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Changes in the Expression of the Prolactin Receptor (PRLR) Gene in Different Physiological Stages in the Mammary Gland of the Iranian Adani Goat

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Contents

The actions of prolactin hormone are mediated by prolactin receptor (PRLR), and proliferation and differentiation of secretory mammary epithelium are dependent on the presence of its receptors. To understand the PRLR expression pattern in mammary gland of dairy goat during different lactation stages, in this study, we first estimated the milk yield breeding value by multitrait random regression model and then compared the expression of the gene in different physiological stage of mammary gland between high- and low-breeding value groups. We assayed the transcription level of the gene by quantitative real-time PCR method, and its outcomes were analysed by a statistical model containing breeding value groups, sampling times and their interactions as fixed effects. The results indicated that the expression levels of PRLR gene were significantly upregulated in the drying stage ($p < 0.01$). The transcription pattern of the gene was significantly different between the two breeding value groups ($p < 0.01$), so that the amount of PRLR mRNA was significantly higher in the low-breeding value groups of animals in the lactation stage ($p < 0.01$). Based on the results of this study, it could be suggested that the abundance of PRLR transcripts in mammary gland of goat might be changed by some physiological, environmental and genetic factors. Nucleotide variations in the promoter region might be resulted in various transcription activities of the gene which should be studied in a complementary research.

Introduction

Prolactin is a single-strand peptide hormone that has a potential role in different biological process such reproduction, immune system, development, metabolism and osmoregulation (Ben-Jonathan et al., 2006; Bole-Feysot et al., 1998). Prolactin is an essential hormone for lactation which stimulates the absorption of amino acids and glucose, as well as affecting the synthesis of casein, α-lactalbumin, lactose and milk fat particles in the epithelial cells of the mammary gland (Ben-Jonathan et al., 2006). Furthermore, in initiation of milk yield, the transfer and transport of lipids towards mammary gland is directed by prolactin, and consequently prevents depositing the lipids in the adipose tissue (Ben-Jonathan et al., 2006). These roles of prolactin are carried out by the receptor-mediated STAT signalling pathway (Gallego et al., 2001). However, it has been previously reported that the response of the mammary gland to prolactin during lactation period is limited by its receptor abundance (Plaut et al., 1989).

Prolactin receptor is a single-pass transmembrane receptor and is a member of the class I cytokine receptor superfamily that is located on the cell membrane. Prolactin receptor mediates the effect of prolactin in the target tissues (Bole-Feysot et al., 1998). The prolactin receptor (PRLR) gene was mapped on goat chromosome 20 (Hayes et al. 1996) and was studied from different viewpoints. Several lines of evidence have shown that the genetic variations of PRLR are associated with phenotypic variations of prolificacy-related traits in swine (Putnova et al., 2002; Tomas et al., 2006; van Rens et al., 2003). Moreover, four single nucleotide polymorphisms were observed in the coding region of PRLR gene, of which two SNPs have amino acid substitution (G401R and T452I) in the cytoplasmic region of prolactin receptor (Zidi et al. 2010). In dairy cattle, it has been reported that genotypes of PRLR are correlated with milk yield and fat content (Viitala et al. 2006). Nevertheless, no significant association was detected between PRLR genotypes of goat and milk yield, content and yield of fat, protein, lactose and dry matter (Zidi et al. 2010). However, the significant association was identified between PRLR alleles and fatty acid composition of milk in goat (Zidi et al. 2010).

Investigations demonstrated that the mRNA and protein of PRLR have expressed in epithelia and stroma of the rat mammary gland at different physiological stages (Camarillo et al., 2001). The amount of prolactin receptor was varied between mature, pregnant and lactescent animals (Buck et al. 1992; Jahn et al. 1991; Maes et al., 1983). The transcription of the PRLR gene in mammary gland tissue is induced by prolactin and oestrogen hormones (Trott et al. 2009). On the other hand, the amount of prolactin receptor in the mammary cell membrane is a factor for limiting the mammary gland to respond to the prolactin hormone (Plaut et al., 1989). Therefore, it is necessary to study the transcription level of the PRLR gene in mammary gland of animals with high and low milk yield. Hence, we used an *in vivo* approach to investigate whether the expression pattern of PRLR gene is different between two groups of milk yield breeding values at different physiological stages of the mammary gland. In this research, three different physiological stages of prenatal, lactation and drying were selected for sampling and investigation of the abundance of PRLR mRNA to understand the

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exact pattern of gene expression during the production of goat and possible application of the findings in the breeding strategies of Adani goats.

