

EXPRESSION PROFILE OF PROLACTIN GENE IN WHITE LEGHORN LAYER AND CONTROL BROILER

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Abstract– Prolactin is an endocrine hormone involved in controlling broodiness in hens. Prolactin also inhibits egg production in chickens. The objective of the study is to see the prolactin expression profile using RT-qPCR during the 42nd week of egg production in the Indian white leghorn layer (IWI, IWK, and IWD) and control broiler. Five from each group are slaughtered and total RNA was isolated from the anterior pituitary glands and cDNA was synthesized. The RT-qPCR studies revealed Prolactin concentration was highest in control broiler chicken compared to all other groups under study. The lowest Prolactin concentration was observed in the IWD layer compared to IWI and IWK layer strains. It is concluded that the prolactin gene of different chickens under experiment showed the presence of lower expression of the prolactin gene, which correlates with high egg production in layers. This experiment will form a base for further study of prolactin expression and chicken egg production in various breeds of poultry.

INTRODUCTION

Riddle *et al.* (1935) noted that the endocrine hormone prolactin, which is secreted from the anterior pituitary gland, has a part in regulating broodiness. A hen typically produces clutches of eggs, with a gap of one to a few days between each clutch. Birds' anterior pituitaries experience an increase in PRL content and plasma levels as egg laying progresses; these levels reach and are maintained at maximum levels during incubation and then decline after the emergence of young (Sharp *et al.*, 1998; Goldsmith and Williams, 1980; Lea *et al.*, 1981; and Lea and Sharp, 1982). The halt of egg production and broodiness during the active period of lay have been linked to an increase in prolactin concentration (Sharp *et al.*, 1998). It is likely that prolactin inhibits reproductive activity at all levels of the hypothalamic-hypophysial-gonadal

axis (Rozenboim *et al.*, 1993). Domestic hens' reproductive function is negatively impacted by a higher blood prolactin level, which leads to lower egg production and broodiness (Sharp *et al.*, 1988). According to certain research (El HaLawani *et al.*, 1988; Nicholas *et al.*, 1988), a rise in plasma prolactin concentration during the incubation phase may inhibit LH secretion and cause gonadal regression. Dopamine controls the anterior pituitary gland's ability to secrete prolactin. Prolactin is released as a result of dopamine's inhibition of VIP's stimulatory activity through the D2 receptors (Youngren *et al.*, 1998). In birds, the prolactin gene has five exons and four introns on chromosome 2 (Kulibaba *et al.*, 2015). In order to form an understanding of the relationship between prolactin expression and egg production, we examined the levels of prolactin expression in layers and broilers during the 42 weeks of egg production. This was done because the

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layer strains used in the experiment are higher egg producers than broilers.

MATERIALS AND METHODS

Experimental Birds and Anterior pituitary gland collection

The present prolactin expression study was undertaken in five birds of White Leghorn layer strains (IWI, IWK, and IWD) and a control broiler at 42 weeks of age to check the prolactin expression which is maintained at the experimental farm of ICAR-Directorate on Poultry Research, Hyderabad. All the birds are slaughtered in a scientific manner under aseptic condition, with the help of a surgical knife, a jugular vein is pierced, and after 2 min of bleeding, the head is decapitated, and a "T" shape incision was given with the help of a small saw. The brain is exposed and the Cerebral hemisphere and cerebrum are removed to expose the pituitary gland which is located at the base of the brain in Sella turcica. With a sterile surgical blade, sella turcica is removed to expose the pituitary gland. With sterile forceps anterior pituitary gland is removed and placed in RNA lather and kept at -80 °C until the isolation of total RNA. The trial was conducted following the guidelines of the Institute Animal Ethics Committee (IAEC) of the ICAR -Directorate of Poultry Research, Hyderabad.

cDNA preparation and expression studies

The cDNA was synthesized from the total RNA by using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific) which follows a method of reverse transcription using random primers. To the master mix, 2 µg RNA was added in order to make up the final reaction volume of 20 µl. The thermal profile for cDNA synthesis is as follows, 42 °C for 60 min, 95 °C for 2min, and 8 °C hold. The resulting cDNA was then stored at -20 °C.

