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# Effect of *PMEL17* Plumage Colour Gene Diversity on Production Performance of Indigenous Chicken Variety of Bangladesh

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## ABSTRACT

**Background:** An adaptive meat and egg type indigenous chicken is crucial for countries those depends on rural poultry production for meeting the protein requirements of the peoples. Genetic characterizations of native chickens have been documented, however, no study has observed the plumage colouration and its potential role in production traits. Thus, the aim of the current study was to know the effect of *PME17*, plumage colour gene diversity on production performance of indigenous chicken varieties.

**Methods:** The plumage colours, comb and body shape of chickens corresponds with the live weight and egg production (clutch size) and the egg characteristics were recorded. Gel electrophoresis and polymerase chain reactions (PCR) were performed from blood cell DNA following standard protocols. The PCR products were sequenced using Sanger sequencing and for molecular analysis MEGA6 software were used.

**Result:** Highest live weight (1400±25.4 g) and egg production (15.3±0.9 /number /clutch) was obtained in spotted-single-round chicken than other varieties. Both external and internal egg characteristics differed between varieties and spotted- single-round variety found to be best than other varieties. The sequence of *PMEL17* gene was 99% homology with the sequence of *Gallus gallus* and *Gallus gallus domesticus*. A mutation was observed at 91bp nucleotide in brownish and at 64bp positional nucleotide and in black-white chicken variety.

**Key words:** Chicken, Gene, Mutation, Production, Traits.

## INTRODUCTION

The genetics of feather colour have been well characterized in the chicken, with several genes (for example, *PMEL17*, *MC1R* and *ASIP*). Of these genes, the gene *PMEL17* affects plumage colour in the chicken with polymorphisms within this gene have been associated with a variety of different colours (Kerje *et al*., 2004). The variation in plumage colouration ranges from the dominant white to dun and smoky, depending on the alleles present in the *PMEL17* gene (Kerje *et al*., 2004). Several studies have seen on phenotypes besides colouration to the *PMEL17* gene, for example, social behaviour (Keeling *et al*., 2004). However, no study found whether production traits are affected by these alleles.

The most commonly distinct chicken varieties of Bangladesh are the Hilly, Naked Neck, Assel and Full-feathered. The plumage colours present in full-feathered chicken are red, white, black, black with red stripes, white with red stripes and brownish (Faruque *et al.,* 2010). Generally, these chickens having single comb with yellowish shank colour (Faruque *et al*., 2010). The yearly egg production of a white indigenous chicken is 90 eggs (Khan *et al*., 2017), that are higher than other chicken varieties.

Genetic characterizations of native chickens have been performed by several researchers (Islam and Nishibori, 2012; Nedup *et al*., 2012), however, no study have observed elsewhere plumage colouration and its potential role in production traits. Therefore, the present study was conducted to know the effect of *PMEL17,* plumage colour gene diversity on production performance of indigenous chicken variety of Bangladesh.

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## MATERIALS AND METHODS

The research work was conducted following the animal ethics rule and the ethical committee of Chattogram Veterinary and Animal Sciences University (CVASU) (Memo no. -CVASU/Dir (R&E) EC/2015/799, Date 05/07/2017).

### Study area, animal and production traits in relation to colour

The field study was conducted in the three locations of Patia upazila (sub-district) of Chittagong district and the molecular study was done in the lab of Poultry Research and Training Centre (PRTC) at CVASU of Bangladesh. The research was conducted under the Department of Genetics and Animal Breeding at Chittogram Veterinary and Animal Sciences University, Khulshi, Chattogram-4225, Bangladesh from March 2017 to May 2018. A total of 240 households (80 household from each location and having at least 3 chickens) from the Badamtal (location 1), Kusumpura (location 2) and Kolagaon (location 3) areas of Potia upazila (sub district) of Chattogram district was directly surveyed by the researchers and field assistant. All the locations had a similar ambient temperature, humidity, bird management protocols and feed used. Colour was recorded and divided into three different colour morph classes (Black and white, brownish and spotted), whilst comb type (predominantly single) and body shape (round and cylindrical) were also selected. Production traits, consisted of mature live weight (g), clutch size (number of days spent laying) and egg weight (g) were recorded. Mature live weight (g) was taken with top loading balance at the chicken's age about 40 to 42 weeks of age of the chicken.

