NHERITANCE OF EXTERNAL GENETIC CHARACTERISTICS IN CHICKEN THROUGH TRIPLE CROSSING MODEL

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NHERITANCE OF EXTERNAL GENETIC CHARACTERISTICS IN CHICKEN THROUGH TRIPLE CROSSING MODEL

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Abstract

Background and objective: *The external genetic inheritance in chickens is very important to know because as a basis for the development of pure race, commercial, and the formation of uniformity in coat color. The purpose of this study was to determine the Inheritance pattern of characteristic external genetic in chicken through the triple crossing model.* ***Materials and methods:*** *Chicken mating uses three types of chickens, namely: Kampung Chicken (K), Broiler Chicken (B), and Leghorn Chicken (L). A total of 257 cross hens were observed from four genetic groups: K♂ x B♀; K♂ x L♀; KB♂ x KL♀; KL♂ x KB♀. External genetic characteristics observed included feather color, feather color pattern, fur pattern, flicker fur, shank color, and comb shape.* ***Results:****External Genetic Characteristics of chickens through the triple crossing model resulted in the External Genetic Constitution in KLB and KBL chickens (generation F2) is iiee ss pp IdId and there are six combination of feather color patterns produced namely Black (E_), Colombian (ee), Wyandotte Columbian (ee), brown/light brown (e^b), dark brown (e^{db}), and wild (e^+). The average Heterozygosity of expectations per individual (H) for Genes of External Characteristic in KLB chickens is0.39428±0.118, and KBL chickens amounted to 0.38074±0.1136.*

Conclusion*: External Genetic Characteristics of chickens through the triple crossing model produce six combinations of feather color patterns which meets the Hardy-Weinberg balance, with External Genetic Constitution (KBLand KLB chicken) in the F2 generation are ii ee ss pp, IdId that have the gene diversity is 0.38074 ± 0.1136 (KBL) - 0.39428 ± 0.118 (KLB).*

Keyword: *Inheritance, The crosses chicken, external genetics, triple crossing.*

INTRODUCTION

Land races or traditional varieties have been found to have higher stability (adaptation over time) in low-input agriculture under marginal environments, thus, their cultivation may contribute farm level resilience in face of food production shocks being recognized as a specific area since exploding population with urbanization and decreasing cultivable lands are the critical factors contributing to food insecurity in developing world[2, 4]. Agricultural scientists realized that PGD can be captured and stored in the form of plant genetic resources (PGR) such as gene bank, DNA library[3], and so forth, in the biorepository which preserve genetic material for long period[5]. Recognizing the importance of PGRFA, the Conference of the Food andAgriculture Organization of the United Nations (FAO), at its Twenty-sixth Sessionin 1991, agreed that a first Report on the State of the World's Plant GeneticResources, for Food and Agriculture should be developed[6]. In Indonesia, the domestic (indigenous) chicken arescattered throughout the archipelago.

Apparently theyhave a lot of diversity with different morphologic characteristics[7]. This diversity is important information for determining policies for the improvement and development of effective breeding programs[8]. The varieties are based on base colour of feather, colour of the plumage, flick feather, pattern of feather, shank colour and comb types[9, 11] Plant and animal breeders introduced desirable genes and eliminateundesirable ones slowly, altering in the process of underlying heredity principle for several decades[10, 12, 13]. Local chickens in Indonesia are very diverse, and spread to rural areas, but these chickens have low productivity[11]. This diversity is important information for determining policies for the improvement and development of effective breeding programs[8],

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for qualitative and quantitative traits[12]. The occurrence of mutations, natural selection and crosses cause genetic drites and gene combinations to change, thus affecting qualitative traits in livestock, besides extreme environmental changes in the region especially in the equatorial region which can affect the work of genes in environmental adaptation. Inheritance of qualitative traits in local chickens is directly related to the ability of adaptation to the environment[8, 13, 14].

