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B Rajith Reddy
Research Associate, Molecular Genetics Lab, ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad, Telangana, India

B Ekambaram
Director of Research, PV Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana, India

P Jaya Laxmi
Professor & Head of Department, Animal Genetics and Breeding, College of Veterinary Science, Mamnoor, Warangal, Telangana, India

CH Harikrishna
Professor, College of Veterinary Science, Department of Livestock Production and Management Rajendranagar, Hyderabad, Telangana, India

TK Bhattacharya
Director, National Research Centre on Equines, Hissar, Haryana, India

Corresponding Author:
B Rajith Reddy
Research Associate, Molecular Genetics Lab, ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad, Telangana, India

Expression profile of prolactin in IWI layer and Nicobari at 60 weeks of age

B Rajith Reddy, B Ekambaram, P Jaya Laxmi, CH Harikrishna and TK Bhattacharya

Abstract
An endocrine hormone called prolactin (PRL) aids in regulating the broodiness of hens. Additionally, prolactin prevents poultry from producing eggs. The study's goal is to examine the prolactin expression profile in female Indian white leghorn layer strain (IWI) and Nicobari chicken birds over a period of 60 weeks using RT-qPCR. Three birds from each group are slaughtered, their anterior pituitary glands' total RNA is extracted, and cDNA is produced. The RT-qPCR tests showed that the IWI layer strain has minimal prolactin expression and Nicobari chicken had the highest prolactin content at 60 weeks. It is possible to draw the conclusion that natural chicken strains produce fewer eggs than strains that are chosen for high egg production due to the higher expression of prolactin.

Keywords: IWI layer, Nicobari, Prolactin, Expression

Introduction
The hormone prolactin (PRL), also referred to as lactotropin and expressed in the pituitary gland at the base of the brain, plays a significant function in birds. The first time prolactin hormone was found to cause broodiness behaviour in birds was by Riddle *et al.* in 1935 [1]. Alipanah *et al.* (2011) [2] showed that birds have a single prolactin-encoding gene with five exons and four introns that is located on chromosome 2. The hypothalamus controls tonic stimulatory prolactin (PRL) secretion in avian species. In birds, vasoactive intestinal peptide (VIP), dopamine (DA), and serotonin (5-hydroxytryptamine-5HT) are the main variables that control prolactin secretion. Thought to be the main prolactin-releasing factor, vasoactive intestinal polypeptide (VIP), which is secreted by the hypothalamus, controls secretion by directly interacting with the anterior pituitary gland. The Passive immunization against VIP limits prolactin secretion in incubating chickens, providing evidence that VIP is a physiologically significant avian prolactin-producing hormone (Sharp *et al.*, 1989) [3]. According to Sharp (1997) [4], an increased blood prolactin level impairs domestic hen reproduction by causing gonadal regression, which in turn causes broodiness and lower egg production. Longer ovulatory sequences and increased egg production have been observed in response to prolactin neutralization (Reddy *et al.*, 2007; Badakhshan and Mazhari, 2014) [5, 6]. According to Nicholas *et al.* (1988) [7], the increase in plasma prolactin levels during the incubation period may inhibit LH secretion and cause gonadal regression. In birds, prolactin binding varies seasonally, including during breeding (Smiley *et al.*, 2020) [10], and prolactin responsiveness to prolactin rises during breeding relative to non-breeding individuals of numerous species (Buntin and Buntin, 2014; Smiley *et al.*, 2021) [8, 9]. Therefore, the focus of our current research was on prolactin expression, which may be connected to avian egg production.

2. Material and Methods
2.1 Experimental Birds and Anterior pituitary gland collection
All of the birds were raised in the same shed under strict supervision, receiving ad-libitum watering and feeding. The present prolactin expression study was undertaken in three birds of White Leghorn layer strains (IWI) and Nicobari at 60 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 conditions, with the help of a surgical knife, a jugular vein is pierced, and after 2 min of bleeding, culling was performed by decapitation, and dissection took place immediately after. The whole brain was removed, and the pituitary was retrieved. With sterile forceps anterior pituitary gland is removed and placed in RNA latter and kept at -80°C until the isolation of total RNA.

2.2 Gene expression analysis

According to the manufacturer's instructions, total RNA was isolated from each sample using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA). The cDNA was synthesized from the total RNA by using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific).To the master mix, 2µg RNA was added in order to make up the final reaction volume of 20 µl. The thermal profile for cDNA synthesis is 42 ºC for 60 min, 95 ºC for 2 min, and 8^0C hold. 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) and GAPDH (GenBank Accession No. NC_006088.5) using DNASTAR software and the primers were F: 5' GGCTGTAGAGATTGAGGAGC 3', R: 5' GCAAAGAGTCTGGAGTCCTC 3' and housekeeping gene primers F: 5'CTGCCGTCCTCTCTGGC3' and R: 5'GACAGTGCCTTGAAGTGT3' respectively were employed for normalization of target gene expression. 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: Forward1 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.). The relative expression of the PRL gene was calculated in comparison with the internal control(GAPDH) at each point i.e. Fold of expression = 2-ΔΔCt Where, ΔCt- average Ct of reference gene (GAPDH), ΔΔCt - average ΔCt of target gene (PRL) - average ΔCt of calibrator gene.