### External and internal egg characteristics

Fifty eggs from each variety of chicken from each location were collected. Eggs were cleansed, measured and weighed using digital electronic balance (Model: DWA-224, Dawer Scales India Private Limited) and recorded. Egg-width and egg-length were measured using Vernier slide calipers (Mitutoyo Corp, Japan). An egg shape index was calculated according to Yakuba *et al.* (2008) as:

$$\text{Shape index} = \frac{\text{Egg width}}{\text{Egg length}} \times 100$$

Eggs shell surface area was determined according to Carter (1975) as, Surface area =

$$3.9782 \times \text{egg weight (g)}^{0.7056}.$$

The thickness of the egg shell was determined using a micrometer screw gauge (Mitotoyo Corp, Japan). The accuracy of shell thickness dimensions was ensured by measuring shell samples at the broad end, the mid-portion and the narrow end of each egg. Each egg was broken and the yolk and the albumen were weighed and separated. The albumen and yolk height were determined using a spherometer (RAC Expots, Haryana, India); the albumen height was measured in the middle of the thick albumen equidistant from the outer edge of the albumen and the yolk using Vernier slide calipers (Mitutoyo Corp, Japan). Both albumen and yolk ratios of each egg were determined as:

$$\text{Albumen or yolk ratio} = \frac{\text{Albumen or yolk height}}{\text{Albumen or yolk width}}$$

In order to correct for the difference in egg weight, the albumen height was converted into Haugh units (Haugh 1937), Haugh unit = $100 \log (H + 7.6 - 1.7W^{0.37})$, where, H = Observed height of the albumen in millimetres and W = Weight of egg in grams.

### Molecular analysis of *PMEL17*

Molecular characterisation of the *PMEL17* gene was performed in three colour morphs: black and white single round (variety 1), brownish single round (variety 2) and spotted single round (variety 3) chickens. Blood samples were collected from 60 laying hens (variety 1, n=20, variety 2, n=10 and variety 3, n=30). DNA was extracted from the whole blood samples using the FavorPrep™ blood genomic DNA extraction mini kit (FAVORGEN biotech corporation, Taiwan) and stored at -20°C. Exon 6 of the PMEL17 gene was then amplified using the following PCR primers (forward primer: GTGGATGTGACACAGCTGGA-3´, reverse primer: R-5´-GGAGCATCACCACCTGA-3´), with a resulting product size of 542bp. PCR products were cleaned using 2µl of ExoSAP-IT (enzyme: ExoASP-IT) per sample.

### Polymerase chain reactions (PCR) and agarose gel electrophoresis

A total of 25µl (12.5µl mastermix, 2.5µl each primers (forward and reverse), buffer 5µl and 2.5µl DNA template) PCR mix was prepared (FavorPrep™). The PCR amplification was conducted in a MJ PTC-200 per litre. Thermal cycler or a Bio-Red C 1000 thermal cycler with an initial denaturation at 95°C for 10 minutes, followed by 50 cycles of denaturation at 95°C for 30s, annealing for 30s at the 65°C, a primary extension at 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. The PCR products were electrophoresed on 2.5% agarose gel (Lonza USA) at 90 V for 1.5 to 2h and stained with ethidium bromide and their sizes were estimated using a 100-bp DNA ladder. The amplified PCR band pattern was visualized by on a UV trans-illuminator and photographed in a computer.

### Gene sequencing, scoring, alignment and detection of mutation

A 5µl aliquot of a post-PCR reaction product was mixed with 2µl of ExoSAP-IT (enzyme: ExoASP-IT). This combined 7µl reaction volume was incubated at 37°C for 15 minutes to degrade the remaining primers and nucleotides. Finally, the ExoSAP-IT enzymatic reaction mixed sample was inactivated by incubation at 80°C for 15 minutes. The purified PCR products were Sanger-sequenced with a big dye

terminator v3.1 sequencing kit and a 3730xl automated sequencer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were thereafter determined on both strands of PCR amplification products at the Macrogen sequencing facility (Macrogen Inc., Seoul, Korea) using an ABI PRISM 3730xl Analyzer (96 capillary type). Eight best sequences from each chicken variety of the *PMEL17* gene, including DNA, were taken and from the NCBI information gene bank and using the tool BLAST on the website http://ncbi.nlm.nih.gov, similar sequences, their similarity score and possible mutations were investigated using MEGA6 software package (Tamura *et al*., 2013).