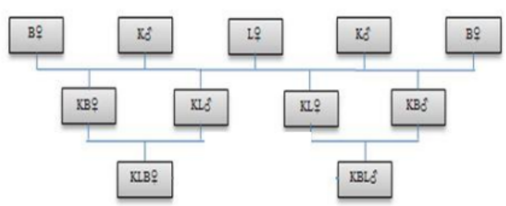
Fur color control gene in Local chickens, example; (ii e + _ bb ss idid, pp), Wareng chicken (I_ E_ bb S_ Id_ pp), White Leghorn (II EE SS BB IdId pp)[15] Qualitative traits in chickens are external genetic characteristics that are controlled by genes[16], the polygenic traits[21], and is a visible external character, with little or no relation to production capability, although qualitative traits describe the existence and characteristics of livestock physically[17]. Qualitative traits are only controlled by a pair of genes and only a few are influenced by environmental factors. Qualitative traits such as color are important in shaping the characteristics of clumps and have been used since domestication as a tool for shaping clumps and selection activities, such as variations in the shape of horns and feather colors, perhaps helping to understand the history of clumps, their demographics and genetic characteristics[18]. recent years, it has become increasingly important to protect national endemic genetic resources and use local breeds to create commercial strains that can adapt to the changing environment[19].

Since the external genetic information of Walik chickens is very limited, therefore, the study on the qualitative traits of such rare indigenous chicken is necessary to support their comprehensive repertoire that would be useful for their preservation efforts and potency development[20]. Improvement of genetic quality of livestock through selection and crossing is determined by the inheritance strength of traits that are enhanced and passed down to the next generation[21, 23], Observation of external genetic diversity in chickens has been widely reported to illustrate the potential of available genetic resources but has not yet described the form of crossing that occurs, and also the genetic balance achieved under what conditions[24]. To improve genetic quality, selection and crossbreeding between animals are carried out, but the genetic balance cannot be achieved in a single cross[18, 25].

This external genetic inheritance is related to pigmentation, the distribution of pigments in primary and secondary feather colors in chickens. Observation of external genetic inheritance through triple crossing activities can provide information about the work of non-allelic genes that are not additive in the next generation[26]. indicate that there is abundant polymorphism in the *MC1R* gene, especially in Hebei chicken, which was associated with its rich plumage colour diversity[27]. Crossbreeding in beef cattle results in a decrease in body weight of 2.5-5.0 kg per 10% increase in cross[28]. This paper will indicate the significance of genetic conservation and its analytic tools and techniques that are made widely available for utilization in postgenomic era.

MATERIALS AND METHODS

The study was carried out at Agriculture Department, Quality Control Lab from July 2017-October 2019.The ingredients used in this study were three types of chicken, namely local male chicken (K), broiler female chicken (B) and Leghorn female chicken (L). This study was carried out of the Poultry Laboratory, Department of Animal Husbandry, Gorontalo State University from August 2017-Oct 2019). A total of 257 cross chickens have been produced from four genetic groups: K♂x B♀; K♂x L♀; KB♂ x KL♀; KL♂ x KB♀.Chicken mating uses a triple crossing model (Figure 1)



(Figure 1. Triple Crossing Model)

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Procedure

Stage 1;Genetic group: K♂ x B♀. Local chicken phenotype (K) hasblack-red feathercolor, single comb, yellow shank color, while Broiler female chicken hasa phenotype of colorless feather color (white), single comb and yellow shank color. The result of the cross is called KB chicken (F1). Genetic group: K♂ x L♀. Leghorn chickens (L) have a brown feather phenotype, a single comb, and a yellow shank color, while local chickens are the same chicken at the first cross. The result of the crossing is called KL chicken (F1).

Stage 2:Genetic group: KB♂ x KL♀ (F1 Generation). KB chickens have a colorless feather color (white), a yellow shank color, a single comb, while KL♀ chicken has a brown/columbian feather color, a single comb, and a yellow shank color. The result of the junction is called KBL chicken (F2). Genetic group: KL♂ x KB♀. KL chicken have brown feathers, yellow shank color, and a single comb. KB chicken has columbian feather color, yellow shank color, single comb. The result of the crossing is called the KLB chicken (F2).

Statistical Analysis

Phenotype proportion of feather color, feather pattern, shank color, feather flicker, comb shape, analyzed descriptively based on the formula[12].

$$Phenotype\ proportin = \frac{\Sigma Phenotype\ A}{N}\ x\ 100$$

Where: Phenotype A: the number of individuals who have phenotype A., N: the number of individuals observed

Frequency of the double allele gene

The frequency of the feather pattern gene which is a double allele is calculated by the formula: P = 1- q - r[18]. because the results of mating F2 produce more than four feather color patterns, so the double frequency of the allele gene is calculated by a mathematical equation: (p + q + r ... + s) = 1, where: p, q, r, and s are the color patterns that result from crossing

$$P = 1 - q - r - s$$

$$s = \left(\frac{\text{Number of individuals with recessive allele (e)}}{} \right)^{1/2}$$

$$r = \left(\frac{\text{Number of individuals with alel e and } (e^b)}{\text{Total number of individuals}} \right)^{1/2} ;$$

$$q = \left(\frac{\text{Number of individuals with alel e and } (e^+)}{\text{Total number of individuals}} \right)^{1/2} - s$$

Where:p = Frequency of gene E; q = Frequency of gene e^+;
r = Frequency of gene e^b; s = Frequency of gene e

The Hardy-Weinberg test was carried out to compare the differences between the phenotype characteristics observed using Chi-square analysis.

