Analysis of Meat Mineral Content in Cemani Chicken with Homozygous (*Fm/Fm*) and Heterozygous (*Fm/fm*$^{+}$) Genotypes

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(received 16–08–2022; revised 13–09–2022; accepted 15–09–2022)

ABSTRAK

Safitry RS, Dharmayanthi AB, Kinoshita K, Akiyama T, Darwati S, Kostaman T, Sopiyana S, Khaerunnisa I, Sumantri C. 2022. Analisa kandungan mineral daging pada ayam Cemani dengan genotipe homozigot (*Fm/Fm*) dan heterozigot (*Fm/fm*$^{+}$). JITV 27(4):195-203. DOI : http://dx.doi.org/10.14334/jitv.v27i4.3075.

Ayam Cemani adalah ayam asli Indonesia dengan hiperpigmentasi hitam pada bulu, kulit, paruh, jengger dan daging. Hiperpigmentasi hitam pada ayam Cemani ini dikenal dengan istilah fibromelanosis. Fibromelanosis pada ayam Cemani bersifat semidominant yang menghasilkan dua genotipe: homozigot (*Fm/Fm*) dan heterozigot (*Fm/fm*$^{+}$). Warna hitam pada daging ayam Cemani dapat menunjukkan adanya kandungan mineral daging yang lebih tinggi dibandingkan daging ayam pada umumnya. Penelitian ini bertujuan untuk mendeteksi genotipe homozigot (*Fm/Fm*) dan heterozigot (*Fm/fm*$^{+}$) serta mengetahui kandungan mineral pada ayam Cemani dengan genotipe homozigot (*Fm/Fm*) dan heterozigot (*Fm/fm*$^{+}$). Pada populasi ayam cemani (n= 32), genotipe alel spesifik *Fm* dideteksi dengan Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR–RFLP) dan enzim restriksi *Mlu*I. Kandungan mineral ayam Cemani yang diuji adalah Fe, Zn, Mn, dan Se. Hasil penelitian menunjukkan bahwa ayam Cemani homozigot (*Fm/Fm*) memiliki kandungan mineral Fe dan Zn yang lebih tinggi. Namun ayam Cemani homozigot (*Fm/Fm*) tidak berbeda kandungan mineral Se dan Mn dengan ayam Cemani heterozigot (*Fm/fm*$^{+}$). Penelitian ini menemukan bahwa genotipe ayam Cemani yang berbeda memiliki komposisi mineral yang berbeda. Di masa depan, analisis ini mendukung pemilihan galur ayam dengan kadar antioksidan tinggi.

Kata Kunci: Ayam Cemani, *Fibromelanosis*, Kandungan Mineral, PCR–RFLP

ABSTRACT

Safitry RS, Dharmayanthi AB, Kinoshita K, Akiyama T, Darwati S, Kostaman T, Sopiyana S, Khaerunnisa I, Sumantri C. 2022. Analysis of meat mineral content in Cemani chicken with homozygous (*Fm/Fm*) and heterozygous (*Fm/fm*$^{+}$) genotypes. JITV 27(4):195-203. DOI : http://dx.doi.org/10.14334/jitv.v27i4.3075.

Cemani chicken is an Indonesian native chicken with black hyperpigmentation on feathers, skin, beak, comb, and flesh. Hyperpigmentation in chickens is called Fibromelanosis. Fibromelanosis in Cemani chickens is semi–dominant, producing two genotypes: homozygous (*Fm/Fm*) and heterozygous (*Fm/fm*$^{+}$). Cemani chicken meat's black color may indicate a higher mineral content than regular chicken meat. The study's is aims are to detect genotype homozygous (*Fm/Fm*) and heterozygous (*Fm/fm*$^{+}$) mutations and to determine the mineral content of Cemani chickens with homozygous (*Fm/Fm*) and heterozygous (*Fm/fm*$^{+}$) genotypes. In the Cemani chicken population (n = 32), the *Fm–specific* allele genotype was detected using a Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR–RFLP) and the *Mlu*I restriction enzyme. The mineral contents of Cemani chicken tested were Fe, Zn, Mn, and Se. The results showed that homozygous Cemani chickens (*Fm/Fm*) had higher Fe and Zn mineral content. However, the homozygous (*Fm/Fm*) and heterozygous (*Fm/fm*$^{+}$) Cemani chicken were not different in mineral content of Se and Mn. This study found that different genotypes of Cemani chicken had different mineral compositions. In the future, this analysis supports the selection of chicken strains with high antioxidant levels.

