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Anterior Pituitary Gland Prolactin Expression Profile of IWI Layer and Kadaknath Chicken during 20 and 40 Weeks of Age

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ABSTRACT: A study was conducted to explore mRNA expression profiles of the prolactin gene in the anterior pituitary glands in two varieties of chicken *viz*., IWI layer and Kadaknath birds both at 20 and 40 weeks of age. In the present experiment, the anterior pituitary glands of three birds from each breed were collected and their total RNA was extracted, and further proceeded for cDNA synthesis. The RT-qPCR study revealed that Kadaknath chicken during 40 weeks had the highest prolactin expression, while the lowest prolactin was observed at 20 weeks in the IWI layer among all the groups. Further, the rise in prolactin expression was minimal in the IWI layer between 20 and 40 weeks with a fold change rise of 1 to 1.35. However, the rise of fold change from 1 to 3.5 at 20 and 40 weeks was observed in Kadaknath chicken which indicates that the rise in prolactin expression is high compared to the IWI layer. It has been concluded that the prolactin expression levels in the pituitary gland are higher in the Kadaknath breed than in the IWI layer, and it increases as age progresses in Kadaknath chickens. According to this expression profile, changes in prolactin levels at different ages may be the reason of high egg production in layers compare to native chickens with lower egg production.

Keywords: IWI layer, Kadaknath, Prolactin, RT-qPCR, Expression.

INTRODUCTION

The egg-laying performance of hens is influenced by the external environment and is controlled by endocrine and genetic mechanisms. The main endocrine factors that regulate egg-laying are gonadotropin-releasing hormone (GnRH), prolactin (PRL), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Advancement of molecular biology makes it possible to change the genetic makeup of birds by editing the genes controlling egg production. Prolactin is such a type of gene that is directly involved in egg production of birds by influencing the broodiness trait. The PRLR receptor, a critical component of the PRL signal transduction cascade, mediates all of the prolactin (PRL) hormone's effects. Mammals have a single prolactin-encoding gene (PRL), which is found on chromosome 6 in humans (Goffin *et al*., 1996), on chromosome 13 in mice (Wiemers *et al*., 2003), on chromosome 7 in pigs (Vincent *et al*., 1998) and on chromosome 2 in birds (Alipanah *et al*., 2011). In chicken, it is located on chromosome 2 with five exons and four introns (Kulibaba *et al*., 2015). This gene contains two regulatory regions, the proximal and distal enhancers. Incubation behavior is induced by increased PRL secretion, which commonly results in regression of the ovary and loss of egg production (Nestor, 1980). The hormone performs a wide range of tasks, such as regulating broodiness and ovarian regression (Talbot and Sharp 1994), and it also contributes significantly to the decline in egg production in chickens (March *et al*., 1994). A hen typically produces eggs in clutches, with a gap of one to a few days between each clutch. It has been explained by the fact that an increase in prolactin concentration limits reproductive activity by contributing to the termination of egg laying and broodiness through the hypothalamic-hypophysial-gonadal axis (Sharp *et al*., 1998; You *et al*., 1995; Youngren *et al*., 1993). In domestic hens, an increase in serum prolactin reduces LH secretion, which in turn causes gonadal regression, resulting in decreased egg production and broodiness (Lea *et al*., 1981; Sharp *et al*., 1988; El Halawani *et al*., 1993; Nicholls *et al*., 1988). Dopamine injections lower blood levels of prolactin, which increases egg production concurrently during the treatment period (Reddy *et al*., 2001). At the farm level, the IWI layer strain produces 260–280 eggs per year, while kadaknath produces 160–175 eggs per year. So, keeping this in view, we examined the prolactin expression levels using real time PCR in the female birds of the IWI layer strain and Kadaknath breed. This experiment may serve to analyze the impact of prolactin on egg production between layers and native chickens.

MATERIAL AND METHODS

Experimental Birds and Anterior Pituitary gland Collection. The prolactin expression in chickens is tested using birds reared in the ICAR-Directorate on Poultry Research experimental farm, Rajendranagar, Hyderabad. All the birds were raised in the intensive sheds receiving ad-libitum watering and feeding. The birds were given a comfortable atmosphere and the temperature ranged from 18 to 44ºC. By spraying water on the roof and utilizing the proper illumination, they can function at their peak during hot weather conditions. The present prolactin expression study was undertaken in three birds each of IWI Layer and Kadaknath female birds during 20 and 40 weeks of age. Each bird's jugular vein is punctured, and after waiting up to two minutes for the blood to cease, it is beheaded in a methodical manner under clean conditions. Following the removal of the skull, the pituitary gland was promptly dissected and obtained. The anterior pituitary gland has been separated out using sterile forceps, placed in RNAlater solution, and kept at -80°C until total RNA is isolated.