Material and Methods

Animals

The animals had been raised by Adani Goat Breeding Centre located in Bushehr Province of Iran. The herd was established by collecting the goats from different regions of Bushehr Province in 2005 as a conservation programme of native genetic resources. The Bushehr climate was usually harsh with an average temperature of 24.4°C, relative humidity of 56–75 per cent and average annual rainfall of 228.5 mm. The goats were raised under local environmental and management conditions and grazed using poor natural pastures whose major plants were Malva sylvestris, Monk's rhubarb and common salt tree. In addition, dairy goats were given supplementary food in autumn. Adani goats were polyoestrous, but their major mating seasons were middle of April to middle of June and early of September to early of November. In the herd, oestrous synchronization and artificial insemination were not used and mating method was natural. The males were introduced to the females only during mating times. The weaning age of kids was at 60th day after birth, and the dairy goats were milked after kid suckling. According to the different milk yield breeding values, 82 nulliparous animals were categorized into two high- and low-breeding value groups for further analysis. The means of milk yield of selected animals on 40th day after kidding (concurrent with the second sampling time) were 0.780 and 0.302 l in the high- and low-breeding value groups, respectively.

Genetic evaluation of animals

To categorize the animals for expression analysis, daily milk records of the first, second and third lactations were considered. The data of 2364 milk test day records from 375 goats were collected during 2006–2012. First, to select the best model, we performed numerous analyses with different degrees of Legendre polynomial function for additive genetic and permanent environmental effects. The model used for genetic evaluation was multitrait random regression with fifth-order Legendre polynomial function for both additive genetic and permanent environmental effects. This model analysed the records of each lactation as different but correlated traits. Residual effects belonging to the observations were divided into 6 days-in-milk (DIM) classes within parity (DIM 5–17, 18–32, 33–47, 48–62, 63–77 and 78–90). The analyses were carried out using WOMBAT program (Meyer 2007). Because the objective of the current research was the gene transcription analysis in the first lactation of the animals, the breeding values of the first lactation were calculated and the coefficients of the first trait according to the following equation were used:

$$u_i = \sum_{5}^{90} \Phi_r \, \alpha_{ir}$$

In this equation, u_i was the aggregated breeding value between 5 and 90 DIM for ith animal, Φ_r was matrix of Legendre polynomials evaluated from 5 to 90 DIM and α_{ir} was the breeding value coefficient of ith animal in the first lactation. In the current study, the gene expression studies were accomplished on the nulliparous goats, so their milk yields were not recorded. To estimate the milk yield breeding values for these goats, the milk yield breeding value of their parents was used according to the following equation:

$$u_{\mathrm{o}} = u_{\mathrm{s}} + u_{\mathrm{d}}$$

where u_{o}, u_{s} and u_{d} were the aggregated milk yield breeding value for offspring, sire and dam, respectively.

Finally, three goats among ten goats with the highest breeding values and three goats among ten goats with the lowest breeding values were selected randomly and attributed to each high- and low-breeding value group, respectively.

Tissue sampling

Mammary gland samples were taken by biopsy gun (needle size 14 g*10 cm, length of sample notch 19 mm; Bard Biopsy Systems) at prenatal (within 2–6 days before calving, in mid-March), milking (40 days after calving, in the end of April) and drying times (115 days after calving in which all goats were dried, in mid-July). The biopsy samples were taken from the posterior part of the mammary gland after pulling the nipple down and then dragging it to the abdomen. Biopsies were immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction.

RNA extraction and cDNA synthesis

Total RNAs were extracted from mammary gland samples with TRIzol reagent (CinnaGen Inc., Tehran, Iran). The concentration of the RNAs was quantified with a spectrophotometer (JENWAY, Staffordshire, England). The cDNA was synthesized from 2 μg of RNA with 2-step RT-PCR kit (Vivantis, Selangor Darul Ehsan, Malaysia).