Key Words: Cemani Chicken, *Fibromelanosis*, Mineral Content, PCR–RFLP

INTRODUCTION

Local chickens in Indonesia comprise native chickens and adapted chickens that can be grouped into broiler, laying, dual–purpose and ornamental types (Nataamijaya 2010; Kartika et al. 2016). Ayam Cemani is a native chicken originating from the Kedu region, Temanggung Regency, Central Java (Habsari et al.

2019). The main locations for cemani chicken are in Kedu village, Beji village and Kahuripan village, Kedu region, Temanggung Regency and its distribution in in Kalikuto, Magelang, Central Java (Sartika et al. 2016) Ayam Cemani is distinguished by the blackness of its entire body and internal organs are black. According to Amelia (2019), Cemani chickens was originated from a population of Black Kedu chickens selected for their black color (feathers, skin, cockscombs, beaks, shanks) and jagged single cockscomb forms. The uniqueness of cemani chicken with black color in whole body and its internal organ increases the selling price in the market. In addition, people also believe that ayam cemani can be used for traditional medicine and rituals (Alfauzi & Hidayah 2020).

Black pigmentation in the birds is called *Fibromelanosis*. Abnormal accumulation of eumelanin in tissues causes the *Fibromelanosis* trait in the chickens body and its internal organ (Hutt 1949; Lukanov & Genchev 2013). The presence of a duplication rearrangement in the genomic region containing the Endhotelin3 gene (*EDN3)* on chromosome 20 cause of *Fibromelanosis* (*Fm*) mutation in Cemani and Silkie chickens (Shinomiya et al. 2012; Dorshorst et al. 2010; Dharmayanthi et al. 2017). *EDN3* plays a role in the formation of melanocyte–producing proteins. *EDN3* causes the excessive expression of melanocyte–forming proteins in Cemani chickens, resulting in a black color in the whole body and internal organs of Cemani chickens (Dorshorst et al. 2010).

Shinomiya et al. (2012) crossed White Silkie (WS) chicken (*Fibromelanosis* traits) with Black Minorca Minorca (BM, wild–type phenotype). They found that the F1 between Black Minorca (BM) and WS resulted in different degrees of pigmentation even though they exhibited the *Fm* phenotype. The pigmentation in the internal tissues of the F1 progeny of BM was significantly lighter than that in WS, indicating that hyperpigmentation is more severe in the *Fm/Fm* homozygote than in the *Fm/fm*$^+$ heterozygote**.** A recent study established a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method that for detecting homozygous (*Fm*/*Fm*) and heterozygous (*Fm*/*fm*$^+$) individuals with the *Fm* mutation in the Cemani population (Dharmayanthi et al. 2017; Dharmayanthi et al. 2022).

Pigmentation of the skin and meat is a complex biological process. Ecological factors, diet, and heredity can all have an impact on skin color (Wu et al. 2021). Cemani chicken possesses a meat color that differs from that of regular chickens in general such as broiler or layer chicken. Cemani chickens have a pale black and deep black meat color. Meanwhile, color of the meat in broiler chickens varies greatly between individuals, ranging from slightly yellow to white (Hidayah et al. 2019). Coloration is a phenotypic trait associated with diverse adaptive functions such as thermoregulation, camouflage, and mate selection (Hamilton et al. 2013). Mitić et al. (2012), in a study of heavy metal content of smoked meat, found that meat with a darker color contains almost twice the iron content compared with light–color meat. The iron content in Cemani chicken, an antioxidant cofactor, has not been explored. Other antioxidant minerals are manganese, selenium, and zinc (Rusli 2016). Antioxidants are essential for the body to neutralize free radicals and prevent damage to normal cells, proteins, and fats (Hasanah 2015).

