TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2011; 35(6): 431-434

doi:10.3906/vet-1003-328

Expression of plasma prolactin and pituitary prolactin mRNA around the broody cycle in Wan-xi White goose

Runshen JIANG, Xingyong CHEN, Ruhua WEI, Zhaoyu GENG*

College of Animal Science and Technology, Anhui Agricultural University, Hefei, 230036 - P.R.CHINA

Received: 31.03.2010

Abstract: A systematic evaluation of the dynamic changes of prolactin (PRL) around the whole cycle of broodiness in domestic fowl has not been performed. We started to investigate the overall changing profile of PRL expression at both protein and mRNA levels from pre- to post-broodiness in Wan-xi White goose, an indigenous breed in East China. The onset of broodiness was recorded, and the plasma PRL concentration and the pituitary PRL mRNA level at 5 stages were measured, in that order, around the first broody cycle. The expression level of pituitary PRL mRNA was highly correlated with the plasma PRL level (r = 0.92), and both changed synchronically around the broody cycle. They significantly increased at the onset of broodiness, but rapidly dropped during the early half period of broodiness, until the later half period of broodiness, and post-broodiness as well, where they reached a similar level as that of pre-broodiness (the laying period). Therefore, intervention on PRL could be a useful tool in regulating goose broodiness, as well as potentially for other domestic fowls.

Key words: Prolactin, mRNA, broody cycle, Wan-xi White Goose

Introduction

Broodiness, observed often in most breeds of domestic fowl, decreases egg production by shortening the laying process (1,2). In chickens and turkeys, increased plasma PRL concentration was associated with the occurrence of broodiness (3,4), and PRL mRNA reached its highest level during incubation (5,6), which indicates that PRL is important in the onset and maintenance of broodiness. Female ducks markedly increased their nest-box occupancy, when a sharp rise in the plasma PRL level occurred during the formation of the final 10%-20% of the clutch (7,8). Laying geese had 5-fold greater level of PRL mRNA than that of the pre-laying geese (9). These facts indicated that the expression of PRL could be associated with reproduction cycle in domesticated and water fowls. However, to the best of our knowledge, no data on the plasma PRL and expression of pituitary PRL mRNA around the entire broody cycle in goose have previously been reported. Therefore, the objective of this study was to reveal the overall changing profile of PRL expression at both protein and mRNA levels from pre- to post-broodiness in goose.

Materials and methods

The experimental stock was Wan-xi White goose, an indigenous breed in East China, which exhibits persistent broodiness. Two thousand geese from 1 single hatch (30 April 2008) were raised under an open floor system in a windowed house from hatch to 28 days, and thereafter were transferred to an open-

* E-mail: gzy@ahau.edu.cn

sided house. At day 210 of age, approximately 30 days before the onset of egg-laying, 600 females and 120 males were matched at random and transferred to another separated house. The house was divided into 30 pens, each with 4 males and 20 females. Each pen was 18 m^2, and on one side equipped with a 3 × 5 m^2 outside ground area and a water bath. Geese were fed with a maize-soybean-based diet (CP 15.5% and ME 10.7 MJ/kg) and forage. The diet and forage for each goose were 250 and 500 g/day, respectively. Geese lay only in cold season, i.e. the ambient temperature between –5 and 20 °C. In the present study, geese were kept under natural day light.

In a full reproduction year, 2 to 4 times of broody cycle are normally observed in this breed, with the first broody cycle occurring usually 1 month after the onset of egg-laying. We recorded the first broody cycle, and divided it into 5 stages to collect samples. Stage I was 20 days after the onset of egg-laying, about 10 days before the onset of first broody cycle; stage II was 3 days after the expression of broody behavior, including box-nesting and aggressiveness, representing for the onset point of broodiness; stage II was 15 days after the onset of broodiness, approximately at the middle of broodiness; stage IV was 30 days after the onset of broodiness, close to the end of broody cycle; stage V was 7 days after the end of the first broodiness, which was in the laying cessation period after the first broody cycle. At each stage, 6 female geese were slaughtered for blood samples (5 mL) and pituitaries.

