IJAS Iranian Journal of Applied Animal Science

Polymorphism of Prolactin and Prolactin Promoter Genes and Its Association with Broodiness and Body Weight in Indonesian Chicken Lines

Research Article

A.B.I. Perdamaian[1] and B.S. Daryono[1*]

[1] Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sleman 55281, Yogyakarta, Indonesia

Received on: 18 Apr 2019
Revised on: 22 Jul 2019
Accepted on: 30 Jul 2019
Online Published on: Jun 2020

*Correspondence E-mail: bs_daryono@mail.ugm.ac.id

Online version is available on: www.ijas.ir

ABSTRACT

In the world of poultry farming, female chicken positioned as parent stock must exhibit excellent laying performance without broodiness and inherit body weight related traits to her offspring. Prolactin is widely accepted to have an important role in the onset and maintenance of broodiness event. The aim of this study is to identify a valuable genetic marker for studying the polymorphism of prolactin (Prl) and prolactin promoter (Prl-P) in Indonesian chicken lines using morphology and molecular data. Experimental chicken lines were produced from broiler (non-broodiness chicken line) and pelung (Indonesian native chicken; broodiness chicken line) selective breeding for five generations. The broodiness event and body weight of each generation of chicken lines were observed and compared with Brown Leghorn as a non-broodiness outgroup. The body weight of chickens were measured weekly from hatch to 49 days of age. Molecular data were archived using restriction fragment length polymorphism (RLFP) and single-strand conformation polymorphism (SSCP). After five generations of genetics selection, at 49 days, the body weight of experimental chicken lines and growth rate were decreased, while broody behavior occurrence was diminished. RFLP and SSCP analysis failed to differentiate broody to non-broody genotype and excellent to poor body weight in each generation due to the non-specific primer. Our conclusion is that broodiness is a recessive trait and can be eliminated by selective breeding.

KEY WORDS broiler, pelung, prolactin, RLFP, SSCP.

INTRODUCTION

Indonesia is widely known for chicken domestication (Fumihito *et al*. 1994; Sulandari *et al*. 2017) having a diverse breed of chicken. Pelung (Iskandar and Susanti, 2007), Java (Ulfah *et al*. 2016; Sulandari *et al*. 2017), Sumatra (Ulfah *et al*. 2016), Cemani (Daryono *et al*. 2016; Dharmayanthi *et al*. 2017), and Nunukan (Tixier-Boichard *et al*. 1997) are the most popular breeds which have anthropologic and economic benefits.

These chicken breeds are rare and expensive; however, they have been massively cultivated. This condition arises due to low productivity of female parent stock. Moreover, in meat-type chicken if an imbalance in demand and supply occurred (Iskandar and Susanti, 2007), it might lead to extinction of chicken breeds. Following the Ethiopian chicken success (Okeno *et al*. 2012), to improve Indonesian local chicken, several prospective local chickens were crossbred to commercial breeds (Puspita *et al*. 2016; Saragih *et al*. 2016; Perdamaian *et al*. 2017).

Despite excellent final stock, the female parent lacks productivity. On the other hand, if large numbers of offspring are produced, their body weights were below the economical threshold at slaughter day.

For building Indonesian meat-type chicken, male pelung (promised meat-typed chicken) was mated to female broiler chicken (Retnoaji *et al*. 2016; Saragih and Daryono, 2016; Utama *et al*. 2018). Crowing-typed native chicken of Indonesia, pelung which also possess heavy body weight among other local breeds is being crossed to broiler to diminish broody behavior in their progeny but keep the body weight. Prolactin (Prl) was used as a molecular marker to differentiate broody genotype among progeny.

Genetics improvement programs are focus to pause the female chicken from laying eggs by selective breeding. Broodiness is not necessary after the introduction of artificial incubation. Chicken Prl is widely known to control avian ability to produce eggs and broodiness (Cui *et al*. 2006; Jiang *et al*. 2009). Plasma prolactin inhibits proliferation of follicle stimulating hormone.

