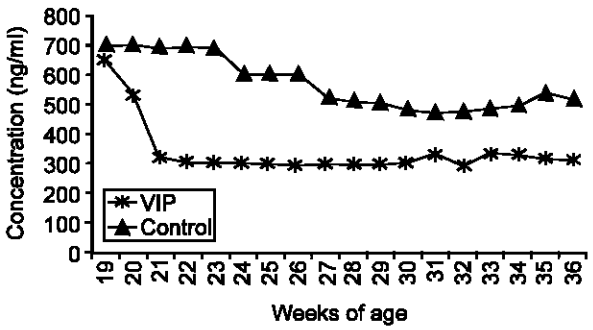

Fig. 1: Plasma prolactin concentration (ng/ml) in control and VIP immunized PB3 bids

limiting hormone in poultry production and control of these factors has a tremendous value in egg production. In dual purpose breeds. It is possible only through active immunization of birds against PRL and VIP may yield better results. Even today, the LH surge intervals and its duration in birds during productive cycle is not well established (Reddy *et al*., 2007). The present study was conducted to investigate the LH surges, its duration, in birds immunized against chVIP during the early stages of productive life. Prolactin secretion is controlled by tonic inhibition via hypothalamus. It is (Harvey *et al*., 1982) reported that control of prolactin release plays a vital role on P_4, E_2ß with meager information on LH surges in domestic hen. It has been reported that active immunization against chVIP 17 to 36 weeks of age decreases prolactin levels with increase in egg production. This study aims at studying the LH surge intervals, PRL, steroid and pause days and egg production during the immunization period from 17 to 36th weeks of age in birds.

Materials and Methods

Animals: At the age of 17 weeks, 42 PB3 birds housed in individual cages (one bird per cage) under two-tier battery system and were divided into two 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 31st week of age the birds in immunized and control group were bled at four hourly intervals. Daily egg production was recorded for each hen at the same time from 21st weeks of age to 36th 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 36 weeks of age. At 31st weeks of age, blood samples from chVIP immunized and control birds were obtained every 4 h for 48h 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 4h 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 6.97% and 9.12%, 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_2ß and P_4 hormones and chemicals used for RIA of hormones. E_2ß 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_2ß were estimated using RIA following the standard method (Hall and Sufi, 1981). Intra and inter assay coefficient of variation for E_2ß were 5.23% and 7.14%, respectively and 5.32% and 9.98% respectively for P_4 and the sensitivity of the method was 1 pg/ml for E_2ß 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_2ß and P_4 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 20to 36 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 79.04±1.54, 70.41±1.01 in chVIP immunized and control hens respectively. The

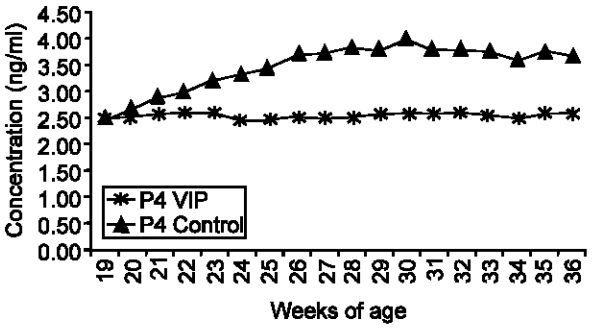

Fig. 2: Plasma progesterone concentration (ng/ml) in control and VIP immunized PB3 birds

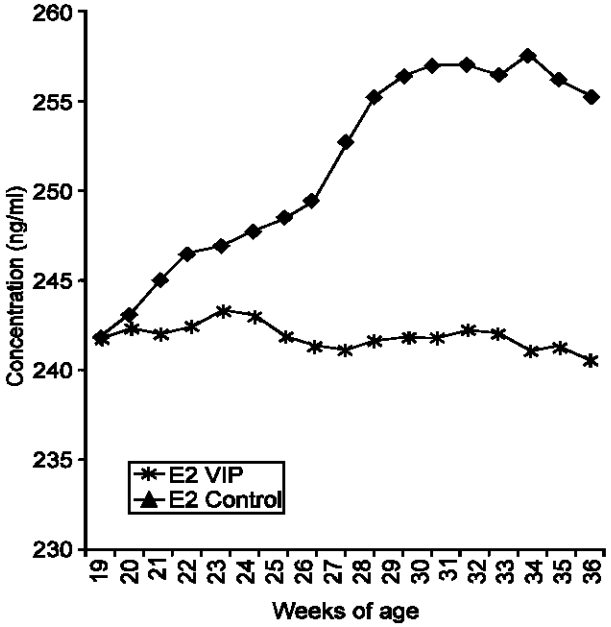

Fig. 3: Plasma estradiol concentration (pg/ml) in control and VIP immunized PB3 birds

difference in egg production was significant between two groups from 20th weeks of age to 36 weeks of age. Egg production in chVIP immunized group between 20th and 36th weeks of age, improved by 9.719% over control group. The plasma PRL level in the control group varied between 649±0.45 ng/ml and in chVIP immunized group to 341.89±3.21 ng/ml during 36 to 46 week of age (Fig. 1). Plasma LH concentration in immunized group varied between 3.50±0.32 ng/ml to 6.94±0.12 ng/ml during 20th to 36 week of age (Fig. 5). Birds immunized against chVIP showed higher concentration of LH plasma LH increased from 4.68±0.12 ng/ml to 5.12±0.02 ng/ml during 20th to 36 week of age. In control group LH levels were fluctuated at around 3.5 to 4.0ng/ml to 5.10 ng/ml, where as in chVIP immunized group the LH levels were significantly ($p<0.01$) higher than the controls. In chVIP