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

Where: X: chi-square;O: Observation;E: expected

The Heterozygosity Value of gene

Calculation of expectation heterozygosity per individual (h) and average expectation heterozygosity per individual (H)based on the formula suggested[4, 10]

$$H = 1 - \sum_{i}^{n} qi^2$$

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Where:h =Average of the expected heterozygosity per individual; qi = Frequency of genetic

RESULTS AND DISCUSSION

The phenotypes of the external genetic characteristics

The proportion of the external genetic characteristics (feather color, color patterns of feathers, feathers flickering, color shank and comb shape from mating results through the triple crossing model are presented in Table 1.

The proportion of colorless feathers (white) in the F2 generation decreased, while the proportion of colored feathers increased by 6.435%, compared to the f1 generation. This is due in marriage between the F2 generation using chickens that have colored feathers.The feather color patterns for F1 generation chickens (KB chickens) produce three color patterns namely Black, wild, columbian and in KL chickens are black, brown / light brown, and Columbian while generation F2 (KL♂ x KB♀ and KB♂ x KL♀) produced six color patterns of black, wild, brown, columbian, wyandotte columbian and dark brown, with different percentages for each crossing group (table 1).

The columbian color pattern in the F1 and F2 generations has the highest proportion of 38.78 - 68.75%, followed by the wild type, brown/light brown, and dark brown color pattern. The dominance of the ee (columbian) allele in the results of this crossing.Illustrates the role of this allele very strongly, and together with the e^+, e^b, e^{db} alleles inhibit the formation of black at the E_ locus. Columbian alleles (ee) limit the work of the allele from locus E in the distribution of melanin pigments, further stated some alleles that limit the distribution of eumelanin in primary feathers, namely alleles e^{db} (dark brown), Co (Columbian), and Mh(mahogany), the work of these three alleles will have an effect when interacting with the locus E on the back, wings, legs, and tail feathers. Plain black gene (E) locus black feathers in all parts of the body. Wild color genes (e+) can be seen with the presence of a black line extending to the back, and columbian gene (e) there is a black color on the surface of the fur which is restricted to the neck, wings, and tail[17]. The brown/light brown color (e^b) is a mutation of the allele at the E locus which inhibits the work of the locus E allele resulting in a brown color. Carotene pigments give a yellow, and orange color, while the melanin pigment gives black, and red color to the feathers of birds and if the two pigments are not found then plain white feathers are raised[19].Stated at locus E show a significant correlation between MC1R polymorphisms and the presence of different alleles at locus E[2]. All populations carrying alleles are responsible for activation of receptors in produce eumelanin, which is needed to express the expanded black phenotype. The columbian White Wyandotte pattern results from mating KLB and KBL chickens, there may be the cooperation of overlapping alleles at the E locus, the eb allele inhibits the E gene at the E locus, whereas ii alleles inhibit the formation of buff in the E locus. Wyandotte white, such as columbian and genes are heterozygous for buff restriction[8].

Based on the chi-square test for external genetic characters (table1): feather color, feather pattern, flickering feather, and shank color in the F1 generation are not in Hardy-Weinberg balance ($p > 0.05$), except the comb shape ($p < 0.05$) because of the form of a recessive homozygous comb (pp). The F2 generation for fur color patterns and comb shapes are in Hardy-Weinberg balance ($p < 0.05$), while other characters are not reached ($p > 0.05$). The feather color pattern is in a balanced state (Hardy-Weinberg balance) because triple crossing causes genes and alleles to work together and form diverse feather color patterns, and spread evenly in the population, where alleles affect each other at the E_ locus expressed in the next generation.