As a result, research into the mineral content of meat in Cemani chickens with homozygous (*Fm/Fm*) and heterozygous (*Fm/fm*$^+$) genotypes, particularly Fe, is required to understand the differences in mineral composition in Cemani chickens with homozygous (*Fm/Fm*) and heterozygous (*Fm/fm*$^+$) genotypes. The study's aims are to detect homozygous (*Fm/Fm*) and heterozygous (*Fm/fm*$^+$) genotypes in the Cemani population and to determine the mineral composition of Fe, Zn, Mn, and Se in each Cemani chicken genotype.

MATERIALS AND METHODS

Blood Sampling

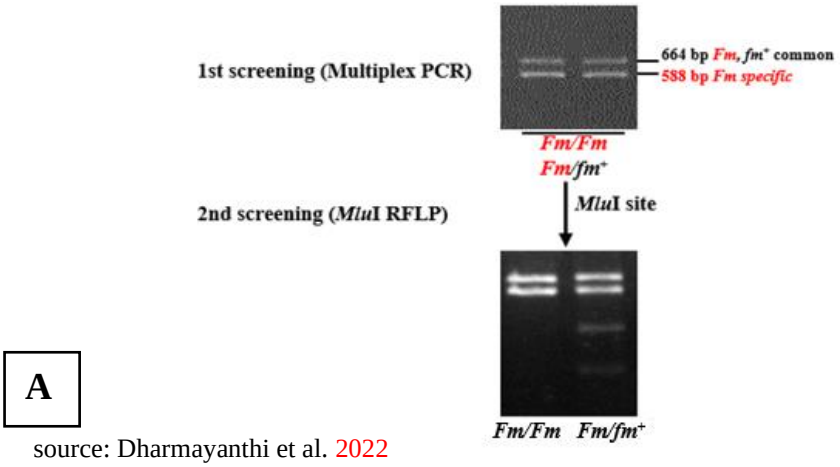
In total, we used 32 blood samples from Cemani chickens; 16 blood samples from PT. Sumber Unggas Indonesia (SUI), Parung–Bogor, Indonesia and 16 blood samples from the Indonesian Research Institute for Animal Production (IRIAP), Ciawi–Bogor, Indonesia. We applied 70% alcohol to the axillary vein on the wing before blood was taken. Blood samples (as much as ±3 mL) were taken using a syringe. The blood samples were placed into a tube containing EDTA and stored in a refrigerator at 4°C before DNA extraction.

DNA Extraction

DNA extraction from Cemani chicken samples was performed using the Genomic DNA Mini Kit (Blood/Cultured Cell) following the kit's protocol.

DNA amplification

The amplification of *Fm*-specific allele was carried out by multiplex PCR technique combining three primers. Three primers used in this study followed previous study (Dharmayanthi et al. 2022) on non-coding genes of Fibromelanosis region: forward primer WT_F : 5'-TTCAGCAGCATTCACTGAAGGC-'3 and reverse primer FMdupl_664_R: 5'-TTCAGCAGCATTCACTGAAGGC-'3 used to amplify 664 bp length of *fm*$^+$ common; forward primer WT_F: 5'-TTCAGCAGCATTCACTGAAGGC-'3 and reverse



source: Dharmayanthi et al. 2022

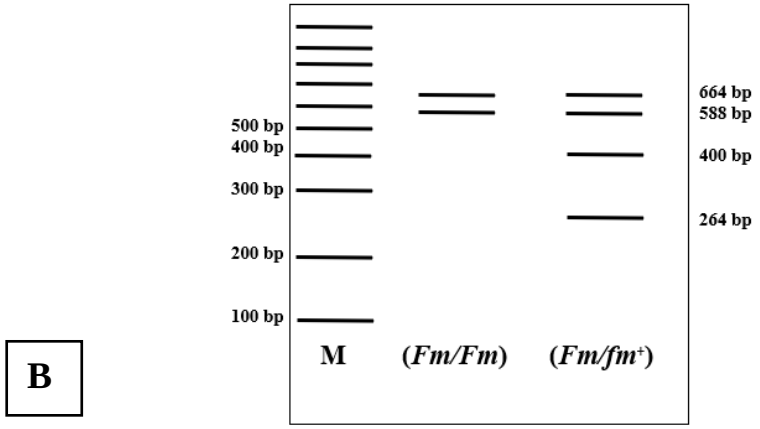


Figure 1. Determination of homozygous (*Fm*/*Fm*) and heterozygous (*Fm*/*Fm*$^+$) genotypes with restriction enzymes *(MluI).* (A) PCR–RFLP amplification workflow to identify genotypes (source: (Dharmayanthi et al. 2017; Dharmayanthi et al. 2022) (B). Visualization results from PCR–RFLP