Plasma was separated by centrifugation (3000 rpm for 15 min), and then assayed for the concentration of PRL using radioimmunoassay (RIA). The chicken standard PRL and RIA assay kits were provided by Dr. A. P. Parlow (NIADDK, USA.). The intra-assay coefficient of variation for PRL was smaller than 5%.

Pituitaries were frozen in liquid nitrogen, and entire pituitary of each individual was used to extract the total RNA using TRIZOL kit according to the manufacturer's protocol (Qiagen, German). RNA was checked by a spectrophotometer, and diluted to 1 μg/μL in diethylpyro-carbonate (DEPC)-treated water and stored at –80 °C.

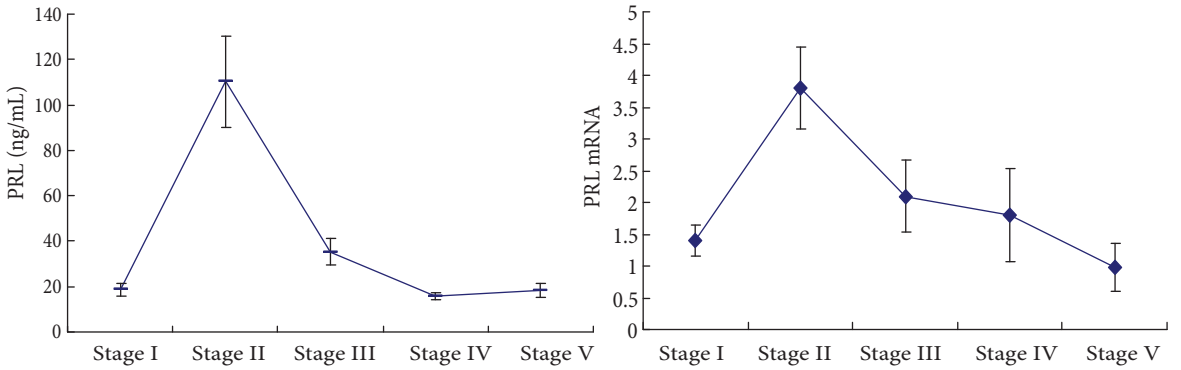
Complementary DNA (cDNA) was produced in a mixture containing 4.0 μL of buffer, 1.6 μL of dNTP (250 μM), 0.5 μL of oligo dT-primer, 1.0 μL of M-MLV, 2.0 μL of total RNA, and 10.9 μL of DEPC ultrapure water. The reactions were carried out in a thermocycler (PTC-100, USA) at 37 °C for 1 h and the reaction products containing cDNA were stored at –18 °C.

Temporal expression of PRL in the pituitary was measured by semi-quantitative real-time PCR (RT-PCR) (RotorGene 6200, USA). PCR primers for PRL gene was Forward 5' GTGGTGCTAAGCGTGTCATCATCT 3' and Reverse 5' GTGGTGCTAAGCGTGTCATCATCT3', and for the house-keeping gene GAPDH was Forward 5'TCATGATTGATCTGCTGAGCTTGC3' and Reverse 5'-TAGAACGCTATGCTCAGGGTCG3'. The reaction mixture included SYBR^R Premix Ex Taq™ 12.5 μL, Forward primer (10 μM) 0.5 μL, Reverse primer (10 μM) 0.5 μL, cDNA 2.0 μL, and ultrapure water 9.5 μL. The reaction was performed under the following condition: 95 °C for 10 min and 40 cycles of 95 °C for 5 s, 55 °C for 25 s, and 72 °C for 15 s. The levels of the transcript were derived in comparison to that of a standard internal control, which was simultaneously amplified with the samples. The levels of gene expression were then normalized against the GAPDH. Negative controls without templates were included.

Comparisons of the plasma PRL as well as the PRL mRNA among the 5 stages of the broody cycle were made by one-way ANOVA using the GLM procedure of the SAS Institute (10). Significant differences among means of stage points were calculated using the Duncan's multiple-range test. A correlation analysis was conducted between plasma protein and the mRNA level using the Factor Analysis and Regression Analysis methods supported by the SAS 9.1.

