Identification of single nucleotide polymorphisms and restriction enzym on prolactin gene in Alabio and Mojosari duck

I Damayanti¹, D Maharani² and S Sudaryati³

¹Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak Pelaihari, Kalimantan Selatan, Indonesia
²Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada Yogyakarta 55281, Indonesia
³Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada Yogyakarta 55281, Indonesia

Corresponding author: d.maharani@ugm.ac.id

Abstract. Prolactin plays important roles in avian reproduction as it induces broody behavior and regulates follicular development. The objectives of this study were to detect Single Nucleotide Polymorphisms (SNPs) of prolactin gene in Alabio and Mojosari ducks and determine the restriction enzymes for genotyping. Genomic DNA was isolated from 50 blood samples per each Alabio and Mojosari ducks. PCR amplification and sequencing were carried out to identify the SNP. In this study, SNP T-6068C was found based on three GenBank sequences alignment. Two SNPs (C-5796A and T-5817C) in intron 4 region were detected based on sequences of Alabio and Mojosari PCR products. Enzymes Fok1, BtsCI, BsrI were detected to recognize the SNP C-5796A. SNP T-6068C can be digested by enzym TspDTI. However no enzym was detected to recognize SNP T-5817C. In conclusion the SNPs detected from this study may be used in future studies to investigate the association of prolactin gene and egg production traits in Alabio and Mojosari ducks.

1. Introduction
Ducks play important role in egg production in Indonesia. Alabio and Mojosari are Indonesian local ducks which mainly raised for egg production in some regions in Indonesia. Because of their potential and importance as egg producers, as well as becoming a part of maintaining livestock biodiversity, an effort to increase the duck productivity is a need. Genetic improvement of the ducks is an option for increasing their productivity, because genetic information is inherited. Recently, the use of molecular genetic technology is an important parts of the genetic quality improvement program for animals. A popular molecular genetic technology is marker assisted selection (MAS), which is a selection method based on phenotypic data information combined with genetic markers. Since prolactin has association with egg production, selection based on prolactin genetic variation may increase the egg production [1].

Prolactin is involved in many biological functions in all vertebrates. This hormone is also known as a negative regulator in the reproductive activities of birds, which induces broody behavior and suppresses gonadal hormone secretion. However, prolactin is also reported to be an important hormone that regulates the follicular formation [2].
Prolactin gene in ducks was cloned and sequenced by [3] and registered with genbank number AB158611. This gene consists of 5 exons and 4 introns, encoding 229 amino acids. Some studies showed prolactin gene polymorphisms were associated with egg production and reproductive traits in poultry. Polymorphism SNP C-5961T in exon 5 was significantly associated with egg production and egg weight in China native ducks [4], with CC genotypes possessed higher egg production than CT genotypes. Meanwhile [5] found six SNPs on Brown Tsaiya ducks (T213C, T295CG, G309T, C381A, G3941T, A3957C) located in non-coding regions. All of the identified SNPs were associated with egg weight at 40 weeks of age and fertility rate except for SNP T295C.

The objective of this study was to investigate the polymorphisms of prolactin gene in Alabio and Mojosari ducks and determine the restriction enzyme for genotyping. The result then could be used for further studies on the association of prolactin gene and egg production in ducks.

2. Materials and methods

2.1. DNA isolation and PCR

Blood samples were collected from 50 Mojosari Duck and 50 Alabio Duck in BPTU-HPT Pelaihari South Kalimantan. The ducks have been selected for egg production in six generations. Genomic DNA was isolated from blood sample using SYNC™ DNA Extraction Kit (Geneaid, Taiwan).

PCR was performed to amplify the Prolactin Gen using primer forward: TGCAAACCATAAAAGAAAAGA and reverse: CAATGAAAAGTGGCAAAGCAA [4]. PCR was carried out under this following protocol 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature 52°C, 30 s at 72°C, and a 10 s final extension at 72°C. The 400 bp PCR-products were electrophoresed on 1.5% agarose for visualisation and stained using Ethidium bromide. The result then documented using digital camera.

2.2. SNP identification

Alignment on three GenBank sequences (AB 158611.1 as target sequence, NC_040047, JQ677091.2) from www.ncbi.nlm.org and comparing DNA sequences of Alabio and Mojosari ducks were carried out to detect the polymorphism of prolactin gen. Sequencing was performed by LPPT UGM Yogyakarta. Restriction Enzymes were detected by Bioedit and Nebcutter (http://nc2.neb.com/NEBcutter2/).

3. Result and discussion

3.1. Result

One SNP T-6068C (fig.1) was detected based on genbank sequences alignment. Furthermore two SNPs C-5796A and T-5817C (fig 2) located in intron 4 region were detected based on DNA sequencing of Alabio and Mojosari ducks. All SNPs found were located in non-coding region.

Figure 1. Multiple alignment of three genbanks accounts prolactin gene
Figure 2. SNP identification of prolactin gene in Mojosar and Alabio duck based on electrophoregram

Restriction enzymes were determined for the three SNPs detected in this study. SNP C-5796A can be recognized by FokI, BtsCl and BsrI restriction enzymes. TspDTI enzyme was detected to digest SNP T-6068C. However no enzyme found to digest SNP T-5817C (table 1.)