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 PRL	95 ºC	95 ºC	60 ºC	95 ºC	60 ºC	95 ºC
	10 minutes	15 seconds	1 minute	15 Seconds	1 Minute	15 Seconds

3. Results

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 (Figure 1) and GAPDH with 119 bp. The results revealed that the highest expression is seen in Nicobari female birds at 60 weeks compared to the IWI layer strain under study. The fold change and expression of all birds with mean and standard error (SE) are presented in Table 2 in which the IWI layer has taken as control due to low expression of PRL gene. The fold change of IWI layer strain was 1 in the IWI layer and 7.1 in Nicobari chicken at 60 weeks of age. The amplification and the melt curve of RT-qPCR results as shown in Figures 2 and 3.

4. 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) [11] who explained that during incubation, granules and cells in the cephalic lobe grow, which raises. prolactin levels in the blood. Crisostomo *et al.* (1998) [12] noted increased egg production for the GST-tPRL immunized hen during the 25th week, while Chu *et al.* (2008) [13], and Eltayeb *et al.* (2010) [14] 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) [15]. According to Karatzas *et al.* (1997) [16] radioimmunoassay and dot blot hybridization results, mRNA levels in the pituitary gland peaked during incubation compared to the egg-laying and immature groups. Talbot *et al.* (1991) [17] 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. Prolactin levels decreased after antiprolactin or neutralization treatments performed by Parvez *et al.* (2017) [18], Dawod *et al.* (2021) [19], 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 other native breeds. 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.

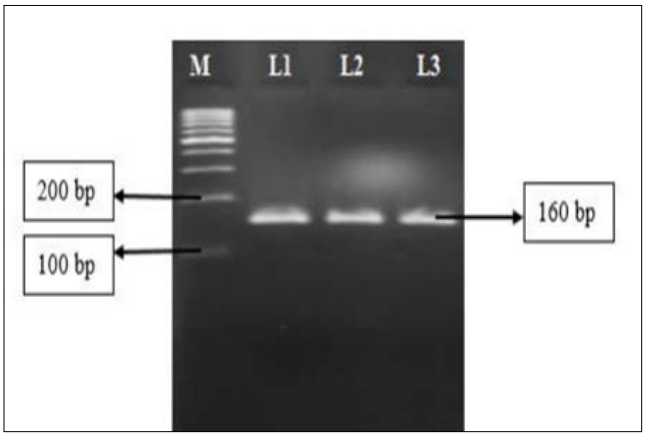

Fig 1: Agarose gel electrophoresis showing RT-qPCR amplification of PRL gene, Lane M: 100bp ladder, Lanes: L1-L3: PRL (160 bp)

Table 2: mRNA expression profile of PRL gene in the pituitary gland of IWI Layer and Nicobari

S. No	Breed	Mean C_t ± SE		Fold change ($2^{-\Delta\Delta Ct}$)
		PRL gene	GAPDH	
1	IWI	12.33±0.38	15.73±0.34	1
2	Nicobari	9.86 ±0.39	16.0±0.15	7.1

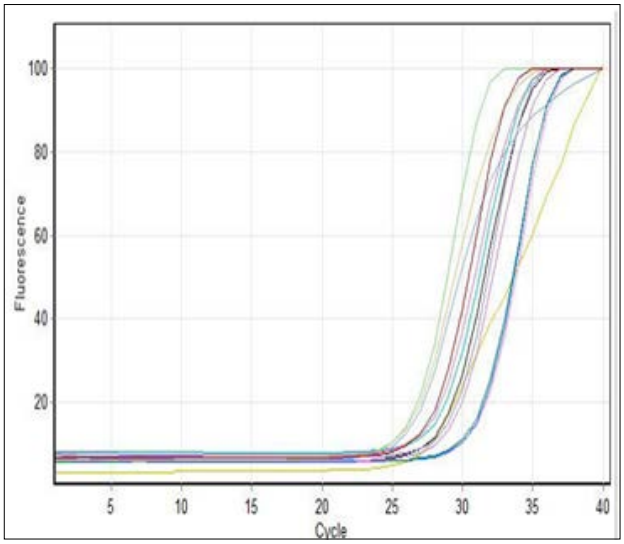

Fig 2: Amplification curve of RT-qPCR

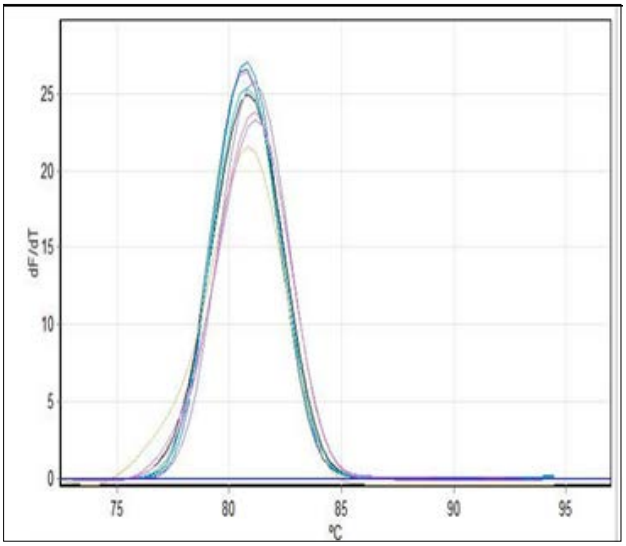

Fig 3: Melting curve of RT-qPCR

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