Primer design and relative real-time PCR

Real-time PCR was performed in a total volume of 20 μl including 5× HOT FIREPol® EvaGreen® qPCR Mix Plus with ROX (Solis BioDyne, Tartu, Estonia), forward and reverse primers, cDNA and nuclease-free water, using a MiniOpticon real-time PCR system (Bio-Rad Laboratories, Berkeley, CA, USA). Glyceraldehyde-3-phosphate

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dehydrogenase (GAPDH) was used as the reference gene, because it is a suitable reference gene for quantitative gene expression studies in mammary cells (Bougarn et al. 2011; Manjarin et al. 2011; Varshney et al. 2012). The oligonucleotide sequences of forward and reverse primers for the genes were as follows: PRLR: 5′- AGGAGGCTCTG GTTCAACTAT, 5′- ACCACAAGGAAGGAGAAA-CAC; GAPDH: 5′-AGTCAAGGCAGAGAACGG GAA, 5′-ACAAACATGGGGGCATCAGCA. Real-time PCRs were performed at 95°C for 15 min, followed by 40 cycles at 95°C for 15 s, 60°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 5 min. Logarithmic serial dilution (5 serial dilutions) with three replicates was used to obtain the efficiencies of PCRs.

Statistical analysis

The real-time PCR data were analysed as the difference (ΔCt) between the threshold cycle (Ct) of GAPDH and that of the gene of interest (PRLR). The following statistical model was used for the analysis of the data:

$$y_{ijk} = S_i + B_j + (S * B)_{ij} + a(B)_k + (S * a(B))_{ik} + e_{ijko}$$

Here, y_{ijk} was the difference (ΔCt) between the threshold cycle (Ct) of GAPDH and interest gene. In this model, the sampling times (S_i), breeding value groups (B_j) and their interactions were used as the fixed factors, and individual animal within breeding value groups ($a(B)_k$) and its interactions with sampling times ($(S*a(B))_{ik}$) represented the random effects. Data were tested by GLM procedure using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Analysis of variance for gene expression indicated that all factors considered in the statistical model had a significant effect on the amount of PRLR mRNA in mammary gland tissue ($p < 0.01$). As it is shown in Fig. 1a, the overall average of transcriptional levels of PRLR gene varies between breeding value groups. Moreover, comparison of the mRNA expression levels based on the main effect of the physiological stages in the mammary gland showed that the overall average of the transcriptional levels of PRLR at drying time was significantly upregulated compared ($p < 0.01$) with prenatal and lactation times (Fig. 1b). This analysis identified that transcription level of the PRLR gene at drying time was upregulated by more than two- and sixfold compared with prenatal and lactation stages, respectively. The expression levels of lactation time were significantly downregulated compared with prenatal time (the change was more than threefold). In general,