Table 1. List of restriction enzymes

| No. | SNP       | Restriction Enzyme | Genotype and fragment size                       |
|-----|-----------|--------------------|--------------------------------------------------|
| 1.  | SNP T-5817C | No.                | -                                               |
| 2.  | SNP C-5796A | FokI               | CC = 73,103,224; AA = 176,224; CA= 73, 103, 176, 224 |
|     |           | BtsCl             | CC = 86,103, 211; AA= 189, 211; CA = 86, 103, 189, 211 |
|     |           | BsrI              | CC = 29, 61, 310; AA = 61, 339; CA = 29, 61, 310, 339 |
| 3.  | SNP T-6068C | TspDTI            | TT=20, 26, 40, 42, 46, 89, 157; CC= 26, 40, 46, 52, 89, 157; TC= 20, 26, 40, 42, 46, 52, 89, 157 |

3.2. Discussion

Prolactin is involved in many biological functions in all vertebrates [4] and there are reported to be 300 functions of this hormone [6]. Prolactin is a single chain polipeptide hormone that belongs to the growth hormone group and is mainly synthesized in the anterior pituitary in all vertebrates. Prolactin plasma concentration in chickens increases during laying period and reaches its peak level during incubation and then decreases after the hatching of the offsprings. The level of PRL mRNA in the anterior pituitary is correlated with plasma prolactin in the anterior pituitary [3]. The level of hormone prolactin has a negative correlation with egg production, estradiol and progesterone [7].

Since PRL gene was cloned and sequenced, some studies focused on the screening the polymorphisms in this gene. Some polymorphisms of this gene have been found to have association with egg production traits. Three SNPs (A-401G, G-268A and T-266A) located in non-coding region were found to be associated with egg production in geese [8]. Recently [4] found the SNP C-381A in intron 1 was significantly associated with shell strength in China native ducks. This SNP was recognized by enzyme XbaI. Furthermore, two SNPs were detected based on PCR products sequences both in Alabio and Mojosari ducks, namely SNP C-5796A and T-5817C located in intron 4 region. Whether SNPs are associated with egg production traits in Alabio and Mojosari need to be investigated. Intron is part of gene that does not code for amino acids. However intron involved in every step of mRNA process, including transcription initiator, transcription terminator and RNA stabilizer [9]. Only SNP T-6068C was found based on three genbanks sequences alignment located in flanking region.

We detected restriction enzymes for SNP C-5796A and SNP T-6068C. No enzymes were detected to digest SNP T-5817C. Three restriction enzymes FokI, BtsCl, BsrI were detected for SNP C-5796A. All these enzymes are available commercially. Fok 1 digests SNP C-5796A in to genotypes CC(73,103,224 bp), AA(176, 224bp) and CA(73, 103, 176, 224 bp). This enzyme is produced by Flavobacterium okanokoites. Enzym BtsCl and BsrI also cut SNP C-5796A into three genotypes (table 1.).
BtsCI was *Bacillus thermosphaericus*. This enzyme will be inactive if treated to a temperature of 80°C for 20 min. Enzym TspDTI was detected to recognize SNP T-6068C.

4. Conclusion

In conclusion, two synonymous polymorphisms of prolactin gene in Alabio and Mojosari ducks located in C-5796A and T-5817C. Restriction enzymes Fok1, BtsCI, BsrI could be used for genotyping animal by PCR-RFLP in the future study.

Acknowledgement

This research was supported by the Indonesian Ministry of Finance through the LPDP scheme and Universitas Gadjah Mada through the RTA scheme (No.3294/UN/DITLIT/DIT-LIT/LT/2019). The authors thank the BPTU-HPT Pelaihari South Kalimantan for assisting in data collection.

References

[1] Kansaku N, Hiyama G, Sasanami T and Zadworny D 2008 *J. Poult. Sci.* **45** 1–6
[2] Li W L, Liu Y, Yu Y C, Huang Y M, Liang S D and Shi Z D 2011 *Domest. Anim. Endocrinol.* **41** 57–66
[3] Kansaku N, Ohkubo T, Okabayashi H, Guémené D, Kuhnlein U, Zadworny D and Shimada K 2005 *Gen. Comp. Endocrinol.* **141** 39–47
[4] Wang C, Liang Z, Yu W, Feng Y, Peng X, Gong Y and Li S 2011 *South African J. Anim. Sci.* **41** 63–9
[5] Chang M T, Cheng Y S and Huang M C 2012 *Anim. Reprod. Sci.* **135** 91–6
[6] Bole-Feyos C, Goffin V, Edery M, Binart N and Kelly P A 1998 *Endocr. Rev.* **19** 225–68
[7] Reddy I J, David C G, Sarma P V. and Singh K 2002 *Gen. Comp. Endocrinol.* **127** 249–55
[8] Jiang R S, Zhang L L, Geng Z Y, Yang T and Zhang S S 2009 *South African J. Anim. Sci.* **39** 83–7
[9] Chorev M and Carmel L 2012 *Bioinforma. Comput. Biol.* **3** 1–15