primer FMspec_588_R: 5'-TGTCCATCTCACATTGC-'3 used to amplify 588 bp length of *Fm* spesific. DNA samples were mixed in a total volume 15ul consisted of primer WT_F 0.4 µL; primer FMdupl_664_R 0.2 µL; primer FMspec_588_R 0.2 µL; nuclease–free water 5.7 µL; and My Taq^{TM} HS Red Mix 7.5 µL. The sample is put into a PCR machine. The amplification process process comprised predenaturation (95°C) for 1 min, followed by 35 cycles of denaturation (95°C) for 15 s, annealing (60°C) for 10 s, and extension (72°C) for 10 s.

PCR–RFLP and genotyping

To identify the *Fm* homozygous (*Fm*/*Fm*) and heterozygous (*Fm*/*fm*$^+$) genotypes of Cemani chickens, a PCR–RFLP genotyping assay was performed using a restriction enzyme (*Mlu*I). The multiplex PCR products were digested at 37°C for 16 h with 10 U of the *Mlu*I restriction enzyme. The digested product was loaded into electrophoresis was performed in 2% agarose gel with a voltage of 50 V for 50 min and then the digested product was visualized using a UV transilluminator. We identified the Cemani chicken genotype based on a previous study by Dharmayanthi et al. 2022 (Figure 1A), and the visualization results are presented in Figure 1B.

Mineral content analysis

Mineral content analysis was performed using the Atomic Absorption Spectrophotometer (AAS) method based on the AOAC (2015) on a Cemani chicken's thigh. The meat's mineral contents tested were Fe, Zn, Mn, and Se. This test was conducted using the analysis services of the Laboratorium Terpadu LSSMKP IPB (Ilfa IPB).

Data analysis

Genotyping data were analyzed by calculating the genotype and allele frequency, Hardy–Weinberg Equilibrium, observed heterozygosity (Ho), and expected heterozygosity (He) using the PopGene 32 program. Allele and genotype frequencies were

calculated using the Nei & Kumar (2000) formula as follows:

$$x_i = \frac{(2n_{ii} + \sum_{i \neq j} n_{ij})}{2N} \qquad x_{ii} = \frac{n_{ii}}{N}$$

where x_i is the frequency of the i–th allele, x_{ii} is the frequency of the i–th genotype; n_{ii} is the number of individuals with genotypes ii, n_{ij} is the number of individuals with genotypes ij, and N is the sample number of individuals.

The χ^2 test was used to examine the Hardy–Weinberg Equilibrium, which was calculated using the Nei & Kumar (2000) formula as follows:

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

where χ^2 is the chi–square test, O is the observation value, and E is the expectation value

Genetic diversity was determined from the observed (Ho) and expected (He) heterozygosity values. Observed heterozygosity (Ho) was calculated using the Weir (1996) formula and expected heterozygosity was calculated using the Nei & Kumar (2000) formula as follows:

$$H_0 = \sum_{i \neq j}^{N} \frac{N_{1ij}}{N} \qquad H_e = 1 - \sum_{i=1}^{q} x_i^2$$

where H_0 is the observed heterozygosity value, N_{1ij} is the number of individuals with heterozygous, N is the observed number of individuals, H_e is the expected heterozygosity value, x_i is the frequency of allele, and q is the allele number.

T–test was used to compare the genotypes pure Cemani chicken with homozygous *Fm/Fm* and Cemani chicken with incomplete *Fm* trait (heterozygous *Fm/fm*$^+$) with a 95% confidence interval were calculated using the Mattjik & Sumertajaya (2013) formula as follows:

$$t = \frac{(\bar{x}_A - \bar{x}_B)}{\sqrt{\frac{S_{A\,B}^2}{n_A} + \frac{S_B^2}{n_B}}}$$

where $\bar{x}_A$is the mean of sample A, $\bar{x}_B$ is the mean of sample B, n_A is the number of individual sample A, n_B is the number of individual sample B, S_A is the standard deviation of sample A, S_B is the standard deviation of sample B.