Prolactin is produced by the anterior pituitary gland (Jiang *et al*. 2009), and is distributed to the entire body via circulation. Most of research on broodiness focus on Prl (Cui *et al*. 2006; Bhattacharya *et al*. 2011; Li *et al*. 2013). Avian Prl consists of five exons and four introns (Watahiki *et al*. 1989), and is located on chromosome 2 (Alipanah *et al*. 2011) whereas Prl receptor (Prl-R) consists of 15 exons and 14 introns (Rashidi *et al*. 2012). Quantitative traits loci are growth rate and laying performance, which is measured by quantitative genetics assay. Single-strand conformation polymorphism (SSCP) and random fragment length polymorphism (RFLP) were performed as described previously (Kalvatchev and Draganov, 2005; Fathi *et al*. 2014) targeted specific region contains alternative nucleotide. Single nucleotide polymorphism (SNP) is the most common variation in the genome caused by mutation and is widely used in candidate gene association study. These accumulated or standalone SNPs might later contribute to an emergence of new phenotypes (Patnala *et al*. 2013). Previous studies reveal a positive correlation of SNPs with growth traits and body measurements, e.g. number, day old chick (DOC) body weight, and age of sexual maturity (Rashidi *et al*. 2012). Recently, there were several research attempts to identify an association of Prl and Prl-P polymorphism and inbreeding depression to broody and body weight related trait in Indonesian chicken lines.

MATERIALS AND METHODS

Animal materials

Pure genetic lines of male pelung chicken were obtained from a reputable chicken breeder and were confirmed by crowing voice analysis, while female broiler was obtained from a commercial broiler breeder. Chicken mating took place at the breeding facility in the university farm (Pusat Inovasi Agroteknologi Terpadu) Bantul district, Yogyakarta special province of Indonesia. The breeding facility was open-sided pen which accommodated 12-hours of natural light. All chickens were given free access to feed and water and all produced eggs were artificially incubated using an incubator machine. Progeny were differently mated to produce third backcrossed chicken (BC_3) and forth filial (F_4) as two chicken lines (Figure 1). Recorded from 2012 to 2018 hatched DOC first filial (F_1) and its descent were raised following standard chicken care sheet to 49 days of age and were weighted every week (Fuller, 2009). The individual inbreeding coefficient was assessed as follows:

$$F(x) = \frac{1}{2}\sum\left[\left(\frac{1}{2}\right)^{n+1}(1+Fa)\right]$$

Fx: inbreeding coefficient.
Fa: inbreeding coefficient of shared ancestor.
n: amount of trace from shared ancestor to desired individual.

Chickens were given free access to accumulate egg and exhibit broody behavior. After laying eggs, female chicken was tagged as non-broody if they ignored to cover the accumulated egg on her nest more than one week and the otherwise for broody traits.

DNA extraction and analysis

The blood sample from each hen was collected by intravenous injection on the wing branchialis artery using 3 Gauge disposable syringes and stored in vacuum (DB VACOTAINER®). Whole genome chicken DNA was extracted according to the manufacture's protocol (ROCHE®). All DNA quantification was done using spectrophotometer and 1% agarose gel before amplified on thermal cycler (Biorad®).

Specific primers for Prl were; forward 5′-GCCTCAATTTCCAAACCAGAC-3′ and reverse 5'-TGCCTTCCAACCCCTATGAC-3′ and for Prl-P was: forward 5′-ATAACAATGGCCTGTCTTGC-3′ and reverse 5′-CCACTGATCCTCGAAAACTC-3′ according to previous research (Cui *et al*. 2006). The 25 µL of polymerase chain reaction (PCR) cocktail contained 50 ng of DNA template and 10 µL of PCR mix (Fast Start PCR Kit, ROCHE®), 0.25 mM forward primer, 0.25 mM reverse primer. The PCR protocol was 94 ˚C for 5 min followed by 30 cycles of 94 ˚C for 45 second, 50.6 ˚C (Prl-P) or 57 ˚C (Prl) for 1 min, and 72 ˚C for 1 min and a final extension at 72 ˚C for 10 min. The PCR products were electrophoresed on 1.5% agarose gel.