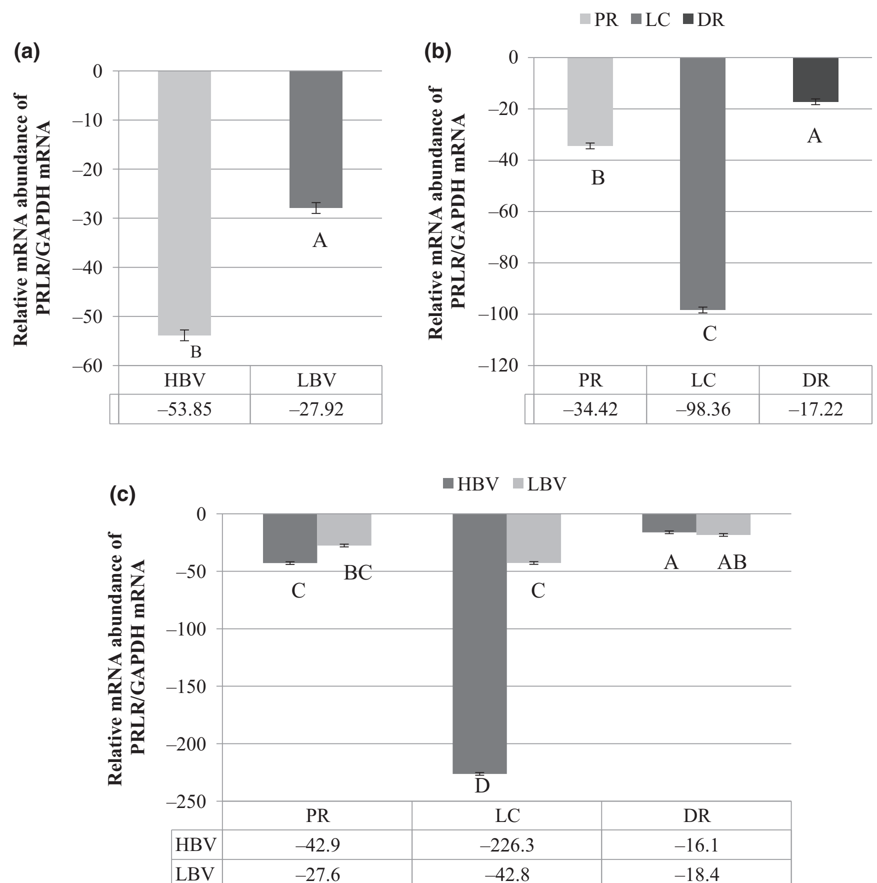

Fig. 1. Comparison of the PRLR gene transcription ($p < 0.01$): (a) between high (HBV)- and low (LBV)-milk-yield breeding value groups (mean ± SEM); (b) among prenatal (PR), lactation (LC) and drying (DR) times (mean ± SEM); and (c) interaction between milk yield breeding value groups and different physiological stages (mean ± SEM)

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the expression pattern of PRLR fell from prenatal time to reach the lowest at the lactation time, and increased to maximum at drying time. As given by different studies, the pattern of expression of the PRLR gene in the mammary gland is different between species (Jahn et al. 1991; Buck et al. 1992; Cassy et al. 1998), although similar pattern was observed between goat and sheep (Cassy et al. 1998). In rat mammary gland tissue, the PRLR transcription level raised from parturition up to lactation (Jahn et al. 1991). The amount of the PRLR gene transcripts in the mammary gland is unchanged between virgin, pregnant and lactating mice (Buck et al. 1992). However, in previous studies, only the PRLR expression pattern during lactation was investigated in goat and it was indicated that the expression fell to lowest at the peak of lactation, and slightly elevated at the end of lactation, and the expression levels of the peak and late lactation were significantly downregulated compared with the early lactation (Ji et al. 2015). In this study, the other physiological stages of mammary gland are considered, and the results proposed that the increased mammary tissue PRLR mRNA expression during the drying stage is very important and may be associated with mammary development and remodelling for subsequent lactation. The highest levels of PRLR mRNA in drying stage were reported for other species (Cassy et al. 1998; Varas and Jahn 2005; Trott et al. 2009). Taken together, these results are in accordance with an essential role of prolactin in the development of the mammary gland (Cassy et al. 1998).

Because the interaction among breeding value groups and physiological stages was significant for expression of PRLR gene in the mammary gland tissue of goats, we further discuss the results of interactions of the fixed factors. Based on the interaction between breeding value groups and physiological stages of mammary gland, the PRLR expression pattern for each breeding value groups at the different physiological times can be uncovered and also the transcription level of the PRLR gene can be compared between the breeding value groups at the prenatal, lactation and drying times.

Figure 1c demonstrates the expression pattern of the PRLR gene in the mammary gland of the high- and low-breeding value groups. Although the expression pattern of the PRLR gene was almost the same for both breeding value groups, but the levels of transcriptions of the PRLR gene were more constant in the low-breeding value group compared with the high-breeding value group. The pattern of the PRLR gene expression in the low-breeding value group revealed that no significant differences of mRNA were observed between prenatal time with the other physiological stages of mammary glands ($p > 0.05$), but the results showed that lactation time showed a significantly lower expression level than drying time ($p < 0.01$; the change was more than twofold). The mode of transcription of the PRLR gene was not constant in the high-breeding value group, and at least expression was considered in the lactation time. Comparison of the transcription levels of PRLR gene in mammary glands of the high-breeding value group at the different physiological stages revealed that this gene was upregulated in drying time compared with prenatal and lactation times, and the changes were more than 2- and 16-fold, respectively. Furthermore, comparison of the amount of PRLR transcripts between lactation and prenatal stages in the high-breeding value group demonstrated that the expression was downregulated in lactation stage less than fivefold. These data indicate that the transcription of the PRLR gene in mammary gland tissues of Adani goat is differentially regulated during different physiological stages and between breeding value groups. The differential expression that observed between different physiological stages may be caused by many endocrine environment (Trott et al. 2009). Several studies demonstrated that oestrogen, progestin and prolactin hormones differentially regulate PRLR expression in the mammary glands (Cassy et al. 2000; Varas and Jahn 2005; Trott et al. 2009). Moreover, other findings have suggested that the expression of PRLR mRNAs is differentially regulated by testosterone (Sakaguchi et al. 1994; Yasui et al. 1999) and growth hormones (Norstedt et al. 1981).