Gene Expression Analysis. Total RNA was isolated from each individual sample using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. 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°C for 60 min, 95°C 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 (GAPDH) primers F: 5'CTGCCGTCCTCTCTGGC3' and R: 5'GACAGTGCCTTGAAGTGT3' respectively were employed for normalization of target gene expression. A thermal cycler of Step One Real-Time PCR and Maxima SYBR Green/ROX qPCR master mix ((Thermo Scientific) containing cDNA template1 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. The relative expression of the PRL gene was calculated in comparison with the internal control(GAPDH) at each point i.e. Fold of expression= $2^{-\Delta\Delta Ct}$ Where, ΔCt- average Ct of reference gene (GAPDH), ΔΔCt- average ΔCt of target gene (PRL) - average ΔCt of calibrator gene. Thermal cycling conditions of qPCR of GAPDH and PRL were initial denaturation at 95ºC 10 min,40 cycles of denaturation and annealing at 95ºC and 60ºC for 15 sec and 1 min, followed by dissociation/melt curve at 95ºC for 15 sec, 60ºC for 1 Min and 95ºC for 15 Sec.

RESULTS AND DISCUSSION

The RT-qPCR amplified products were visualized in 2% agarose gel electrophoresis along with a 100 bp DNA ladder, revealing the Prolactin (PRL) gene has been amplified and represented by 160 bp (Fig. 1) and GAPDH with 119 bp (Fig. 2). The results revealed that the highest expression is seen in the Kadaknath bird at 40 weeks compared to all other groups under study. The fold change and expression of all birds with mean and standard error (SE) are presented in Table 1 in which the 20 weeks of both the breeds are taken as control due to low expression of the PRL gene. The comparison made between the same breed showed a rise in fold change from 1 to 1.35 in the IWI layer and 1 to 3.5 in Kadaknath chicken at 20 and 40 weeks of age. The amplification and the melt curve of RT-qPCR results are shown in Fig. 3 and 4 respectively.

Because there are few reports of the prolactin gene's mRNA expression, we built a logical argument that focuses on controlling prolactin expression while also increasing chicken egg production. However, our findings and the analysis presented here may be useful in boosting egg production or researching the effects of egg production in chickens, particularly in native chickens. Our findings are quite comparable to Rangneker and Bagalkote (1978) explanation of the increase in prolactin cell granules beginning at 14 weeks and reaching a maximum between 22 and 28 weeks. Additionally, the amount of granules decreased during the course of 36 weeks, although they increased the most during broodiness. This suggests that broodiness is the time of greatest granulation. In the current study, we discover that prolactin expression has increased in both layers and kadaknath chicken between two different ages. The prolactin levels in the plasma of two lines of commercial broiler chickens and two non-selected random-bred broilers were compared (Burke *et al*., 1982), and it was found that the unselected lines had higher levels of prolactin than the selected lines. This suggests that bird selection may be useful in reducing the effect of prolactin. Dot-blot hybridization and its connection with plasma concentrations, which were the focus of a few research on various hybridization techniques and radioimmunoassay to examine the influence of prolactin in the pituitary gland of birds, revealed that incubating hens had higher prolactin levels than laying hens (Talbot *et al*., 1991; Karatzas *et al*., 1997; Edens, 2011). Similarly, reverse hemolytic plaque assay revealed an increase in PRL secretion by inducing incubation behavior in hens (Lopez *et al*., 1996). There are few findings showed a clear increase inprolactin (PRL) mRNA expression during the incubating period (Wong *et al*., 1991; Kansaku *et al*., 1994; Tong *et al*., 1997; Chu *et al*. 2008; Eltayeb *et al*., 2010). The active immunization against prolactin on the expression of incubation behavior in turkey hens showed higher egg production during the 25th week (Crisostomo *et al*., 1998). Hence, this will be helpful to study the increment of egg production in native chickens. Commercial layers lay about 300 eggs annually compared to broilers with fewer eggs because of more expression of prolactin in

broilers compared to layers (Farooq *et al*., 2002). Neutralization experiments with antiprolactin agents showed decreased concentration of prolactin levels (Parvez *et al*., 2017; Dawod *et al*., 2021) and rise in egg production in treated birds compare to control groups (Reddy *et al*., 2002) showed a strong relationship between prolactin levels and the egg production in chicken.