### Statistical analysis

The least square means of the different recorded parameters on the basis of the chicken variety and location using PROC GLM and PROC MIXED of SAS (2008) followed by randomized block design (RBD). Mean differences were compared using least significant difference (lsd) test at the 5% level of significance.

## RESULTS AND DISCUSSION

### Production traits in relation to colour morphs

The different production traits of the indigenous chicken varieties and locations are presented in Table 1. The spotted single round variety chicken was found to be best for egg production (number/ clutch in days, 15.3±0.9 days) and also have the heaviest live weight compared to other variety chickens (Table 1).

The clutch size of the different indigenous chicken variety was higher than the Nigerian native chicken (Daikwo *et al.,* 2011). On the other hand, a similar live weight of the different varieties of indigenous chicken was observed by Khan *et al.* (2017). The variation of clutch size and live weight of this study might be due to differences between regions, genetics and feeding of chickens. Similar factors were described by other researchers elsewhere (Grobbelaar *et al.,* 2010; Khan *et al.,* 2017: Sarma *et al.,* 2018).

**Table 1:** Production traits (Mean ± Standard Error) of different variety of indigenous chicken in three locations of Chittagong district of Bangladesh.

| Location | Types | No of observation | Traits | | | |
|---|---|---|---|---|---|---|
| | | | Clutch size (days) | Location average clutch size (days) | Live weight (g) | Location average live weight (g) |
| | BWSR | 12 | $11.0^{b}$±0.5 | | $1150^{c}$±8.3 | |
| | BWSC | 10 | $7.9^{c}$±0.8 | | $1080^{d}$±12.5 | |
| Location-1 | BRSR | 15 | $11.3^{b}$±0.9 | 10.7±0.8 | $1150^{c}$±40.1 | $1188^{b}$±20.4 |
| | BRSC | 8 | $8.8^{c}$±0.6 | | $1170^{c}$±20.3 | |
| | SPSR | 9 | $15.3^{a}$±0.9 | | $1360^{a}$±13.2 | |
| | SPSC | 9 | $10.0^{bc}$±0.9 | | $1220^{b}$±27.7 | |
| | BWSR | 10 | $11.8^{b}$±0.8 | | $1300^{a}$±35.9 | |
| | BWSC | 16 | $10.0^{bc}$±0.9 | | $990^{d}$±8.5 | |
| Location-2 | BRSR | 12 | $11.7^{b}$±0.3 | 11.4±0.6 | $1190^{b}$±28.2 | $1185^{b}$±15.9 |
| | BRSC | 10 | $7.9^{c}$±0.4 | | $1140^{c}$±4.7 | |
| | SPSR | 13 | $15.1^{a}$±0.8 | | $1330^{a}$±8.6 | |
| | SPSC | 7 | $12.0^{b}$±0.4 | | $1160^{bc}$±9.6 | |
| | BWSR | 10 | $10.8^{b}$±0.9 | | $1280^{c}$±19.3 | |
| | BWSC | 9 | $9.9^{bc}$±0.5 | | $1100^{d}$±14.9 | |
| Location-3 | BRSR | 13 | $13.0^{a}$±0.3 | 11.2±0.7 | $1250^{c}$±45.7 | $1254^{a}$±22.4 |
| | BRPC | 10 | $9.0^{c}$±0.9 | | $1320^{b}$±14.2 | |
| | SPSR | 8 | $14.0^{a}$±0.9 | | $1400^{a}$±25.4 | |
| | SPSC | 11 | $10.3^{b}$±0.9 | | $1175^{cd}$±14.8 | |
| | BWSR | | $11.1^{b}$±0.7 | | $1243^{ab}$±21.2 | |
| | BWSC | | $9.3^{bc}$±0.7 | | $1057^{c}$±11.9 | |
| Overall | BRSR | | $12.0^{b}$±0.5 | | $1197^{bc}$±38.0 | |
| variety | BRSC | | $8.6^{c}$±0.6 | | $1210^{b}$±13.1 | |
| | SPSR | | $14.8^{a}$±0.9 | | $1363^{a}$±15.7 | |
| | SPSC | | $10.8^{b}$±0.7 | | $1185^{a}$±17.3 | |

**N.B.** BWSR= Black and white single round, BWSC= Black and white single cylindrical, BRPR= Brownish single round, BRSC= Brownish single cylindrical, SPSR=Spotted single round, SPSC=Spotted single cylindrical.

