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**GENETIC DIVERSITY OF THE MAJOR HISTOCOMPATIBILITY  
COMPLEX BY USING LEI0258 MICROSATELLITE MARKER  
ASSOCIATED WITH PRODUCTIVE PERFORMANCE AND  
VIRAL DISEASES IN BROILER BREEDERS**

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**ABSTRACT:** The chicken Major Histocompatibility Complex (MHC) is closely related to resistance to several diseases. Moreover, selection for genetic resistance to diseases is a critical goal in the broiler breeding companies, which should be carefully balanced with different economic traits, including live body weight (LBW) and meat yield. The microsatellite marker LEI0258 is used to evaluate the effect of selection for 12 generations for high LBW at 6 weeks of age (LBW6) with natural selection against Marek disease (MD) on genetic component associated with resistance to MD and Newcastle diseases (ND) in local broiler breeders, Cairo B-2 line, compared to commercial broiler breeder line (AA line). Results indicated that, Cairo B-2 line had lower carcass parts with higher internal organs percentages compared to AA line. In addition, there was a positive association between 473 bp allele of LEI0258 with regard to LBW6 and carcass traits in two lines. Moreover, LEI0258 alleles 357 bp and 295 bp were present with high content in Cairo B-2 line only, which increased the genetic resistance to Marek's disease and increased the humoral immune response against ND vaccination in Cairo B-2 line compared to AA line. The correlations between these valuable productive traits and LEI0258 microsatellite will effectively assist the selection process by applying Marker-Assisted Selection in Cairo B-2 line breeding program in the future.

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**Keywords:** Broiler Breeder; Body weight; Immune response; Marek's disease, Selection

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## **INTRODUCTION**

Newcastle disease (ND) and Marek's disease (MD) are the most worldwide-recognized problem and are defined as the major cause of great economic loss for meat and egg in the poultry production. Newcastle Disease Virus (NDV) widely differs regarding the severity of the disease and strain, and it is widely considered as an acute infectious epidemic disease to chicken flocks (Zhang et al., 2018). In addition, MD is reported to cause annual loss of more than \$1 to \$2 billion (Morrow and Fehler, 2004).

Using recent biotechnology in poultry breeding programs for disease resistance plays a critical role in the control of different diseases. Moreover, the Key technology of molecular breeding is determined the genes associated with disease resistance (Wang et al., 2014). For instance, different genes of the chicken major histocompatibility complex (MHC) plays a critical role in disease resistance to several diseases including Marek's disease virus (Hansen et al., 1967, Briles et al., 1977 and Bacon et al., 2000) and Newcastle disease virus (Lwelamira et al., 2008). Thus, the chicken MHC genes considered as suitable marker genes in the immune genetics in broiler breeding programs (Pinard et al., 2002).

On the other hand, selection for enhancing the immune response is a critical trait in the poultry breeding programs that should be balanced with different productive traits (Fulton, 2004). Thus, selecting for improving genetic resistance to MD is controlled mechanism that can augment vaccination control measures and to control portions of the phenotypic variance (MacEachern et al., 2012). Additionally, selection for

enhancing genetic resistant chickens to MD is a critical mechanism for genetic development of disease resistance in meat and egg chicken type (Luo et al., 2013).

Cairo B-2 line is developed at Poultry Farm, the Experimental Agricultural Station, Faculty of Agriculture, Cairo University, Egypt, to become the first Egyptian broiler female line (Nassar et al., 2012). Selection improvement program was initiated to develop its productivity from 2003 until now. In addition, Cairo B-2 line was individually selected based on high LBW at 6 weeks of age (LBW6) as phenotypic selection method. Nevertheless, Cairo B-2 line was not vaccinated against MD and natural selection program against MD was implemented to increase genetic resistance against MD in this line. Thus, the current study was conducted to investigate the effects of selection programs after twelfth generation on genes associated with MHC, LBW, carcass traits, and immune response against ND by using LEI0258 marker in Cairo B-2 line Compared with Commercial broiler breeder line.

## **MATERIAL AND METHODS**

### **History of Cairo B-2 line as a local broiler breeder Female line**

A selection improvement program was started at the Poultry Farm, Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt, to develop Cairo B-2 line as a local broiler female line intended for meat production (Nassar et al., 2012). This line was originated by crossing between Arbor Acers grandparent-female-line males with females from the native Egyptian chickens breed, i.e. the Native White Baladi chickens. The highest LBW6 males and females were selected as parents for the next generation in Cairo

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B-2 line. Besides, natural selection against MD was performed in all generations without using vaccination against the disease. The mortality rate was more than 36 % during the first three generations, then decreased from generation to the next, attaining less than 0.01% from the sixth up to the twelfth generations.