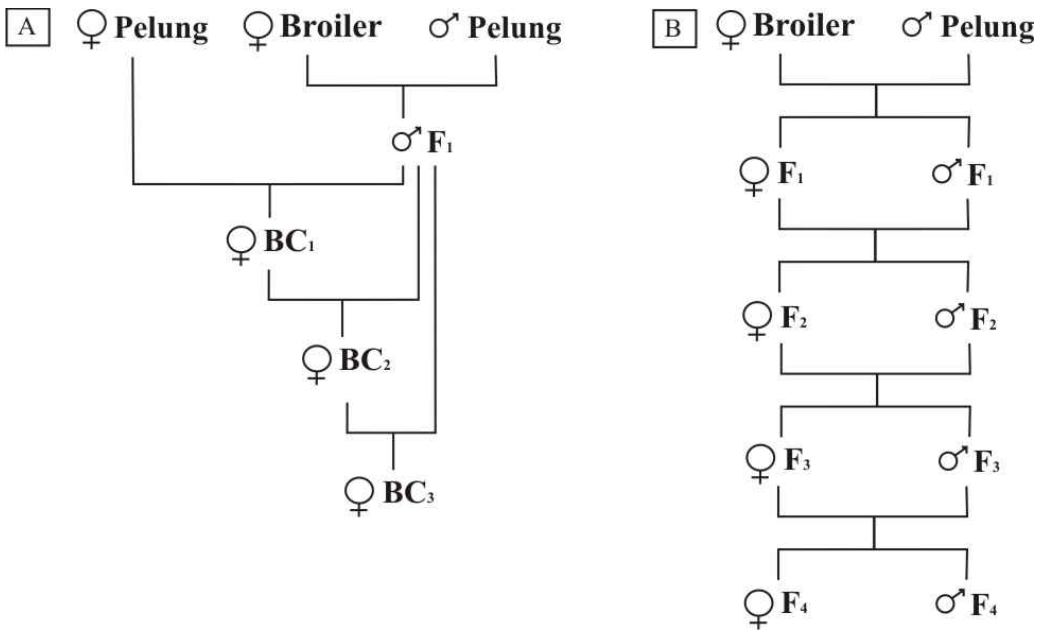

Figure 1 Representative chicken mating scheme to produce (A) third backcrossed (BC_3) and (B) forth filial (F_4) as two difference lines

The protocol for SSCP is as follows: 4 µL of PCR product was diluted to 16 µL loading buffer (95% formamide, 0.005% xylene cyanol FF, 0.005% bromphenol blue, 20 nM EDTA, 2 M urea) subsequently heat denatured using water bath then immediately snap chilled using ice block. The mixture was loaded to wells and running on vertical non-denaturing 8% polyacrilamide (37.5:1) gel electrophoresis. Gel was stained by ethidium bromide after electrophoresis. Restriction fragment length polymorphism (RFLP) protocol is as follows: A 10 µL PCR product was mixed to 18 µL H_2O, 2 µL 10× buffer R and 2 µL HindIII (Fermentas) and subsequently incubated for 4, 8, 12, 16, 20, and 24 hours at 37 ˚C. After incubation, mixtures were run in 2% agarose gel to visualize enzyme digestion. All acquired molecular data were analyzed using ANOVA one way (SPSS, 2011).

RESULTS AND DISCUSSION

Body weight inheritance in pedigreed chicken

Prolactin (Prl) is a member of growth hormone super family (Brooks, 2012), which has a broad range of effects on the organism. It was well known that prolactin contributes to growth and development (Kulibaba, 2015), female reproduction (Sangeeta Devi and Halperin, 2014), fish osmoregulation (Seale *et al.* 2013), and immunomodulation (Costanza *et al.* 2015). In poultry species, prolactin is reported to be closely associated with growth and biometrical traits in ducks (Mazurowski *et al.* 2016) turkey (Fathi *et al.* 2014) and chicken (Zerehdaran *et al.* 2004).

Repeated backcrossing and inbreeding mating were used to generate a fine introgression line. Chicken line which produce eggs continuously without broodiness, derived from repeated backcrossing and inbreeding. Backcrossed to its pelung parent, F_1 chicken presumed inherit growth characteristics and morphological characters to its progeny (BC_1, BC_2, BC_3, respectively).

Similarly, full-sib mating (F_1×F_1; F_2×F_2; F_3×F_3) were purposed to generate a morphologically uniform generation. It has been shown that F_1 gains more than 1300 g, while its descent BC_3 and F_4 had gained 750 and 850 g at seven weeks of age (Figure 2). Based on our research, weight gain was decreased due to the inbreeding depression caused by individual inbreeding coefficient and allel segregation (Figure 2). Inbreeding coefficient of evaluated chicken was 0.4375 (BC_3) and 0.422 (F_4).