Means with different superscripts in the same row differ significantly ($P < 0.05$).

### External and internal egg characteristics in relation to colour

The highest egg weight was observed for the spotted single round and the lowest was for the same colour with a cylindrical body type (Table 2). In addition to varying by colour morph, a significant (P<0.05) effect of location on egg weight was also observed. Similar values of egg weight of different genotypes of chicken were reported by several researchers (for example, Khan *et al*., 2017; Sarma *et al.,* 2018). The variation in egg weight might be due to the differences in genetics, feeding and management of the chicken and these factors was also reported by other researchers (Khan *et al.,* 2004; 2017).

No effect of colour morph on egg shell thickness was observed. Iqeobi *et al*. (2004) reported a higher average shell thickness value in normal plumage genotype than the current study. An effect of colour morph was found on shape index and the highest values was observed in the spotted single round colour morph than others (Table 2). The shape index was similar with Khan *et al.* (2004) and variation of shape index may be due to variation of the breed and management. The surface area of different varieties of chicken eggs did not differ between location, but differed (P <0.05) among varieties in a location (Table 2).

An effect of colour morph was found for albumin and yolk weight, but not albumin and yolk ratio (Table 3). The Haugh units varied significantly between colour morphs, but not between locations (Table 3). The albumen and yolk weight, haugh unit observed in the current study agreed with those reported by Nonga *et al*. (2012) and Yakubu *et al*. (2008) and this variation may be due to variety differences of chicken.

### Molecular analysis of *PMEL17*

The scoring of similarity and matching rate of three different varieties of chickens was compared for the sequenced plumage colour gene (*PMEL17*) of *Gallus gallus* are presented in Table 4. The sequences revealed a 99% homology (NCBI accession no: AY 636126.1, AY636129.1, respectively) with the sequence of *Gallus gallus,* these were concord with Kerje *et al*. (2004) as well as the sequence of the domestic chicken (Kerje *et al*., 2004; Kuliawa *et al*., 2009).

**Table 2:** External characteristics (Mean ± Standard error) of eggs of different variety of indigenous chicken in three locations of Chittagong district, Bangladesh (N = 50 eggs per variety).