Results

We found that the concentration of plasma PRL increased before the onset of broodiness ($P < 0.01$), and decreased during the early half of broodiness ($P < 0.01$) (Figure 1A). In the later half of broodiness and post-broodiness, i.e. laying cessation period after the broody cycle, plasma PRL maintained a level approximately the same as that in the laying period of pre-broodiness ($P > 0.05$).



(a) Plasma concentration of PRL

(b) Pituitary PRL mRNA Level

Figure 1. The expression of PRL around the broody cycle in Wan-xi White goose.

Note: The data were shown as means ± SE. Stage I: 20 days after the initial of egg laying; Stage II: 3 days after the expression of broody behavior; Stage III: 15 days after the onset of first broodiness; Stage IV: 30 days after the onset of broodiness; Stage V: 7 days after the end of broody cycle.

We found also a significant linear correlation between plasma protein and the mRNA level of PRL with $r = 0.92187$ ($F = 16.98$, $P < 0.05$), which indicates that PRL mRNA level in the pituitary synchronically changed with the plasma protein level, shown as follows.

The level of pituitary PRL mRNA also increased before the onset of broodiness ($P < 0.01$), and decreased during the early half of broodiness ($P < 0.05$) (Figure 1B). In the later half of broodiness and post-broodiness, PRL mRNA maintained the same level as that in the pre-broody laying period ($P > 0.05$).

Discussion

In this study, it is shown that the pituitary PRL mRNA expression exhibited a similar trend as plasma PRL, which suggests that plasma PRL could be derived mainly from the pituitary. The concentration of plasma PRL and pituitary PRL mRNA dramatically increased before the onset of broodiness indicating that the mechanism of onset of broodiness in geese was similar to chickens and turkeys (11). Plasma and pituitary levels of PRL and its mRNA subsequently declined to reach a nadir. This is also in agreement with results reported on chicken and turkeys (6). In this experiment, the plasma concentration level of PRL reached the peak only at the onset of broodiness, and rapidly decreased in the following 2 or 3 weeks to a normal level as in the laying period and maintained until the end of broodiness. This could be species-specific, since it differed from chickens and turkeys, in which higher PRL level maintained during the most part of incubation (5,6). In addition, the plasma PRL and PRL mRNA did not differ significantly between stage I and stage V, that is the plasma PRL still maintained a relatively high level in geese, during laying cessation period of post-broodiness, which was different from that in turkey, chicken, and Japanese quails (15-19).

Though research on broodiness could provide novel data on different species as demonstrated in this study, it is no longer required for hatching in modern poultry production due to the adoption of artificial incubation technology. Therefore, intervention on poultry broodiness can be adopted to increase egg production, such as active immunization against PRL (12,13) and passive immunization of the vasoactive intestinal peptide, a PRL-releasing stimulator (14). Considering the same mechanism of onset of goose broodiness as in other avian species, the immunization related to PRL may be adopted to reduce the incidence of broodiness, and thus increase egg production in geese.

Acknowledgments

This work was supported by the grant from China S & T Pillar Program (No. 2008BADC1B00) and the Bureau of Science and Technology of Anhui Province (No. 06013039A). We would like to thank Dr. Caixia Yang for her valuable comments and assistance in revision of this manuscript.