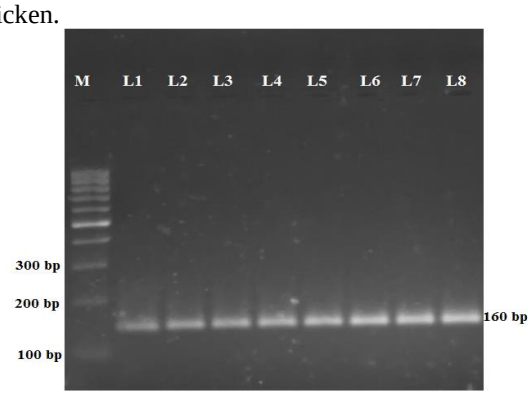

Fig. 1. Agarose gel electrophoresis showing RT-qPCR amplification of White Leghorn layer and kadaknath, Lane M: 100bp ladder, Lanes: L1-L8 PRL (160bp).

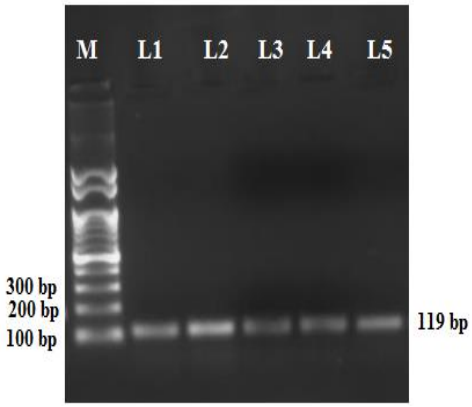

Fig. 2. Agarose gel electrophoresis showing RT-qPCR amplification of GAPDH, Lane M: 100bp ladder, Lanes: L1-L4 GAPDH (119bp) of IWI layer and Kadaknath birds.

Table 1: Fold change within IWI layer and Kadaknath at 20 and 40 weeks.

Sr. No.	Breed	Mean C_t ± SE		Fold change ($2^{-\Delta\Delta Ct}$)
		PRL gene	GAPDH	
1.	IWI Females at 20 weeks	13.5±0.47	15.4±0.15	1
2.	IWI Females at 40 weeks	10.69±0.40	16.43±0.24	1.35
3.	Kadaknath Females at 20 weeks	14.9±0.0.08	16.54±0.04	1
4.	Kadaknath Females at 40 weeks	14.1±0.17	16.2±0.12	3.5

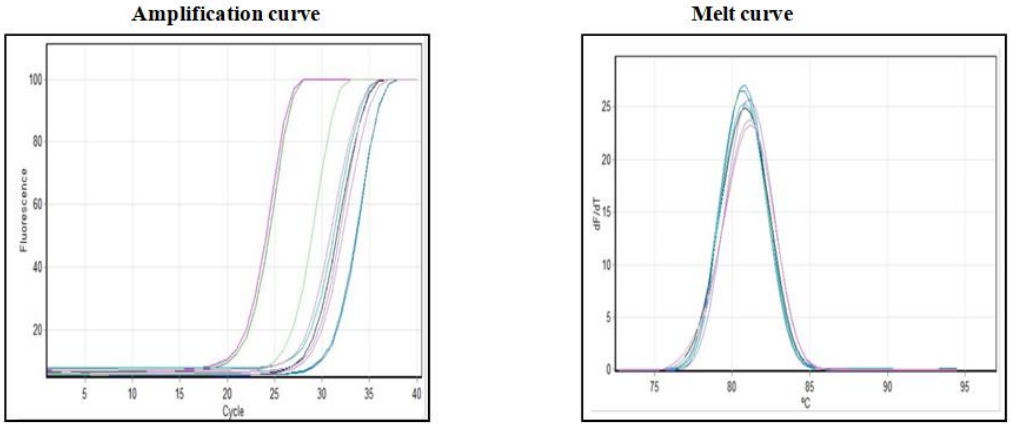

Fig. 3. The amplification and melt curve of real-time PCR for detecting prolactin expression in the chicken anterior pituitary gland.

CONCLUSIONS

The present investigation revealed low levels of prolactin expression inlayers compared to kadaknath chicken and the increment of prolactin is more in kadaknath chicken than IWI layers as age advances.

FUTURE SCOPE

The levels of prolactin expression in the pituitary gland affect the ovary and show a direct effect on egg production and the commercial output of the farm. Different aspects such as immunization, antiprolactin agents, and photo stimulation might increase egg production besides selection. However, stress also plays a key role on the output of egg production in chicken. Hence a multidimensional approach is needed to increase the egg production in native chicken along with nutritional intake of bird.

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Conflicts of Interest. None.

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