Inbreeding depression might appear within progeny due to the expression of deleterious homozygous alleles (Panetto *et al.* 2010). Precisely, the weight gain trend through generation was alternately decreased. Not only in chicken, the evidence of inbreeding depression was found in a large number of both domestic (Panetto *et al.* 2010) and wild species (Christie *et al.* 2012) and indeed share similar symptoms. Under artificial selection process, major genes are segregated through generation. A long-term genetic selection might result in accumulation of homogeneity and frequencies of unfavorable alleles. The impact of additive gene action which originated from non-additive genes might collaborate in final phenotypes.

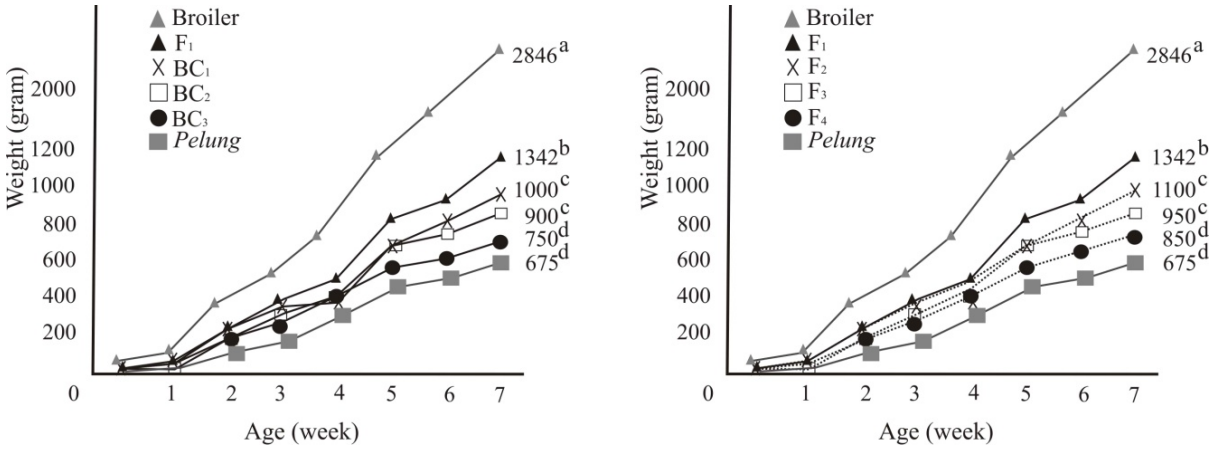

Figure 2 Growth rate of pedigreed chicken each generation after genetic selection through five generation
Backcross line (left) and Inbreed line (right)

Broody inheritance in pedigreed chicken

Prolactin plays has an important role in chickens during egg production since the onset of broodiness is initiated by the elevation of plasma prolactin which results in ovarian regression and stops egg production (Li *et al*. 2013). In our study, breeding facility exploited 8 to 10 hours of natural light to chicken photoperiodicity.

While local breeds (pelung, kampong, bantam) used as control exhibit broodiness in the same places in which several pedigreed Ayam progeny not show due to intrinsic factor as shown in Table 1.

Based on recent observations, the broody traits were disappeared after three serial backcross mating ($F_1 \times BC_2$) or two serial inbreed mating ($F_1 \times F_1$).

Furthermore, in backcross mating, BC_1 used as parents which broody traits inherit the behavior to third BC_2 progeny. Presumed, F_1, BC_1, and BC_2 were heterozygote and BC_3 was homozygote despite less objective due to small population.

Similarly, inbreeding lines which use same male F_1 as parent, generated F_2 and beyond (F_3, F_4, respectively) were homozygote for broody trait. Limited to our pedigree, a simple Punnet square can be applied to explain broody inheritance. Broody is a recessive trait, and controlled by one gene and one inhibitor gene. Broodiness is when the hen stops laying eggs and stays on nest for a long time. During broodiness, hen will show defensive act when something or someone approach and minimize feed intake (Romanov *et al*. 2002). Hen also routinely rotates her eggs to distribute heat evenly. Plasma prolactin was detected at highest during broodiness. There are several intrinsic (polymorphism, epigenetic) and extrinsic (humidity, temperature, feed availability, and photoperiodicity) (Geng *et al*. 2014) factors which affect broodiness. Polymorphism and epigenetic status (DNA methylation, histone acetylation) on regulating genes like prolactin, progesterone, and luteinizing hormone influence body metabolism which definitely alter organism behavior toward broodiness.