References

1. Sockman, K.W., Schwabl, H., Sharp, P.J.: The role of prolactin in the regulation of clutch size and onset of incubation behavior in the American kestrel. Horm. Behav., 2000; 38: 168-76.
2. Reddy, I.J., David, C.G., Sarma, P.V., Singh, K.: The possible role of prolactin in laying performance and steroid hormone secretion in domestic hen (*Gallus domesticus*). Gen. Comp. Endocrinol., 2002; 127: 249-55.
3. Burke, W.H., Dennison, P.T.: Prolactin and luteinizing hormone levels in female turkeys (*Meleagris gallopavo*) during a photoinduced reproductive cycle and broodiness. Gen. Comp. Endocrinol., 1980; 41: 92-100.
4. Bacon W.L., Burke, W.H., Nestor, K.E., Brown, K.I.: Influence of genetic increases in egg production on traits associated with broodiness in turkeys. Poult. Sci., 1983; 62: 2460-73.
5. Talbot, R.T., Hanks, M.C., Sterling, R.J., Sang, H.M., Sharp, P.J.: Pituitary prolactin messenger ribonucleic acid levels in incubating and laying hens: effects of manipulating plasma levels of vasoactive intestinal polypeptide. Endocrinology, 1991; 129: 496-502.
6. Karatzas, C.N., Guemene, D., Zadworny, D., Kuhnlein, U.: Changes in expression of the prolactin and growth hormone gene during different reproductive stages in the pituitary gland of turkeys. Reprod. Nutr. Dev., 1997; 37: 69-79.
7. Hall, M.R.: External stimuli affecting incubation behavior and prolactin secretion in the duck (*Anas platyrhynchos*). Horm. Behav., 1987; 21: 269-287.
8. Hall, M.R.: Endocrinological and behavioral changes associated with the onset of incubation in the duck. Physiol. Behav., 1991; 50: 311-6.
9. Yen, C.F., Lin, H.W., Hsu, J.C., Lin, C., Shen, T.F., Ding, S.T.: The expression of pituitary gland genes in laying geese. Poult. Sci., 2006; 85: 2265-69.
10. SAS Institute: SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC, 2001.
11. Lea, R., Dods, A., Sharp, P., Chadwick, A.: The possible role of prolactin in the regulation of nesting behavior and the secretion of luteinizing hormone in broody bantams. J. Endocrinol., 1981; 91: 89-97.
12. March, J.B., Sharp, P.J., Wilson, P.W., Sang, H.M.: Effect of active immunization against recombinant-derived chicken prolactin fusion protein on the onset of broodiness and photoinduced egg laying in bantam hens. J. Reprod. Fertil., 1994; 101: 227-33.
13. Crisostomo, S., Guemene, D., Garreau-Mills, M., Morvan, C., Zadworny, D.: Prevention of incubation behavior expression in turkey hens by active immunization against prolactin. Theriogenology, 1998; 50: 675-90.
14. Crisostomo, S., Guemene, D., Garreau-Mills, M., Zadworny, D.: Prevention of the expression of incubation behavior using passive immunization against prolactin in turkey hens (*Meleagris gallopavo*). Reprod. Nutr. Dev., 1997; 37: 253-66.
15. Sharp, P.J., Scanes, C.G., Williams, J.B., Harvey, S., Chadwick, A.: Variations in concentration of prolactin, luteinizing hormone, growth hormone and progesterone in the plasma of broody bantams (*Gallus domesticus*). J. Endocrinol., 1979; 80: 51-7.
16. Goldsmith, A.R., Hall, M.: Prolactin concentrations in the pituitary gland and plasma of Japanese quail in relation to photoperiodically induced sexual maturation and egg laying. Gen. Comp. Endocrinol., 1980; 42: 449-54.
17. Bluhm, C.K., Phillips, R.E., Burke, W.H.: Serum levels of luteinizing hormone (LH), prolactin, estradiol, and progesterone in laying and nonlaying canvasback ducks (*Aythya valisineria*). Gen. Comp. Endocrinol., 1983; 52: 1-16.
18. Wong, E.A., Ferrin, N.H., Silsby, J.L.: Cloning of a turkey prolactin cDNA: Expression of prolactin mRNA throughout the reproductive cycle of the domestic turkey (Meleagris gallopavo). Gen. Comp. Endocrinol., 1991; 83: 18-26.
19. Kansaku, N., Ohkubo, T., Okabayashi, H., Guémené, D., Kuhnlein, U., Zadworny, D., Shimada, K.: Cloning of duck PRL cDNA and genomic DNA. Gen. Comp. Endocrinol., 2005; 141: 39-47.