A comparison of PRLR mRNA abundance was also made between breeding value groups at different physiological stages (Fig. 1c). The outcome demonstrated that no significant differential expression exists between the two groups during prenatal and drying stages. Only during the lactation stage, the gene was expressed at a significantly different level between the high- and low-breeding value groups, and the expression levels of the high-breeding value group were significantly downregulated compared with the low-breeding value group. Because during lactation, prolactin binding sites on cellular membrane limit the response of the mammary gland to prolactin, the downregulation of PRLR transcription in a high-breeding value group is notable and may be caused by genetic background. Milk breeding values are additive effects of variations of the genome that correlate with milk production. Therefore, these results indicated that there may be a relationship between PRLR transcription and DNA variations in Adani goats. Consequently, the polymorphism in PRLR gene is the priority to be investigated specially in the regions effect on PRLR transcription. To date, many genetic variations have been reported for PRLR gene. From an animal breeding perspective, these polymorphisms have an association with milk production traits in dairy cattle and goat (Viitala et al. 2006; Zidi et al. 2010; Lu et al. 2011) as well as with reproductive traits in goat, pigs and sheep (Terman 2005; Chu et al. 2007; An et al. 2015). Previous studies have revealed that the amount of prolactin receptors limits the response of the mammary gland to prolactin hormone, so the selection of Adani goats based on milk yield breeding value may limit the response of the mammary gland to prolactin hormone after several generations. Besides, because of

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the low level of PRLR transcription in high breeding value in milking time, the selection based on milk breeding value may affect the absorption of amino acids and glucose, and the synthesis of casein, α-lactalbumin, lactose and milk fat particles in lactation period (Ben-Jonathan et al., 2006) after numerous generations. On the other hand, although development and differentiation of the mammary gland occur primarily during pregnancy, PRLR involved in the development and differentiation of epithelial cell (Kelly et al. 2002); thus, the low level of PRLR mRNA in the high-breeding value group may affect the development and differentiation of epithelial cell in the lactation stage as a result of animal breeding based on milk breeding values. In general, nucleotide variations in the promoter region of the PRLR gene might be resulted in various transcription activities of the gene in lactation time. Identification of the polymorphisms in the 5′ upstream region of the PRLR gene can shed light on how the molecular mechanism of the transcription activity of the gene is related during different physiological stages as well as between breeding value groups of Adani goats.

Conclusion

Generally, the abundance of PRLR mRNA was significant between physiological stages in the high-breeding value groups of Adani goats, but no significant differences were observed in the low-breeding value groups. The greatest amount of PRLR transcription was considered in drying time in both breeding value groups. Based on these results, we can suggest that: (i) the regulation of the amount of PRLR transcription is dependent on the stage of mammary gland, (ii) the expression pattern of PRLR gene is affected by breeding values that are cumulated additive effects of DNA polymorphisms and (iii) the transcription levels were different only in lactation stage between the two breeding value groups. Therefore, identification of the polymorphism(s) that are correlated with PRLR transcription, especially the polymorphism(s) that are located in 5′ upstream region of PRLR gene, should be considered to find a possible causative SNP related to differential expression of PRLR gene in Adani goat population.

Conflict of interest

None of the authors have any conflict of interest to declare.

Author contribution

Morammazi carried out the research and made the draft of the manuscript. Masoudi designed the study and developed the interpretations of the results and also modified the draft of the manuscript. Vaez Torshizi contributed in the analysis of the data. Pakdel contributed partly in the analysis of the data.

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