RESULTS AND DISCUSSION

Genotyping the Ayam Cemani population

The PCR amplification results for identifying the *Fm* allele in Cemani chickens showed that two fragment lengths, namely, 664 and 588 bp, were amplified in all Cemani chicken samples (Figure 2A). The results of RFLP using the *Mlu*I restriction enzyme showed two different genotypes found in the Cemani chickens (Figure 2B). PCR–RFLP identified 21 Cemani chickens that were homozygous for the *Fm*/*Fm* genotype and produced two undigested bands (664 bp and 588 bp), and 11 Cemani chickens with heterozygous *Fm/fm*$^+$ genotype produced four bands of 664, 588, 400, and 264 bp (Table 1).

Genotype frequency, allele frequency, and heterozygosity values

The PCR–RFLP results showed that there were two genotypes (homozygous *Fm/Fm* and heterozygous *Fm/fm*$^+$) and two alleles (*Fm* allele and wild type *fm*$^+$) in this study. The values of genotype frequency, allele frequency, heterozygosity, and Hardy–Weinberg test on the *Fm*–specific alleles shown in Table 2. The genotype frequency of 11 individual SUI Cemani chickens out of 16 SUI Cemani chickens showed that half of the Cemani chicken population possessed an incomplete *Fm* trait or heterozygous *Fm/fm*$^+$ (69%) and 5 individual SUI Cemani chickens were pure Cemani chickens or homozygous *Fm/Fm* (31%). Meanwhile, all individual Cemani chickens in the IRIAP were pure Cemani chickens or homozygous *Fm/Fm* (100%). The frequency of alleles in the Cemani chicken population in SUI is was less than 0.99, indicating that the Cemani population is polymorphic. Meanwhile, the Cemani chicken population in IRIAP was 1, and population of Cemani chickens in IRIAP is valued at 1, the population can be assumed to be monomorphic. According to Allendorf et al. (2013), polymorphism is a population with an allele frequency of less than 0.99. Thus, polymorphism in SUI Cemani chickens indicated a population, whereas monomorphism in IRIAP Cemani chickens indicated a nondiverse population.

Hardy–Weinberg equilibrium analysis is used to determine the presence of evolution in a population (Panggabean 2016). This analysis was calculated using the chi–square test (χ^2). The Hardy–Weinberg results showed that the IRIAP Cemani chickens in IRIAP had a value of 0.000, which means that that they cannot be analyzed because only one allele in existx the population. By contrast, SUI Cemani chickens were not significant (P>0.05), indicating gene mutations, migration, and selection in the population. Factors influencing Hardy–Weinberg's condition include random mating, no gene mutations, no migration, and no selection (Nugroho et al. 2016). In a population, He and Ho calculations are used to estimate genetic diversity and to select livestock for the next generation (Putri et al. 2021). In this study, the Ho value was 0.68 and that of He was 0.46 in SUI Cemani chickens, whereas in IRIAP Cemani chickens, the Ho and He values were 0. Populations with heterozygosity of below 0.5 or closer to the margin indicate a low

