



Molecular insights into PRL and PRLR genes' influence on milk obtaining – a review

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Abstract. The prolactin (PRL) gene, mapped on chromosome 23 in cattle, is usually considered in association studies with various milk traits. Although contradictory results of genotypes associations can be reported, they are generally justified if taking into account the variability of individuals (differences in investigated breeds), the number of individuals, and the investigated milk trait. The PRL gene is not a part of any Quantitative Trait Locus (QTL), but the gene of its receptor (PRLR) is. This fact is important, considering the role of PRLR protein in some steps of PRL action. This study is a review that debates important and up-dated aspects of PRL and PRLR genes' location and structure, physiological mechanisms of action of PRL hormone, and some associations of a well-studied polymorphism (a point mutation) with some milk traits.

Key Words: associations, milk, prolactin, traits.

Introduction. Milk production is of high importance both for its consumption and for cattle management. Various loci have become candidates in the process of marker assisted selection, some of them being part of linkage groups that make up so-called Quantitative Trait Loci (QTLs) (He et al 2006; Dong et al 2013; Patel & Chauhan 2017). Smaragdov (2012) reviewed several genes with strong and weak effects on milk production traits, where genes with strong effects included DGAT1 (the acyl-CoA: diacylglycerol acyltransferase), GHR (growth hormone receptor), and ABCG2 (ATP binding cassette subfamily G member 2), while the PRLR (prolactin receptor), PPARGC1A (peroxisome proliferator-activated receptor gamma coactivator 1-alpha), casein genes and about 50-100 other genes were considered as having a weak effect. Although the prolactin gene (PRL) does not belong to a QTL, the gene of its receptor (PRLR) has been mapped within QTLs (Smaragdov 2012).

The aim of this paper was to review the most important molecular aspects of PRL and PRLR genes' influence on milk obtaining process. Some physiological aspects of prolactin hormone involvement in milk synthesis and secretion were also discussed. This review study is based on 35 scientific papers selected for their scientific relevance for the subject matter.

Basic Approaches of PRL and PRLR Genes' Location and Structure. As reviewed up to now, the bovine PRL gene is located in the 23rd chromosome. It spans about 10 kb (9.4 kb), including here five exons and four introns (Dybus 2002; Wojdak-Maksymiec et al 2008; Sodhi et al 2011; Alfonso et al 2012; Sonmez & Ozdemir 2017; Thuy et al 2018; Oğuzkan & Bozkurt 2019). It was firstly mapped by Hallerman et al (1988), as reviewed by Alipanah et al (2007b) and He et al (2006), its content in exons and introns being reported by Camper et al (1984), as reviewed by Dybus (2002). Moreover, Patel & Chauhan (2017) reported the location of the bovine PRL gene at 43 cM close to the chromosomal QTL.

Regarding the PRLR gene in cattle, it is mapped on chromosome 20 (20q17) and consists of 10 exons (Cosenza et al 2018). Based on the information on human and mouse genomes, it seems to be located about 7 Mb from the GHR gene (Viitala et al

2006). The PRLR gene is 62 kb long and the encoded transmembrane receptor belongs to the superfamily (class 1) of cytokine receptors (triggering signaling pathways) (Smaragdov 2012). Hu et al (2009) described the ability of this receptor not only to activate the JAK2 pathway and of STAT5, but also of other two members of the STAT family, STAT1 and STAT3.

The PRLR Protein and the Starting Point of PRL Hormone Action. The importance of PRLR lies in the coding of a protein molecule, a specific membrane receptor, which is able to be bound by the PRL hormone in its first step of action, a fact that contributes to the activation of the JAK/STAT signaling pathway (JAK - Janus Kinases; STAT - Signal Transducer and Activator of Transcription) (Lü et al 2010). In fact, 3 of the 10 exons of the PRLR gene can be alternatively spliced with at least two different known isoforms (long and short) (Cosenza et al 2018). Accordingly, in cattle, two distinct prolactin receptor isoforms were reported, the long form, with 557 amino acids, and the short one, with 272 amino acids (Brym et al 2005), whereas Cosenza et al (2018) found 581 amino acids and 296 amino acids for the long and short isoforms in ruminants, respectively. In the same report of Cosenza et al (2018), the short protein was found to originate from an alternative splicing of 39 bp (in exon 10) that generates two stop codons. However, the short PRLR form is unable to activate JAK2 and to contribute to the subsequent phosphorylation of STAT5 transcription factors, Brym et al (2005) reviewing even an inhibitory effect via formation of heterodimers. There were also reported other interspecific actions since the STAT5 factors are able to recognize sequences located in promoters of milk protein genes and the long PRLR isoform binds PRL and contributes to their activation (Cosenza et al 2018). Interestingly, PRL exerts its physiological functions in the process of inducing lactation, by acting through PRLR dimerization (Lü et al 2010; Uddin et al 2013).