The gene-specific primers for RT-qPCR were designed based on the coding sequence of the chicken PRL gene (NCBI Accession No. NC_052533.1, Chromosome 2) using DNASTAR

(Lasergene Inc) software and the primers were F: 5' GGCTGTAGAGATTGAGGAGC 3' and R: 5' GCAAAGAGTCTGGAGTCCTC 3'. A thermal cycler by HIMEDIA® Step One Real-Time PCR (Life Technologies) and Maxima SYBR Green/ROX qPCR Master Mix((Thermo Scientific) containing cDNA template 1 µl, SYBR Green ROX 5 µl, Primer: forward 1 µl, Primer reverse 1µl and nucleus free water 2 µl were set in duplicates each containing a final volume of 10 µl was used to perform the RT-qPCR thermal profile (Table 1). All samples of the housekeeping gene GAPDH (GenBank accession no.NC_006088.5) primers F: CTGCCGTCCTC TCTGGC and R: GACAGTGCCTTGAAGTGT were employed for normalization of target gene expression. The specificity of amplification was ensured by performing the melting curve analysis which is given in Table 1.

The C_t value i.e. the threshold value of the target gene (PRL) and the reference gene (GAPDH) for each reaction of qPCR were obtained. By employing the comparative C_t method of relative quantification, the expression levels of the target were thus analyzed in relation to the internal control used, the expression of the target gene was quantified as "n-fold up/down-regulation of transcription". Thus the relative expression of the gene was calculated in comparison with the internal control at each point, i.e. Fold of expression= $2^{-\Delta\Delta C_t}$ Where, ΔC_t - average C_t of reference gene (GAPDH), $\Delta\Delta C_t$ - average ΔC_t of target gene (PRL) - average ΔC_t of calibrator gene.

RESULTS

The expression of the PRL gene was studied in the pituitary gland tissues of female birds during the egg-laying stage. The RT-qPCR amplified products were visualized in 2% agarose gel electrophoresis along with a 100 bp DNA ladder, revealing the PRL gene has amplified and represented by 160 bp and GAPDH with 119 bp shown in Figure 1. The results revealed that the highest expression is seen in lines, IWD had the lowest expression and the expression of the IWD layer is taken as standard for estimating

Table 1. Thermal cycling conditions of qPCR of GAPDH and PRL

Gene	Initial denaturation	PCR stage (40 cycles)		Dissociation/Melt curve		
		Denaturation	Annealing			
GAPDH and	95 °C	95 °C	60 °C	95 °C	60 °C	95 °C
PRL	10 minutes	15 seconds	1 minute	15 seconds	1 minute	15 seconds

the fold change in different breeds. The fold change study revealed the control broiler 2.74 and among commercial layers IWK has 1.14 folds and IWI has 1.31. The fold change and expression of all breeds with mean and standard error (SE) are presented in Table 2.

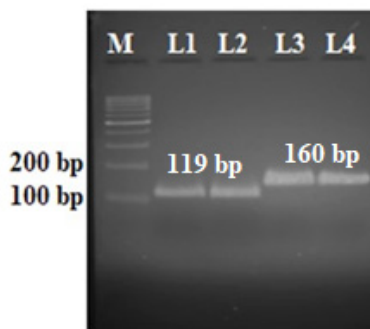


Fig. 1. Agarose gel electrophoresis showing RT-qPCR amplification, Lane M: 100bp ladder, Lanes: L1-L2 GAPDH (119bp), L3-L4: Prolactin PRL (160 bp)

The amplification and the melt curve of RT-qPCR results as shown in Figure 2.

DISCUSSION

Since there are very few reports on RT-qPCR

expression investigations on chicken, comparisons with pertinent studies are done in order to connect the current findings. The current results may be similar to those of Rangneker *et al.* (1978) and Lopez *et al.* (1996) who explained that during incubation, granules and cells in the cephalic lobe grow, which raises prolactin levels in the blood. Prolactin levels decreased after antiprolactin or neutralization treatments performed by Parvez *et al.* (2017), and Dawod *et al.* (2021), who also concluded that prolactin levels are high during the incubation period. These findings may compare with the current findings because layers take fewer breaks than the broilers being studied. Control layers express prolactin at lower levels than commercial broilers, and these results are similar when compared to broiler birds, which lay 150 to 180 eggs annually, and layer birds, which lay about 300 eggs annually (Farooq *et al.*, 2002).