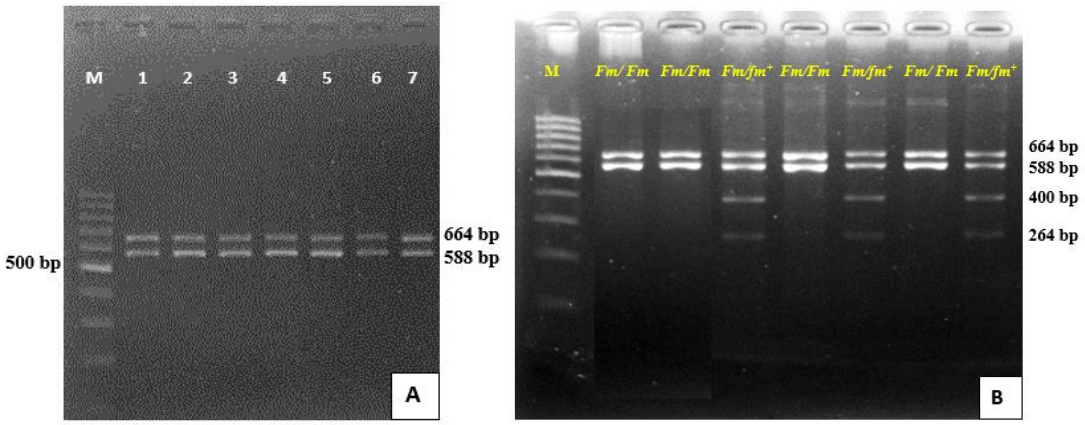


Figure 2. Gel electrophoresis of the PCR–RFLP of the *Fm*–specific allele in Cemani chickens. (A) PCR products amplified by multiplex–PCR primers. (B) *Mlu*I–digested pattern of multiplex–PCR primer–amplified products

Table 1. The result of Cemani chickens genotyping using PCR-RFLP

No	IRIAP Cemani chickens (n = 16)			SUI Cemani chickens (n = 16)		
	Sample Code	Phenotype (skin color)	Genotypes	Sample Code	Phenotype (skin color)	Genotypes
1	47	Deep black	*Fm/Fm*	CM1	–	*Fm/fm*$^+$
2	84	Pale black	*Fm/Fm*	CM2	–	*Fm/Fm*
3	85	Pale black	*Fm/Fm*	CM3	–	*Fm/fm*$^+$
4	88	Deep black	*Fm/Fm*	CM4	–	*Fm/Fm*
5	192	Deep black	*Fm/Fm*	CM5	–	*Fm/fm*$^+$
6	193	Deep black	*Fm/Fm*	CM6	–	*Fm/fm*$^+$
7	194	Deep black	*Fm/Fm*	1A	–	*Fm/Fm*
8	199	Deep black	*Fm/Fm*	5A	Pale black	*Fm/fm*$^+$
9	200	Pale black	*Fm/Fm*	12A	Pale black	*Fm/fm*$^+$
10	1019	Pale black	*Fm/Fm*	14A	Pale black	*Fm/fm*$^+$
11	1037	Pale black	*Fm/Fm*	21A	Pale black	*Fm/fm*$^+$
12	1061	Pale black	*Fm/Fm*	25A	Pale black	*Fm/fm*$^+$
13	1072	Deep black	*Fm/Fm*	26A	Pale black	*Fm/fm*$^+$
14	1096	Deep black	*Fm/Fm*	27A	Deep black	*Fm/Fm*
15	21018	Pale black	*Fm/Fm*	30A	Pale black	*Fm/fm*$^+$
16	21077	Pale black	*Fm/Fm*	33A	Deep black	*Fm/Fm*

Table 2. Genotype, allele frequency, heterozygosity, and Hardy–Weinberg test of the *Fm*–specific allele

Types of Chickens	N	Genotype Frequency		Allele Frequency		H_o	H_e	χ^2
		Fm/Fm	*Fm/fm*$^+$	*Fm*	*fm*$^+$			
SUI Cemani chickens	16	0.3125 (5)	0.6875 (11)	0.66	0.34	0.6875	0.4657	3.9^{ns}
IRIAP Cemani chickens	16	1 (16)	0	1	0	0	0	na

Fm/Fm = homozygous Cemani, *Fm/fm*$^+$ = heterozygous Cemani, Ho= observed heterozygosity; He= expected heterozygosity; χ^2= chi–square test ; ns= non–significant (P > 0.05), na= not analyzed