| Location | Varieties | Traits | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Egg weight (g) | Average | Egg shell thickness (mm) | Average | Shape index | Average | Surface area $cm^2$ | Average |
| | BWSR | $39.9^{b}$±0.4 | | 0.7±0.3 | | 79.6±1.8 | | $53.6^{a}$±2.1 | |
| | BWSC | $37.5^{c}$±0.6 | | 0.7±0.3 | | $73.2^{b}$±0.6 | | $51.3^{b}$±0.6 | |
| Location 1 | BRSR | $41.4^{a}$±0.4 | 39.2±0.5 | 0.8±0.3 | 0.8±0.3 | $78.7^{a}$±2.4 | 76.7±2.6 | $55.1^{a}$±2.3 | 52.9±1.1 |
| | BRSC | $37.3^{c}$±0.4 | | 0.8±0.2 | | $73.4^{b}$±0.9 | | $51.2^{b}$±0.9 | |
| | SPSR | $41.7^{a}$±0.8 | | 1.0±0.5 | | $79.9^{a}$±1.8 | | $55.3^{a}$±0.1 | |
| | SPSC | $37.4^{c}$±0.7 | | 1.0±0.2 | | $75.3^{b}$±2.1 | | $51.3^{b}$±0.4 | |
| | BWSR | $42.1^{a}$±0.8 | | 1.0±0.6 | | $78.2^{a}$±0.4 | | $55.7^{a}$±0.5 | |
| | BWSC | $35.4^{d}$±0.7 | | 0.5±0.3 | | $68.0^{c}$±0.8 | | $49.3^{b}$±0.7 | |
| Location 2 | BRSR | $38.3^{b}$±0.7 | 38.4±0.9 | 0.6±0.8 | 0.7±0.4 | $76.5^{ab}$±1.3 | 74.8 ±1.1 | $52.1^{ab}$±1.1 | 52.2±0.7 |
| | BRSC | $37.3^{c}$±0.4 | | 0.7±0.2 | | $74.4^{b}$±0.9 | | $51.2^{b}$±0.9 | |
| | SPSR | $39.6^{b}$±0.9 | | 0.8±0.1 | | $77.9^{a}$±0.8 | | $53.4^{a}$±0.6 | |
| | SPSC | $37.4^{c}$±0.7 | | 0.8±0.1 | | $73.9^{b}$±0.8 | | $51.3^{b}$±0.6 | |
| | BWSR | $38.7^{b}$±0.6 | | 0.6±0.1 | | $73.1^{b}$±2.2 | | $52.5^{b}$±0.7 | |
| | BWSC | $37.9^{bc}$±0.6 | | 0.6±0.1 | | $70.6^{b}$±2.0 | | $51.7^{b}$±0.6 | |
| Location 3 | BRSR | $37.8^{bc}$±0.7 | 38.3±0.6 | 0.5±0.9 | 0.7±0.3 | $76.9^{a}$±2.1 | 74.7±1.7 | $51.6^{b}$±0.6 | 52.2±0.7 |
| | BRSC | $37.3^{bc}$±0.4 | | 0.7±0.2 | | $73.4^{b}$±0.9 | | $51.2^{b}$±0.9 | |
| | SPSR | $42.2^{a}$±0.8 | | 0.9±0.1 | | $78.8^{a}$±2.6 | | *55.8a±0.5* | |
| | SPSC | $36.5^{c}$±0.5 | | 0.7±0.1 | | $75.1^{b}$±0.1 | | $50.4^{b}$±0.6 | |
| | BWSR | $40.1^{a}$±0.6 | | 0.8±0.3 | | $77.0^{a}$±1.5 | | $53.8^{a}$±1.1 | |
| Overall | BWSC | $39.9^{ab}$±0.6 | | 0.6±0.2 | | $70.6^{c}$±1.1 | | $53.6^{b}$±0.6 | |
| variety | BRSR | $39.2^{ab}$±0.6 | | 0.6±0.6 | | $77.4^{a}$±2.5 | | $52.9^{a}$±1.3 | |
| | BRSC | $37.3^{b}$±0.4 | | 0.7±0.2 | | $73.7^{b}$±0.9 | | $51.2^{b}$±0.9 | |
| | SPSR | $41.1^{a}$±0.7 | | 0.9±0.1 | | $78.9^{a}$±2.5 | | $54.8^{a}$±0.5 | |
| | SPSC | $37.1^{c}$±1.1 | | 0.8±0.2 | | $74.8^{b}$±2.3 | | $50.9^{b}$±0.4 | |

**N.B.** Chickens definition is shown under Table 1.

Means with different superscripts in the same row differ significantly (p< 0.05).

Of the sequences, no polymorphisms were found in the spotted colour genotype, but polymorphisms were identified at 64bp (C to T) and 91bp (G to A) in the black-white and brown genotypes, respectively (Fig 1a, b). The nucleotide changes for the black-white morph is predicted from a protein-coding changes. Therefore, the sequence analysis showed that the dominant white and the black alleles were exclusively associated with an insertion and deletion of amino acids in the gene, *PMEL17* transmembrane region. Vaez *et al*. (2008) also found the mutation was together with the recessive silver polymorphism in the mouse, the only *PMEL17* gene. The present results show that the different colour morphs have different production characteristics.

**Table 3:** Internal characteristics (Mean ± Standard error) of eggs laid by different variety of indigenous chicken in three locations of Chittagong district, Bangladesh (N=50 eggs per variety).