Feathers color is a polygenic trait[13], whereas different fur colors (Black, yellow, Black-red) in the MC1R gene show a lot of polymorphism[21].Hardy-Weinberg's rule states that in large populations no selection, mutation and migration and marriages occur randomly (at least in one generation), the frequency of genes remains the same from generation to generation[7]. The population is said to be in Hardy-Weinberg balance[22].

The Frequency of external characteristic controlling genes in cross chickens

The frequency of genes feather color, feather patterns, flickering fur, shank color, and comb shape in the triple crossing model is presented in Table 2. The Gene of Controlling External Characteristics formed on KB chickens (F1 Generation) are I_E_bbS_Id_pp while KL chicken (F1

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generation) are iieebbssId_ pp. The KLB and KBL chickens (F2 generation) have a controlling gene external characteristics are ii ee bbssIdId pp. Constitution controlling gene external characteristics the KB chicken has similarities with wareng chicken, while the KL chicken, KLB and KBL have the same constitution controlling gene Rhode Island Red chickens, Merawang chickens and local chickens. Constitution genes controlling external characteristics of wareng chickens is I_ E_ bb S_ Id_ pp, Native chickens is ii ee^{+} bb ss idid PP, Rhode Island Red chickens are ii ee ss IdId pp, while Merawang chicken is ii ee ss bb IdId pp[11,15].

Genetic variability of crossbred chickens

Genetic variability (diversity) of crossbreeding chickens (KB, KL, KBL, and KLB) is based on the value of heterozygosity per individual (h) and the average heterozygosity per individual (H) is presented in table 3.

These results illustrate the comb shape on F1 and F2 generations are uniform, which is indicated by the value of the expected heterozygosity of individual (h) is 0. This is in accordance with who stated that a population has low gene diversity if it has a value of heterozygosity less than 0.5[6].

The color pattern of feathers in F1 and F2 chickens shows high gene diversity, indicated by the expected heterozygosity value (h) is relatively high that is equal to 0.61509 ± 0.125-0.74148 ± 0.118, although, in a limited population. Local chickens have variations in feather color patterns with an individual's expected heterozygosity (h) of 0.6596 and of 0.35-0.45[14]. The average expected heterozygosity per individual is the average proportion of heterozygosity per locus in the population that randomly mating[10].

Suggested heterozygosity values ranging from 0 (zero) to 1 (one), and if the value of heterozygosity is 0 (zero) than among the population measured has a very close genetic relationship and if the value of heterozygosity is 1 (one) then the population measured does not have a genetic relationship[10].State that genetic variability in a population is known by calculating the proportion of polymorphic loci (Poly), the average expected heterozygosity per individual (H) in the number of effective alleles per locus (Ne)[4].Suggested that genetic variability can increase or decrease in the population and depends on the condition of the livestock[9].

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Table 1. The proportion of qualitative traits in KB, KL, KBL and KLB chickens through the crossing and hardy-Weinberg balance test

No	Fenotipe	Crossing-group				Hardy-Weinberg Test			
		K♂ x B♀ (KB)	K♂ x L♀ (KL)	KL♂x KB♀ (KLB)	KB♂ x KL♀ (KBL)	X^2count/X^2 table KB	X^2count/X^2 table KL	X^2count/X^2 table KLB	X^2count/X^2 table KBL
		n = 71	n = 57	n = 69	n = 70				
1	Feather Color								
	White (I)	66.20	15.79	39.13	30.00	7.45/3.841	26.68/3.841	7.81/3.841	11.20/3.841
	Colored(i)	33.80	84.21	60.87	70.00				
		100.00	100.00	100.00	100.00				
2	Feather pattern								
	Balack (E_)	8.33	2.08	19.05	8.16	11.58/7.815	52.11/7.815	4.72/9.48	4.44/9.48
	Wild (e^+)	37.50	0.00	16.67	24.49				
	Brown/ Light Brown (e^b)	0.00	29.17	14.29	20.41				
	Columbian (ee)	54.17	68.75	42.86	38.78				
	W.W Columbian(ee)*	0.00	0.00	4.76	4.29				
	Drak brown (e^{db})	0.00	0.00	2.38	2.04				
		100.00	100.00	100.00	100.00				
3	Fur pattern								
	Barret (B)	18.31	17.54	33.33	31.88	11.45/3.841	24.02/3.841	12.95/3.841	11.85/3.841
	Non-barret (b)	81.69	82.46	66.67	68.12				
		100.00	0.00	100.00	100.00				
4	Fur Flicker								
	Silver (S)	11.11	0.00	26.67	17.65	6.45/3.841	4.00/3.841	3.60/3.841	3.00/3.841
	Gold (s)	88.89	100.00	73.33	82.35				
		100.00	100.00	100.00	100.00				
5	Shank Color								
	Yellow/white (Id)	87.32	70.18	81.16	75.36	39.56/3.841	9.28/3.841	30.22/3.841	19.06/3.841
	Black/Gray (id)	12.68	22.81	13.04	15.94				
	Green (id)	0.00	7.02	5.80	8.70				
		100.00	100.00	100.00	100.00				
6	Comb Shape								
	Single (pp)	100.00	100.00	100.00	100.00	0	0	0	0
		100.00	100.00	100.00	100.00				