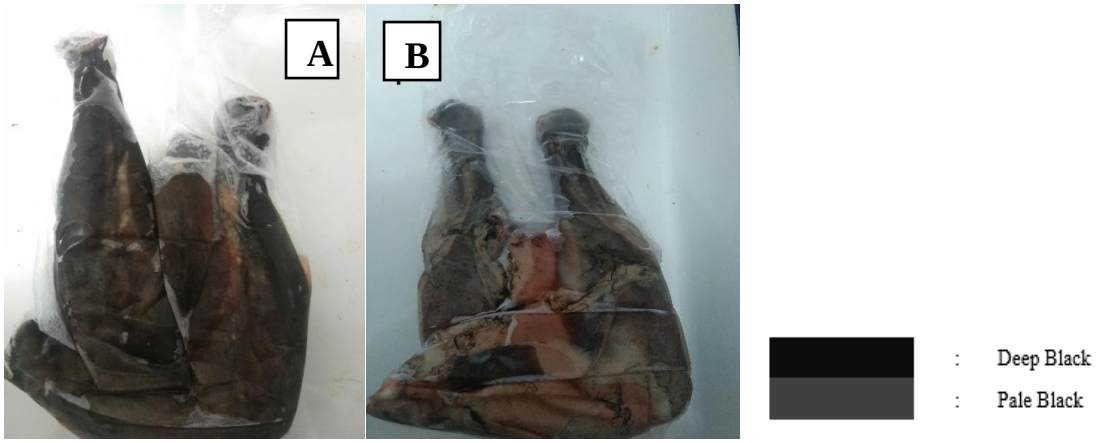


Figure 3. Degree of blackness in the meat of Cemani chickens. (A) Deep black. (B) Pale black

Table 3. Mineral content of Cemani chicken meat

Mineral (mg/kg)	Genotype		P–value
	Pure Cemani (homozygous *Fm/Fm*) (n = 21)	Cemani chickens incomplete *Fm* trait (heterozygous *Fm/fm*$^+$) (n = 11)	
Iron	22.94±9.67	11.55±2.31	0.001*
Zinc	16.43±5.86	14.60±4.66	0.000*
Manganese	0.26±0.19	0.52±0.61	0.378
Selenium	0.40±0.64	2.43±1.88	0.092

*= significant (P<0.05)

variation (Sheriff & Alemayehu 2018). Unlike IRIAP Cemani chickens, SUI Cemani chickens have a higher level of heterozygosity, indicating that the Cemani population in SUI has a high genetic variation. In addition, the significant difference between Ho and He shows that the population has genetic imbalances (Tambasco et al. 2003; Harbison & Nguyen 2017). The population mated randomly because Ho was higher than He (Sharma et al. 2016). Random mating in the Cemani population in SUI suggests that a large number of Cemani chickens have incomplete *Fm* traits.

Degree of the blackness of Cemani chicken meat

In this study, we divided the blackness level of Cemani chicken's into two, namely, deep black and pale black (Figure 3). The results of the observations of 25 Cemani chicken meats indicated that Cemani chickens with deep black and pale black were 40% and 60%, respectively. The black color difference in Cemani chickens is caused by the semi–dominant *Fibromelanosis* gene (Shinomiya et al. 2012). Gene mutations control color expression in chickens (Zhang et al. 2015). In addition, a recent study discovered that the different genotypes in the Cemani population, i.e. homozygous *Fm/Fm* and heterozygous *Fm/fm*$^+$, have differing levels of blackness (Dharmayanthi et al. 2017; Dharmayanthi et al. 2022). Pigmentation in poultry caused by the amount of melanin and muscle size. The higher level of melanin pigments in the skin could darken the skin because the skin color was primarily controlled by the melanin content (Yamaguchi et al. 2007). There are parts of Cemani chicken with a pale black color that have dark black meat, particularly the meat under the skin. The different level of melanin pigment probably responsible for this different deep black color in the meat. In the skin, The higher level of melanin pigments in the skin could darken the skin because the skin color was primarily controlled by the melanin content (Yamaguchi et al. 2007). In the study of Nganvongpanit et al. (2020), the melanin pigmentation in Thailand black bone chicken was present in all of the tissue layers of most organs. However, the distribution of melanin pigmentation in the organs was found to be different except in some tissue samples, such as those obtained from the liver. In the muscle, the uneven distribution of darkness was also due to the accumulation of the different pigment melanin (Kriangwanich et al. 2021). Moreover, different the age of chicken is also caused the different melanin content in breast, drumstick, wing and skin samples (Buasap et al. 2021). This dark–colored chicken meat had higher total pigment, myoglobin, iron and redness (Buasap et al. 2021).

Mineral content of Cemani chicken meat

On the basis of the PCR–RFLP results, the meat mineral content in Cemani chickens was divided into pure Cemani chicken with homozygous *Fm/Fm* and Cemani chicken with incomplete *Fm* trait (heterozygous *Fm/fm*$^+$). Table 3 shows the results of the mineral content analysis of iron (Fe), zinc (Zn), manganese (Mn), and selenium (Se) in the Cemani chicken thigh meat.