Chu *et al.* (2008), and Eltayeb *et al.* (2010) noted the highest PRL expression during the incubation period. A surge in PRL transcription was observed, with the incubation phase showing the greatest increase (Tong *et al.*, 1997). According to Karatzas *et al.* (1997)'s radioimmunoassay and dot blot hybridization results, mRNA levels in the pituitary

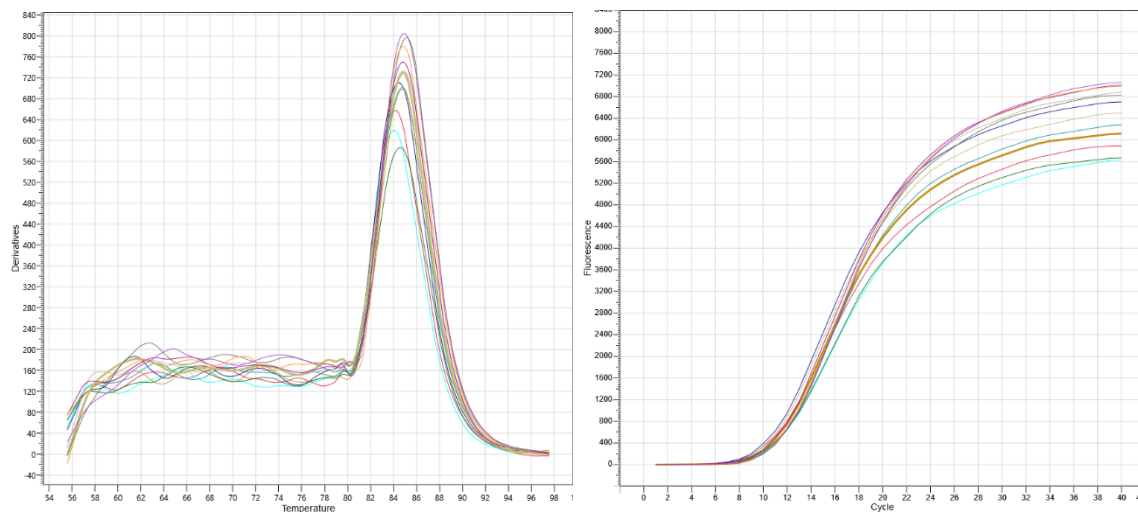


Fig. 2. Amplification curve and Melting curve of RT-qPCR

Table 2. mRNA expression profile of PRL gene in pituitary gland of control layer and control broiler

S. No.	Breed	Mean $C_t \pm SE$		$\Delta C_t \pm SE$	Fold change ($2^{-\Delta\Delta C_t}$)
		PRL gene	GAPDH		
1	IWD female (IWD)	14.30±0.27	19.51±0.18	-5.21±0.43	1
2	Control broiler	14.95±0.53	19.82±0.92	-6.01±1.45	2.74
3	IWI female (IWI)	11.38±0.80	16.62±0.04	-5.24±0.46	1.31
4	IWK female (IWK)	14.75±0.12	20.00±0.44	-5.25±0.46	1.14

gland peaked during incubation compared to the egg-laying and immature groups. Talbot *et al.* (1991) used dot-blot hybridization to quantify the PRL mRNA levels in pituitary glands and found that the incubation chickens had a 3 fold higher PRL level than the laying bird. The investigation found that high egg production in layers may be related to low levels of prolactin expression in the pituitary gland, which in turn affects the ovary. The comparison made here is based on different dimensions, but the experiment's findings are still relevant.