Information:

- *Genetic Group K(♂) x B(♀) : KB chicken⁾*
- *Genetic Group K(♂) x L(♀) : KL chicken⁾*
- *Genetic Group KB(♂) x KL(♀) : KBL chicken⁾*
- *Genetic Group KL(♂) x KB(♀) : KLB chicken⁾*

KB), KL⁾ : F1 Generation

KBL),KLB⁾ : F2 Generation

** W. whydonett Columbian (ee) : Columbian group (ee)*

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Table 2. Gene Frequency in feather colors, feather patterns, flickering feathers, shank colors, and comb shapes in the triple crossing model

No	Genotipe	Frequency genes			
		K♂ x B♀(KB)	K♂ x L♀(KL)	KL♂ x KB♀(KLB)	KB♂ x KL♀(KBL)
		n = 71	n = 57	n = 67	n = 61
1	Feather color				
	q^I	0.8310	0.0833	0.1957	0.1094
	q^i	0.1690	0.9167	0.8043	0.8906
		1.000	1.000	1.000	1.000
2	Feather pattern				
	q^E	0.2614	0.2317	0.1834	0.0346
	q^{e+}	0.2213	0.0000	0.2252	0.2673
	q^{eb}	0.0000	0.3169	0.2085	0.2928
	q^{ee}	0.5173	0.4513	0.3612	0.4053
	q^{ee} (C. wynadotte)*	0.000	0.000	0.0145	0.0214
	q^{db}	0.000	0.000	0.0071	0.0072
		1.000	1.000	1.000	1.000
3	Fur pattern				

Table 3. Expected heterozygosity per Individual (h) and the average of expected heterozygosity per Individual (H) in KB, KL, KLB and KBL chickens

Phenotype	KB Chicken	KL Chicken	KLB Chicken	KBL Chicken
Feather Color	0.28090±0.101	0.15278±0.119	0.31477±0.110	0.19842±0.073
Feather Color Pattern	0.61509±0.115	0.64215±0.118	0.74148±0.125	0.67686±0.122
Feather Pattern	0.42604±0.125	0.47502±0.125	0.49964±0.126	0.47877±0.125
Feather Flicker	0.48000±0.125	0.00000	0.49837±0.125	0.48960±0.125
Shank color	0.17382±0.072	0.41644±0.122	0.31148±0.107	0.44422±0.123
Comb shape	0.00000	0.00000	0.00000	0.00000
Average	0.320312±0.107	0.28106±0.096	0.39428±0.118	0.38074±0.1136

	1.000	1.000	1.000	1.000

Information:
q^I: colorless feathers, q^i: colored, q^{ZB}: Barred, q^{Zb}: non-barred, q^{ZS}: silver, q^{Zs}: gold, q^E: black, q^{e+}: liar, q^e: columbian, q^p: single, IdId: yellow/white, idid: black / gray / green
* *White Whydonett Columbian: Columbian group (ee)*

Significant statement: This study finds through Triple Crossing Models, that can be beneficial for native chicken breeders and produce more varied chicken quality. This study will help the researcher to uncover the critical areas of Poultry that many researchers were unable to explore. Thus a new theory on crossing chicken models local chicken may be arrived at.

CONCLUSION

External Genetic Characteristics of chickens through the triple crossing model produce six combinations of feather color patterns are Black (E_), columbian (ee), Wyandotte columbian (ee), brown / light brown (e^b), dark brown (e^{db}), and wild (e^+) which meets the Hardy-Weinberg balance, with External Genetic Constitution (KBLand KLB chicken) in the F2 generation are ii ee ss pp, IdId that have the gene diversity is 0.38074 ± 0.1136 (KBL) - 0.39428 ± 0.118 (KLB).

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