Supporting Mechanisms for Physiological Action of PRL Hormone. The PRL hormone is involved in the processes of lactation and reproduction in mammals, the supportive role in cattle reproduction being even confirmed by the presence of PRLRs in corpus luteum and granulosa cells (Looper et al 2010).

The PRL synthesis and secretion is mainly ensured by the lactotropic cells of the anterior pituitary, although the mRNA product of the gene was also detected in the brain and several other tissues such as mammary gland, placenta, amnion, decidua, uterus, and lymphocytes (Dybus et al 2005; Alipanah et al 2007a; Wojdak-Maksymiec et al 2008; Mehmannaavaz et al 2009; Lacasse et al 2016; Thuy et al 2018). However, the most important site of its synthesis was highlighted by Sasavage et al (1982), claiming that bovine PRL mRNA constitutes about 60% of polysomal mRNA from the bovine anterior pituitary gland.

As proteins, the PRL hormone and its receptor may be involved in more than 100 different functions (Dybus et al 2005; Ghasemi et al 2009; Looper et al 2010). The PRL protein precursor has 229 amino acids; of these, 30 amino acids constitute the signal peptide, and 199 amino acids represent the mature bovine PRL molecule, with a molecular mass of about 23 kDa (He et al 2006; Alipanah et al 2007a; Wojdak-Maksymiec et al 2008; Mehmannaavaz et al 2009; Thuy et al 2018). However, in most mammalian species, this hormone consists of 197-199 amino acids (Dybus et al 2005; Ghasemi et al 2009).

The primary effects of PRL are on the mammary gland, including here the process of mammatogenesis, of lactogenesis, and galactopoiesis (He et al 2006; Alipanah et al 2007a,b; Wojdak-Maksymiec et al 2008; Looper et al 2010; Bukhari et al 2013).

Raven et al (2014) reviewed a stadial process of mammatogenesis, starting with the formation of a rudimentary structure before puberty, when several signaling proteins and transcriptional factors are involved, such as *Wnt*, *notch*, and *hedgehog*, which usually regulate developmental processes. Afterwards, when the mammary structure begins to form, genes for growth hormone and for proteins which constitute the basement membrane start to be expressed. Alveolar buds are formed at puberty as a result of several hormones. Although the PRL hormone is important for lobulo-alveolar

development and establishment of lactation, it seems to be less important after the teat formation in cattle (Raven et al 2014). PRL plays an important role in the epithelial cell differentiation of the mammary gland (Oztabak et al 2008). In fact, during subsequent lactations, there is a cycle of several rounds of mammary epithelium proliferation, differentiation and cell removal via their detachment and apoptosis. However, investigating various gene sets supposed to be involved in the mammary development pathway, the prolactin signaling and the involution pathway, Raven et al (2014) found 64, 27, and 40 genes involved, respectively, among which PRL and PRLR were reported only in the first two pathways. Other genes of interest and associated with milk traits were CSN2, GH1 and GHR for the mammary development pathway, and CSN1S1, CSN2, and GH1 for PRL signaling, with four genes of the mammary gland development being reported in a well-known QTL for milk production, located on BTA20 and including FGF10, MSX2, PRLR, and GHR.