| Location | Variety | Traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Albumen weight (g) | Average | Albumen ratio | Average | Yolk weight (g) | Average | Yolk ratio | Average | Haugh unit | Average |
| | BWSR | 20.2$^{a}$±0.9 | | 0.6±0.1 | | 16.5$^{a}$ ± 0.8 | | 0.7±0.1 | | 79.3$^{a}$±3.6 | |
| | BWSC | 19.1$^{ab}$±1.5 | | 0.5±0.1 | | 15.1$^{ab}$ ± 2.3 | | 0.7±0.2 | | 76.9$^{ab}$±4.1 | |
| Location-1 | BRSR | 21.1$^{ab}$±1.1 | 20.2±1.1 | 0.6±0.1 | 0.6±0.2 | 16.8$^{a}$ ± 0.3 | 15.3$^{a}$±1.8 | 0.6±0.1 | 0.6±0.1 | 82.4$^{a}$±1.1 | 78.9±3.8 |
| | BRSC | 18.9$^{b}$±1.1 | | 0.4±0.2 | | 14.7$^{ab}$± 1.8 | | 0.5±0.1 | | 75.9$^{b}$±2.9 | |
| | SPSR | 22.7$^{a}$±3.4 | | 0.7±0.3 | | 17.8$^{a}$±2.7 | | 0.6±0.1 | | 82.6$^{a}$±2.8 | |
| | SPSC | 19.4$^{ab}$±2.5 | | 0.5±0.1 | | 13.9$^{b}$±0.3 | | 0.5±0.1 | | 76.2$^{b}$±1.9 | |
| | BWSR | 21.6$^{a}$±0.8 | | 0.5±0.1 | | 15.4$^{a}$±0.6 | | 0.4±0.1 | | 79.1$^{ab}$±0.9 | |
| | BWSC | 19.1$^{b}$±2.4 | | 0.6±0.5 | | 13.0$^{b}$±0.1 | | 0.4±0.1 | | 78.1$^{ab}$±0.1 | |
| Location-2 | BRSR | 19.8$^{ab}$±0.7 | 20.0±1.3 | 0.6±0.1 | 0.5±0.2 | 15.3$^{a}$±0.1 | 14.2$^{b}$±0.3 | 0.6±0.1 | 0.5±0.1 | 81.2$^{a}$±1.5 | 79.2±1.2 |
| | BRSC | 19.4$^{b}$±0.9 | | 0.5±0.3 | | 13.1$^{b}$±0.1 | | 0.4±0.2 | | 80.4$^{a}$±0.1 | |
| | SPSR | 20.9$^{a}$±2.6 | | 0.6±0.1 | | 14.5$^{ab}$±0.2 | | 0.6±0.1 | | 78.6$^{ab}$±2.3 | |
| | SPSC | 19.3$^{b}$±1.8 | | 0.3±0.2 | | 13.9$^{b}$±0.3 | | 0.5±0.1 | | 77.2$^{b}$±1.9 | |
| | BWSR | 20.4$^{a}$±1.7 | | 0.6±0.1 | | 14.7$^{ab}$±1.0 | | 0.6±0.1 | | 81.1$^{a}$±1.7 | |
| | BWSC | 19.4$^{b}$±1.2 | | 0.5±0.1 | | 14.5$^{ab}$±0.9 | | 0.5±0.1 | | 76.9$^{b}$±4.7 | |
| Location-3 | BRSR | 19.7$^{b}$±0.5 | 20.1±0.9 | 0.6±0.1 | 0.5±0.1 | 14.2$^{ab}$±0.8 | 14.4$^{ab}$±1.0 | 0.6±0.1 | 0.5±0.1 | 84.8$^{a}$±4.1 | 77.8±3.5 |
| | BRSC | 19.5$^{b}$±0.9 | | 0.5±0.3 | | 14.7$^{ab}$±0.1 | | 0.4±0.1 | | 77.4$^{b}$±0.1 | |
| | SPSR | 21.8$^{a}$±0.3 | | 0.5±0.1 | | 15.6$^{a}$±1.2 | | 0.5±0.1 | | 76.7$^{b}$±4.5 | |
| | SPSC | 19.6$^{ab}$±1.5 | | 0.4±0.1 | | 12.9$^{b}$±1.9 | | 0.4±0.3 | | 74.0$^{b}$±9.1 | |
| | BWSR | 20.7$^{a}$±1.1 | | 0.6±0.1 | | 15.4$^{a}$±0.9 | | 0.6±0.1 | | 79.8$^{ab}$±2.9 | |
| Overall | BWSC | 19.3$^{b}$±1.1 | | 0.5±0.2 | | 14.2$^{ab}$±0.9 | | 0.5±0.1 | | 77.4$^{b}$±3.9 | |
| Variety | BRSR | 20.2$^{ab}$±0.7 | | 0.6±0.1 | | 15.4$^{a}$±0.4 | | 0.6±0.1 | | 82.1$^{a}$±1.9 | |
| | BRSC | 19.3$^{b}$±1.0 | | 0.5±0.3 | | 14.2$^{ab}$±0.6 | | 0.4±0.1 | | 73.2$^{b}$±1.2 | |
| | SPSR | 21.8$^{a}$±1.4 | | 0.6±0.2 | | 14.6$^{ab}$±2.5 | | 0.6±0.2 | | 79.3$^{ab}$±4.2 | |
| | SPSC | 19.4$^{b}$±1.2 | | 0.4±0.1 | | 13.6$^{b}$±0.9 | | 0.4±0.2 | | 75.9$^{b}$±2.6 | |

**N.B.** Chickens definition is shown under Table 1.

Means with different superscripts in the same row differ significantly ($p<0.05$).