Table 1 The occurrence of broodiness within progeny of pedigreed chicken compared to positive control (kampong, pelung, bantamn) and negative control (broiler and Brown Leghorn)

Generation	N	Broody behavior (%)
Kampong	3	100
Bantamn	2	100
Pelung	5	100
Broiler	5	0
Brown leghorn	4	0
F_1	6	33
F_2	5	0
F_3	5	0
F_4	5	0
BC_1	5	33
BC_2	5	33
BC_3	5	0

Polymorphism on prolactin and prolactin promtor

The effect of Prl and Prl-P gene polymorphism on growth traits and broodiness were analyzed.

The relationship between body weight and broody event with identified genotypes in two chicken lines (backcross and inbreed) were examined. Since only one haplotype was produced during experiment, Table 1 does not contain data concerning the association of this genotype with growth and broody traits in the evaluated chickens.

In this study, vertical and horizontal gel electrophoresis failed to differentiate two traits. A specific primer amplified nucleotides 395-576 from a total 4391 bp of chicken Prl and Prl-P with presumed variability. According to a previous report by utilizing a sequence primer will be able to detect polymorphism in White Leghorn, Yangshan, Taihe Silke, White Rock, and Nongdahe but monomorphic in Indonesian chicken, it might be Prl and Prl-P conserve region in Indonesian chicken. Current Brown Leghorn, Broiler, and pelung specimen was possess monomorphic sequence but exhibit difference broody traits might be from polymorphism on other region. The inclusion of peppermint and anti-prolactin drug on feed cause diminshed broody behavior (Eltayeb *et al*. 2010). Other report indicated the plasma level of prolactin may vary on different chicken lines in tropical climate (Banu *et al*. 2017). These findings show that broodiness trait might be regulated on proteomic level (Banu *et al*. 2017). Another possibility, recent instrument was not precise to visualize polymorphism. Based on this research, broodiness is a recessive trait which is controlled by a single promoter gene and single inhibitor gene whereas polymorphism on prolactin (Prl) and prolactin promoter (Prl-P) fragment were not detected.

CONCLUSION

Broodiness is a recessive trait can be annihilated by selective breeding program. Prolactin is importance marker for egg production-related traits in chicken.

ACKNOWLEDGEMENT

The authors wish to acknowledge Pusat Inovasi Agroteknologi Terpadu (PIAT) Gadjah Mada University for providing breeding facility and research funding (Grant No: UGM/KP4/224/C/03/06).

REFERENCES

Alipanah M., Kamal S. and Hussein K.B. (2011). The polymorphism of prolactin gene in native chicken Zabol region. *J. Anim. Vet. Adv*. **10(5),** 619-621.

Banu N., Rashid M.B., Hasan M.M., Aziz F.B., Islam M.R. and Haque M.A. (2017). Effect of anti-prolactin drug and peppermint on broodiness, laying performance and egg quality in indigenous hens. *Asian J. Med. Biol. Res*. **2,** 547-557.

Bhattacharya T.K., Chatterjee R.N., Sharma R.P., Rajkumar U., Niranjan M. and Reddy B. (2011). Association of polymorphism in the prolactin promoter and egg quality traits in laying hens. *British Poult. Sci*. **52(5),** 551-557.

Brooks C.L. (2012). Molecular mechanisms of prolactin and its receptor. *Endocr. Rev*. **33(4),** 504-525.

Christie M.R., Marine M.L., French R.A. and Blouin M.S. (2012). Genetic adaptation to captivity can occur in a single generation. *Proc. Natl. Acad. Sci*. **109(1),** 238-242.

Costanza M., Binart N., Steinman L. and Pedotti R. (2015). Prolactin: A versatile regulator of inflammation and autoimmune pathology. *Autoimmun. Rev*. **14(3),** 223-230.

Cui J.X. Du H.L., Liang Y., Deng X.M., Li N. and Zhang X.Q. (2006). Association of polymorphisms in the promoter region of chicken prolactin with egg production. *Poult. Sci*. **27,** 208-214.