The maintenance of milk synthesis and secretion is related to the PRL synthesis. Suckling and milking are the most powerful natural stimuli for PRL release, in a process in which its secretion seems to be primarily regulated by the tuberoinfundibular neurons of the hypothalamus via the inhibitory action of dopamine. However, parturition is associated with a major peak in PRL concentration and the PRL releasing decreases as lactation progresses (Dybus et al 2005; Ghasemi et al 2009; Lacasse et al 2016). PRL secretion was found to not differ between high and low productions, but its metabolism and distribution were reported to increase between days 30 and 150 of lactation (Alfonso et al 2012). Igono et al (1988) found that longer daylight periods and higher ambient temperatures influence the prolactin levels and they also showed that the milk PRL concentration was the lowest during the winter months and highest in summer, with peak levels in July and August, in tandem with the variation of temperature-humidity indices.

PRL is an important chemical mediator, which influences the milk production pathway by its synthesis and secretion both at central and local systems. It is known that PRL is synthesized at the level of epithelial cells of the lactating mammary gland and that it can be found in milk (Wojdak-Maksymiec et al 2008). Interestingly, PRL is also secreted by lymphocytes, with a different mechanism of control compared to the pituitary site, since lymphocytes themselves contain dopamine receptors that may be involved in its synthesis and secretion control at the lymphocytic site. Thus, PRL has supplementary functions such as those of immunostimulation and of autoimmunity (Wojdak-Maksymiec et al 2008; Ghasemi et al 2009; Sonmez & Ozdemir 2017). Other functions in which PRL is involved refers to water and electrolyte balance and osmoregulation, regulation of various functions within the brain, maintenance of maternal behaviour, tegument growth and synergism with steroids (Alfonso et al 2012; Boleckova et al 2012; Dong et al 2013).

A Point Mutation in *PRL* Gene and some Resulting Associations with Milk Traits.

The polymorphism consisting of a silent A→G mutation in the codon for amino acid 103 (A103G) is one of the most well investigated for the PRL gene. Several results are worth to be mentioned, since various associations were found between this PRL-RsaI polymorphism in the third exon and some of the milk traits:

- the AA genotype and the A allele, displayed as an uncut (undigested) 156 bp fragment in the PCR-RFLP technique, were reported as predominant in Holstein Friesian cattle (Oğuzkan & Bozkurt 2019) and in Black and White cattle (Dybus 2002; Dybus et al 2005; Khatami et al 2005), in Black Pied and Red Pied Russian cattle (Alipanah et al 2007a,b; Alipanah et al 2008), in the Gorbatov Red breed and Ayrshire breeds (Udina et al 2001), in American Swiss cattle (Alfonso et al 2012), in East Anatolian Red cattle (Oztabak et al 2008), in Montbeliard cows (Ghasemi et al 2009). In all of these cases, the AB genotype (perceived as three fragments of 156, 82, and 74 bp) was predominant over the BB genotype (82 and 74 bp). Oğuzkan & Bozkurt (2019) reported the following order of genotypes in their milk yield: AA>AB>BB, while Ghasemi et al (2009) confirmed that the AA cows yielded the most milk if compared to other genotypes. In Black Pied Russian cattle, Alipanah et al (2007a,b; 2008) found the AB genotype to be associated with

higher milk yield when compared to AA and BB, while in Red Pied Russian cattle, the BB genotype yielded the most milk (BB>AA>AB).

- Contradictory results were also reported for other milk traits. For example, Alipanah et al (2007b) found the BB genotype to be associated with more fat percentage than AA and AB in Black Pied Russian cattle, while in Red Pied Russian cattle, the AB genotype was associated with more fat content and percentage than AA and AB. Similarly, in Red Pied Russian cattle, the BB genotype yielded more protein than the AA genotype and the latter yielded more than the AB genotype. The AA genotype was associated with higher milk protein content than AB in Black and White cattle (Polish Friesian) (Dybus 2002), while the BB genotype had the highest somatic cell count and AA the lowest in a study performed in Red and White Holstein Friesian cattle by Wojdak-Maksymiec et al (2008).

Conclusions. The bovine PRL gene was mapped on the 23rd chromosome at 43 cM close to a chromosomal QTL. Although is not a part of a QTL, the gene of its receptor is. The PRL hormone has a content of 197-199 amino acids in most mammalian species, being involved in mammogenesis, lactogenesis and galactopoiesis processes. A silent mutation in the codon for amino acid 103 gave rise to a PRL-RsaI polymorphism. Various associations with milk traits were reported here, with contradictory results mostly considering the investigated milk trait.

Conflict of Interest. The authors declare that there is no conflict of interest.

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