**Table 4:** The scoring of similarity and matching rate of different sequences of indigenous chickens.

| Genotypes | Indigenous chicken variety | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Brownish | | | Spotted | | | Black and white | | |
| | Max.score | E value | Identity | Max.score | E value | Identity | Max.score | E value | Identity |
| *Gallus gallus PMEL17*AY 636129.1 | 894 | 0.0 | 99 | 913 | 0.0 | 99 | 904 | 0.0 | 99 |
| *Gallus gallus PMEL17*AY 636126.1 | 892 | 0.0 | 99 | 902 | 0.0 | 99 | 893 | 0.0 | 99 |
| *Gallus gallus* Premelanosome protein (*PMEL17*) mRNANM_205112.2 | 884 | 0.0 | 99 | 904 | 0.0 | 99 | 895 | 0.0 | 99 |
| *Gallus gallus* Premelanosome protein (*PMEL17*) mRNANM_015886909.1 | 634 | $1e^{-177}$ | 88 | 650 | 0.0 | 88 | 641 | $7e^{-180}$ | 87 |

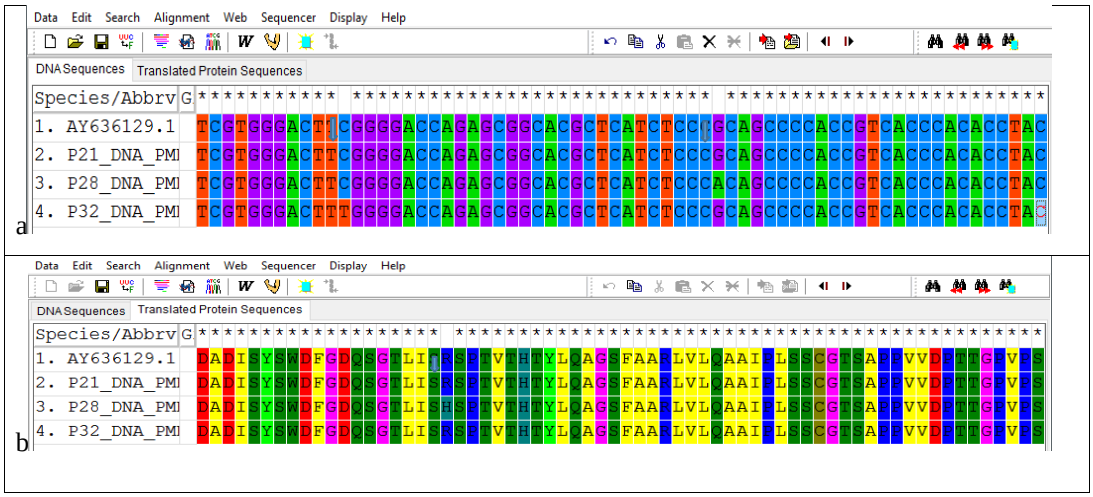


**Fig 1(a):** Sequence alignment of PMEL17 with reference sequence by using MEGA 6 programme **(b)** protein alignment of the studied genotype.

**N.B.** P21 DNA *PMEL17*=Spotted single round, P28 DNA *PMEL17*= Black and white single round, P32 DNA *PMEL17*= Brownish single round, AY636129.1= *Gallus gallus PMEL17* protein gene).

## CONCLUSION

It can be concluded that the spotted single round variety chickens were found to be best for egg production, live weight and external and internal quality of eggs than the other studied variety of chickens. Mutation of plumage colour genes, *PMEL17* was detected in white-black and brownish single round variety chickens and appeared the variability of the production traits. The spotted variety can be used in the traditional systems in rural areas as dual purpose chicken production.

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## Conflicts of Interest

The authors declare no conflict of interest with the funding body.

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