The four mineral contents in Cemani meat were chosen for this study because they function as antioxidants in the body. Antioxidants are the substances that inhibit oxidation or "free radical scavengers" as antioxidants form minor reactive species via radicals (Neha et al. 2019). The function of Iron (Fe) in metabolic processes is to activate the enzymes peroxidase and catalase. The enzyme catalase stimulates the hydrolysis of hydrogen peroxide into oxygen and water molecules, whereas peroxidase is used with hydrogen peroxide to stimulate the oxidation of organic and inorganic compounds (Al–Lamei et al. 2020). Peroxide reactions can be characterized by oxidative halogenation and dehydrogenation, oxygen transport and hydrogen peroxide decomposition (Shivakumar et al. 2017). The rest of the Fe metabolism is stored in the body, including the liver, bone marrow, spleen, and skeletal muscles (Prasetiyo et al. 2014). Meanwhile, Zn plays a role in the body's digestion, carbohydrate metabolism, and nucleic acid production of over 70 enzymes (Imanto et al. 2018). Mn serves as a synthesis of carbohydrates, mucopolysaccharides, and enzyme systems for the growth and reproduction of livestock (Prasetiyo et al. 2014). In addition, the Se also plays an essential role in the body's defence system by helping neutrophil activity in the primary defense to protect the body from bacterial infections (Pratiwi et al. 2018). Table 3 shows that samples of pure Cemani chickens with homozygous *Fm/Fm* were significantly different from Cemani chickens incomplete *Fm* trait with heterozygous *Fm/fm*$^+$ in Fe (22.94 mg/kg) and Zn (16.43 mg/kg). However, Cemani chickens with incomplete *Fm* trait (heterozygous *Fm/fm*$^+$) were not significantly different from Cemani homozygous *Fm/Fm* in Mn (0.52 mg/kg) and Se (2.43 mg/kg) minerals content. The results of this study are in accordance with those Mitić et al. (2012), who revealed that black meat has a high mineral content of Fe and Zn. In addition, iron can be found in muscle tissue (myoglobin) (Wijaya et al. 2015) and affects the Fe value in cemani chickens, whereas mineral Zn accumulates highly in bone tissue. The high Mn and Se mineral contents found in Cemani chickens with an incomplete *Fm* trait and a heterozygous *Fm/fm*$^+$ indicate that the meat contains high antioxidant levels. Mn and Se minerals function as antioxidants in the body. Mn mineral in meat acts as a cofactor enzyme (Wijaya et al. 2015), whereas Se mineral acts as an antioxidant in meat and can increase tocopherol activity (vitamin E) (Wijaya et al. 2015). The factors that affect the composition of meat include genetics, gender, physiology, age and body weight, diet, and meat type.

In the future, mineral content analysis can be used to create pure Cemani chicken strains that are high in antioxidants. Further research should explore feed analysis, chicken age uniformity, physicochemical testing, and antioxidant activity.

CONCLUSION

The analysis of genetic diversity in the *Fm*–specific alleles in the Cemani populations in two different chicken farm, SUI and IRIAP, revealed that Cemani chickens in SUI have high genetic diversity, as evidenced by a higher Ho value compared to Cemani chickens in IRIAP. We found two genotypes of Cemani chicken in this study: homozygous *Fm/Fm* genotype showed deep black and some chicken showed pale black meanwhile all heterozygous *Fm/fm*$^+$ with pale black. Mineral content analysis revealed that Cemani chickens with homozygous *Fm/Fm* have higher content of Fe and Zn minerals than Cemani with heterozygous *Fm/fm*$^+$. However, Cemani chickens with heterozygous *Fm/fm*$^+$ have Mn and Se that were not different from Cemani with homozygous *Fm/Fm*. This study found that different genotypes of Cemani chicken had different mineral compositions. In the future, this analysis supports the selection of chicken strains with high antioxidant levels.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support for this research from the Ministry of Research and Technology/National Research and Innovation Agency, Indonesia DIPA PN IPH–LIPI 2021 and Agro–Maritime Program 4.0 IPB University (contract no: 2490/IT3.L1/PN/2020).

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