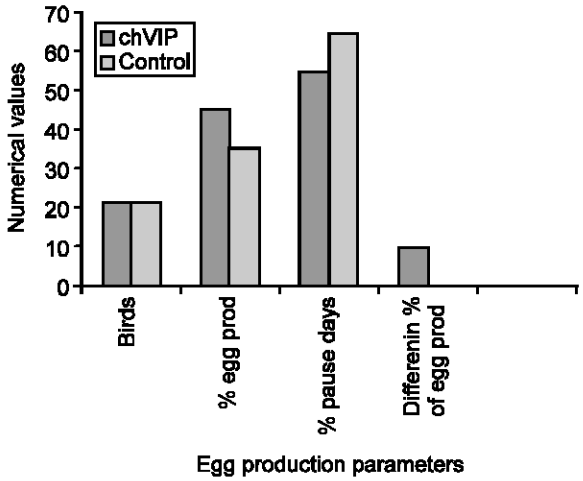

Fig. 4: Egg production, pause days in control and VIP immunized birds

immunized group, the increase in LH level was of greater magnitude in chVIP immunized birds for eleven weeks (Fig. 5). The four hourly preovulatory surges of LH in chVIP immunized birds were occurred mostly between the 8AM 1.00PM with an highest concentration where as these surges were occurred around 4PM to 6PM in the controls with an highest concentration of 5.31ng/ml (Fig. 5). The plasma $E_2ß$ level in birds of control group varied between 245±0.80 pg/ml to 260±0.23 pg/ml during 20 to 36 weeks week of age (Fig. 3). In chVIP immunized group plasma $E_2ß$ increased during 20th to 36th weeks of age. The pattern of progesterone followed the similar pattern (Fig. 2). However, intermittent hormonal fluctuations were observed in both control and treated groups. Egg production, was positively correlated with $E_2ß$ ($r = 0.73$), P_4 ($r = 0.82$) and LH ($r = 0.616475$), P_4 ($r = -0.61$) and LH ($r = -0.77$).

Discussion

Egg production is more in chVIP immunized birds compared to controls (Fig. 4). 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, $E_2ß$ and P_4. Active immunization of birds against chVIP during the initial stages of production associated with an increase egg production with concomitant increase in P4, $E_2ß$ and LH concentration and decrease in 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 domestic hen

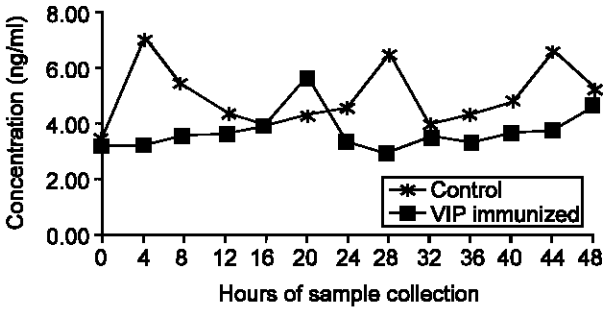

Fig. 5: Plasma LH concentration between control and VIP immunized PB3 birds at hourly intervals

remained same to a high functional state during 19th to 36th weeks of age following chVIP immunization. Elevated levels of plasma concentrations of P_4, E_2ß and LH were observed by inhibiting steroid dehydrogenate enzymes catabolizing P_4 to 20 α-dihydroprogesterone (Vildhius *et al.*, 1981) and E_2ß levels by stimulating precursors, enzymes and receptors required for E_2ß 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_2ß 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 30h with more number of incidences of LH surges) in treated hens as against > 33-38 in control birds (Fig. 5). Further, longer interval of LH surges of >30h 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 hrs), 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 9.71% (Fig. 2). 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 managemental conditions with more profits to poultry industry.

Acknowledgements

The authors are thankful to Director, NIANP, 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.

Harvey, S., A. Chadwick, G. Border, C.G. Scanes and J.G. Phillips, 1982. Neuroendocrine control of prolactin secretion. Aspects of Avian Endocrinology, Practical and Theoretical implications. (eds-I) Texas Tech. Press. USA, pp: 41-69.

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., 2: 167-175.

Kaprowski, J.A. and H.A. Tucker, 1971. Radioimmunoassay of Proalctin. 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.

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.

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.

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.