Daryono B.S., Roosdianto I. and Saragih H.T.S. (2016). Pewarisan karakter fenotip Ayam hasil persilangan Ayam pelung dengan ayam cemani phnotypical characters in hybrids chicken of crossbreeds between pelung and cemani. *J. Veterinar*. **11(4),** 257-263.

Dharmayanthi A.B., Terai Y., Sulandari S., Zein M., Arifin S., Akiyama T. and Satta Y. (2017). The origin and evolution of fibromelanosis in domesticated chickens: Genomic comparison of Indonesian cemani and chinese silkie breeds. *PLoS One*. **12(4),** 1-24.

Eltayeb N.M., Wani C.E. and Yousif I.A. (2010). Assessment of broodiness and its influence on production performance and plasma prolactin level in native chicken of the Sudan. *Asian J. Poult. Sci*. **4,** 1-6.

Fathi M. and Tabriz T., Zarringhobaie E. and Ghorban. (2014). Association of polymorphisms in the promoter region of turkey prolactin with egg performance. *Genetika*. **46(2),** 591-599.

Fuller M.F. (2009). The Encyclopedia of Farm Animal Nutrition. Rowett Research Institute, Aberdeen, United Kingdom.

Fumihito A., Miyake T., Sumi S., Takada M., Ohno S. and Kondo N. (1994). One subspecies of the Red Junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. *Proc. Natl. Acad. Sci. USA*. **91,** 12505-12509.

Geng A.L., Xu S.F., Zhang Y., Zhang J., Chu Q. and Liu H.G. (2014). Effects of photoperiod on broodiness, egg-laying and endocrine responses in native laying hens. *British Poult. Sci*. **55(2),** 264-269.

Iskandar S. and Susanti T. (2007). Karakter dan manfaat Ayam pelung di Indonesia. *Wartazoa*. **17(3),** 128-135.

Jiang R.S., Zhang L.L., Geng Z.Y., Yang T. and Zhang S.S. (2009). Single nucleotide polymorphisms in the 5'-flanking region of the prolactin gene and the association with reproduction traits in geese. *South African J. Anim. Sci*. **39,** 83-87.

Kalvatchev Z. and Draganov P. (2005). Single-strand conformation polymorphism (SSCP) analysis: A rapid and sensitive method for detection of genetic diversity among virus population. *Biotechnol. Biotechnol.Equip*. **3,** 9-14.

Kulibaba R.A. (2015). Polymorphism of growth hormone, growth hormone receptor, prolactin and prolactin receptor genes in

connection with egg production in poltava clay chicken. *Sel Skokhozyaistven. Biol*. **50(2),** 198-207.

Li H.F., Shu J.T., Du Y.F., Shan Y.J., Chen K.W., Zhang X.Y., Han W. and Xu W.J. (2013). Analysis of the genetic effects of prolactin gene polymorphisms on chicken egg production. *Mol. Biol. Rep*. **40,** 289-294.

Mazurowski A., Mazurowski A., Frieske A., Wilkanowska A., Kokoszyński D., Mroczkowski S., Bernacki Z. and Maiorano G. (2016). Polymorphism of prolactin gene and its association with growth and some biometrical traits in ducks. *Italian J. Anim. Sci*. **15(2),** 200-206.

Okeno T.O., Alexander K.K. and Kurt J.P. (2012). Genetic and economic evaluation of alternative breeding objectives for adoption in the smallholder Indigenous chicken improvement program. Pp. 1-4 in Proc. Conf. Int. Res. Food Secur., Hambur, Germany.

Panetto J.C.C., Gutiérrez J.P., Ferraz J.B.S., Cunha D.G. and Golden B.L. (2010). Assessment of inbreeding depression in a Guzerat dairy herd: Effects of individual increase in inbreeding coefficients on production and reproduction. *J. Dairy Sci*. **93(10),** 4902-4912.

Patnala R., Judith C. and Jyotsna B. (2013). Candidate gene association studies: A comprehensive guide to useful in silico tools. *BMC Genet*. **14,** 39-45.

Perdamaian A.B.I., Saragih H.T.S.S.G. and Daryono B.S. (2017). Effect of varying level of crude protein and energy on insulin-like growth factor-i expression level in Indonesian hybrid chicken. *Int. J. Poult. Sci.* **16(1),** 1-5.

