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Molecular cloning and characterization, and prokaryotic expression of the *GnRH1* gene obtained from Jinghai yellow chicken

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ABSTRACT. The gonadotropin-releasing hormone (GnRH) plays an important role in the control of reproductive functions. Recent studies have reported the occurrence of *GnRH* molecular variants in numerous species. In this study, the *GnRH1* gene from Jinghai yellow chicken was cloned by reverse transcriptase-polymerase chain reaction and transformed into BL21 (DE3) competent cells. The *GnRH1* gene and amino acid sequences were subjected to bioinformatic analyses. The *GnRH1* gene nucleotide sequence was discovered to be 352 bp long, containing a coding, promoter, and section of the 3'-regions. The *GnRH1* gene shared 93, 81, 54, 58, 61, 76, 76, 59, 76, and 66% sequence identity with *Meleagris gallopavo*, *Columba livia*, *Homo sapiens*, *Bos taurus*, swines, *Capra hircus*, *Ovis aries*, *Pantholops hodgsonii*, *Equus caballus*, and *Rattus norvegicus*, respectively. The *GnRH1* gene

showed conserved domains. The GnRH1 protein was a secreted protein comprising 92 amino acids, with a molecular weight of 10205.6 Da and a theoretical pI of 5.67. Most of the amino acid residues were observed to be hydrophilic, indicating water solubility. The predicted secondary structures of proteins included α-helices (h; 23.08%), β-extensions (e; 10.92%), and random coils (c; 66.0%). The successful construction of prokaryotic expression vector pET32a-*GnRH1* was confirmed by restriction and sequence analysis. SDS-PAGE analysis showed the successful expression of recombinant plasmid in *Escherichia coli* BL21 (molecular weight = 25-28 kDa). Larger quantities of protein were expressed in supernatant, indicating greater expression in soluble form. Western blot analysis confirmed the expression of the target protein.

Key words: Jinghai yellow chicken; Cloning; Bioinformatic analysis; Prokaryotic expression; *GnRH*