Conflicts of Interest

The authors declare that there are no conflicts of interest

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REFERENCES

- Chu, X. H., Xu, N. Y., Hu, J. P., Lu, L. Z., Chen, W. H. and Wang, Y. Q. 2008. Expression characteristics of prolactin gene in Eastern Zhejiang white geese. *Yi Chuan Hereditas*. 30(8): 1021-1025.
- Dawod, A., Osman, N., Heikal, H. S., Ali, K. A., Kandil, O. M., Shehata, A. A. and Mahboub, H. 2021. Impact of Nano-Bromocriptine on Egg Production Performance and Prolactin Expression in Layers. *Animals*. 11(10): 2842.
- El Halawani, M.E., Fehrer, S., Hargis, B.M. and Porter, T.E., 1988. Incubation behavior in the domestic turkey: physiological correlates. *CRC Critical Rev. Poult. Biol.* 1: 285-31.
- Eltayeb, N.M., Wani, C. E. and Yousif, I. A. 2010. Assessment of broodiness and its influence on production performance and plasma prolactin level in native chicken of the Sudan. *Asian Journal of Poultry Science*. 4(1): 1-6.
- Farooq, M., Mian, M. A., Durrani, F. R. and Syed, M. 2002. Prevalent diseases and mortality in egg type layers under subtropical environment. *Livestock Research for Rural Development*. 14(4) : 1-7.
- Karatzas, C. N., Guémené, D., Zadwomy, D. and Kuhnlein, U. 1997. Changes in expression of the prolactin and growth hormone gene during different reproductive stages in the pituitary gland of turkeys. *Reproduction Nutrition Development*. 37(1): 69-79.
- Lea, R. W. and Sharp, P. J. 1982. Plasma prolactin concentrations in the broody turkey; lack of agreement between homologous chicken and turkey prolactin radioimmunoassay. *British Poultry Science*. 23: 451-459.
- Lea, R.W., Dods, A.S.M., Sharp, P.J. and Chadwick, A. 1981. The possible role of prolactin in the regulation of nesting behaviour and the secretion of Luteinizing hormone in broody bantams. *J. Endocrinol.* 91: 89-97.
- Lopez, M. E., Gregory, C. C. and Porter, T. E. 1996. Cellular basis for elevated prolactin secretion during incubation behaviour in bantam chickens: analysis by reverse haemolytic plaque assay. *Biology of Reproduction*. 54(4): 826-833.
- Nicholas, T.J., Goldsmith, A.R. and Dawson, A. 1988. A Photo refractoriness in birds in comparison with mammals. *Physiol. Rev.* 68: 133-176.
- Parvez, M. M. I. R., Rashid, B., Hasan, M., Mobarak, H., Roy, K. K and Haque, M A. 2017. Effect of serum from laying hen and antiprolactin drug on egg production of indigenous chicken in Bangladesh. *Asian-Australasian Journal of Bioscience and Biotechnology*. 2: 51-54.
- Rangneker, P. V. and Bagalkote, S. G. 1978. Observations on the cyto-morphological changes in the anterior pituitary of the Indian domestic fowl *Gallus domesticus*, during various growth phases including laying and broody periods. *Zoological Journal of the Linnean Society*. 64(4): 331-345.
- Rozenboim, I., Tabibzadeh, J.L. and El Halawani, M.E. 1993. Effect of ovine prolactin administration on hypothalamic Vasoactive Intestinal Peptide (VIP), gonadotrophin releasing hormone I and II content and anterior pituitary VI Preceptors in laying turkey hens. *Biol. Reprod.* 48: 1246-1250.
- sharp, P.J., Dawson, A. and Lea, R.W. 1998. Control of Leutinizing hormone and prolactin secretion in birds. *Comp. Biochem. Physiol. Part C*. 119: 275-282.
- Sharp, P.J., Macnamee, M.C., Sterling, R.J., Lea, R.W. and Pedersen, H.C. 1988. Relationships between prolactin, LH and broody behaviour in bantam hens. *J. Endocrinol.* 118: 279-286.
- Talbot, R. T., Hanks, M. C., Sterling, R. J., Sang, H. M. and Sharp, P. J. 1991. Pituitary prolactin messenger ribonucleic acid levels in incubating and laying hens: effects of manipulating plasma levels of vasoactive intestinal polypeptide. *Endocrinology*. 129(1): 496-502.
- Tong, Z., Pitts, G. R., Foster, D. N. and El Halawani, M. E. 1997. Transcriptional and post transcriptional regulation of prolactin during the turkey reproductive cycle. *Journal of Molecular Endocrinology*. 18(3): 223-231.