Puspita U.E., Utomo R.T., Perdamaian A.B.I., Lesmana I., Arijuddin H., Erwanto Y., Daryono B.S. and Saragih H.T.S.G. (2016). Effect of varying levels of protein and energy in prestater feeds on pectoralis muscle development of kampung super chicks (*Gallus gallus gallus*). *Asian J. Anim. Vet. Adv.* **12(1),** 31-37.

Rashidi H., Ghodrat R.M., Farhadi A. and Gholizadeh M. (2012). Association of prolactin and prolactin receptor gene polymorphisms with economic traits in breeder hens of indigenous chickens of Mazandaran province. *Iranian J. Biotechnol*. **10(2),** 129-135.

Retnoaji B., Wulandari R., Nurhidayat L. and Daryono B.S. (2016). Osteogenesis study of hybrids of indonesia's native chicken pelung (*Gallus gallus domesticus*) with broiler (*Gallus gallus domesticus*). *Asian J. Anim. Vet. Adv.* **11(8),** 498-504.

Romanov M.N., Talbot R.T., Wilson P.W. and Sharp P.J. (2002). Genetic control of incubation behavior in the domestic hen. *Poult. Sci*. **81(7),** 928-931.

Sangeeta Devi Y. and Halperin J. (2014). Reproductive actions of prolactin mediated through short and long receptor isoforms. *Mol. Cell. Endocrinol*. **382(1),** 400-410.

Saragih H.T.S.S.G. and Daryono B.S. (2016). Histological study on the pancreatic beta -cell number of indigenous chicks in first crossbred (F1). *J. Indonesian Trop. Anim. Agric.* **35(3),** 201-205.

Saragih H.T.S.S.G., Utomo R.T., Perdamaian A.B.I., Puspita U.E., Lesmana I. Arijuddin H., Erwanto Y. and Daryono B.S. (2016). The effect of early posthatch local feed in pectoralis muscle of jawa super chicks (*Gallus gallus domesticus*). *AIP Conf. Proc.* **1755(1),** 1-6.

Seale A.P., Yamaguchi Y., Johnstone W.M., Borski R.J., Lerner D.T. and Grau E.G. (2013). Endocrine regulation of prolactin cell function and modulation of osmoreception in the *Mozambique tilapia*. *Gen. Comp. Endocrinol*. **192,** 191-203.

SPSS Inc. (2011). Statistical Package for Social Sciences Study. SPSS for Windows, Version 20. Chicago SPSS Inc., USA.

Sulandari S., Zein M.S.A. and Sartika T. (2017). Molecular characterization of Indonesian indigenous chickens based on mitochondrial DNA displacement (D)-loop sequences. *Hayati J. Biosci*. **15(4),** 145-154.

Tixier-Boichard M., Boulliou-Robic A., Morisson M., Coquerelle G., Horst P. and Benkel B. (1997). A deleted retroviral insertion at the Ev21-K complex locus in Indonesian chickens. *Poult. Sci.* **76(5),** 733-742.

Ulfah M., Kawahara-Miki R., Farajalllah A., Muladno M., Dorshorst B., Martin A. and Kono T. (2016). Genetic features of red and green junglefowls and relationship with Indonesian native chickens sumatera and kedu hitam. *BMC Genom*. **17(1),** 320-328.

Utama I.V., Perdamaian A.B.I. and Daryono B.S. (2018). Plumage uniformity, growth rate and growth hormone polymorphism in Indonesian hybrid chickens. *Int. J. Poult. Sci.* **17(10),** 486-492.

Watahiki M., Tanaka M., Masuda N., Sugisaki K., Yamamoto M., Yamakawa M., Nagai J and Nakashima K. (1989). Primary structure of chicken pituitary prolactin deduced from the CDNA sequence. Conserved and specific amino acid residues in the domains of the prolactins. *J. Biol. Chem*. **264(10),** 5535-5539.

Zerehdaran S., Vereijken A.L.J., Van Arendonk J.A.M. and Van Der Waaij E.H. (2004). Estimation of genetic parameters for fat deposition and carcass traits in broilers. *Poult. Sci*. **83(4),** 521-525.