### **Experimental populations and management.**

The use of animals as well as the experimental design was approved by Cairo University Institutional Animal Care and Use Committee (CU-IACUC), Cairo University, Egypt. The following are the certificate reference numbers: CU/II/F/30/18.

Selected males and females, from the twelfth-selected generation of Cairo B-2 line, were mated to produce the thirteenth generation. Three hundred chicks from Cairo B-2 line and three hundred commercial chicks from Arbor Acres broiler breeder line (AA line) were wing-banded and sexed at hatch, using the vent method. All chicks were reared intermingled, 10 birds/m<sup>2</sup>, in an open house, deep litter system. Birds were provided with a commercial broiler starter (23% CP and 3,050 kcal ME/kg) and a broiler grower (21% CP and 3,100 kcal ME/kg) diets from 1 to 14 days and from 15 days to 6 weeks of age, respectively. Water and feed were provided ad libitum from hatch until 6 weeks of age. Light was provided 24 hours per day. At hatch, chicks were vaccinated against avian influenza virus by using (S/C) injection of H5N2 inactivated vaccine at one week of age. Chicks were also vaccinated against Newcastle disease at 7 days (eye drop, Hitchner, Nobilis®), at 10 days (S/C injection with Newcastle inactivated

vaccine, Nobilis®), and at 21 days (eye drop, La Sota strain, Nobilis®). Chicks were also vaccinated against infectious bursal disease at 14 and 24 days (eye drop) using Gumboro D78 strain (Nobilis®).

### **Experimental measurements for body weight and carcass traits**

For all lines, LBW at hatch, 7, 14, 21, 28, 35, and 42 days were obtained individually by using a digital scale. At 42 days of age, 50 males and 50 females from each line were chosen at random. Birds were weighed and the weights were recorded as LBW, and then slaughtered after 8 hours of fasting (Papa, 1991). Birds were slaughtered by slitting the throat, cutting the carotid arteries, jugular veins, esophagus and trachea without severing the head (Salwani et al., 2016). After slaughtering, each bird was hanged in a bleeding funnel for 3 minutes, weighed, then scalded in a 68<sup>o</sup> C water bath for 30 seconds, and the feathers were removed by an automatic circular feather plucker. Birds were eviscerated and weighed; the head and shanks were removed and weighed; lastly, the carcasses were weighed then chilled. Each chilled carcass was weighed to obtain the carcass weight. The wings with bones were then removed from the front parts and weighed and recorded as wings with bones weight. Also, the skinless pectoralis major and minor muscles were removed to obtain breast muscles weight. The bones from the thighs and drumsticks were removed then the skinless leg muscles were weighed as leg meat weight. Internal organs and abdominal fat pad were removed and then weighed to obtain abdominal fat pad weight, heart weight, gizzard weight empty and without the fat adhering, liver weight, and spleen weight. Moreover, all previous muscles

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and organs weights were also calculated as percentages of the LBW.

### Blood samples and DNA isolation

At 28, 35, and 42 days of age, blood samples (1 ml/bird) were randomly collected from the wing vein of 100 female birds per each line in non-heparinized tubes, and the blood was allowed to clot. These samples were centrifuged at 3000 rpm for 10 minutes and the serum was separated. Serum samples were stored at -20°C until analysis. Serum antibody titers against NDV were determined by using log<sub>2</sub> of the hemagglutination inhibition (HI) test (Hanson, 1972 and Swayne and Halvorson, 2003).

On the other hand, individual genomic DNA was isolated from venous blood collected in anti-coagulate buffer from 100 females from each line at 42 days of age. The extraction was carried out according to the method described by Bailes et al., (2007). Microsatellite LEI00285 were used, which are associated with the immune response against MD and ND (<http://www.ncbi.nlm.nih.gov/genbank/>). The nucleotide sequences of the microsatellites used in this study are (Forward:

CACGCAGCAGAACTTGGTAAGG;

Reverse:AGCTGTGCTCAGTCCTCAG

TGC). The reaction mixture (20 µl) contained 50 ng DNA, 200 µM dNTPs, 1 µM from each primer, 0.5 unit of Red Hot Taq polymerase (AB-gene House-UK) and 10 X Taq polymerase buffer (AB-gene House-UK). The PCR conditions were as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were electrophoresed at 100 V

on a 1.5% Agarose gel and visualized by staining with ethidium bromide.

### Statistical analysis

Data were analyzed as a two-way analysis of variance using the SAS software, general linear model (SAS Institute, 2009). The main effects were line and sex. Traits analyzed were LBW at hatch, 7, 14, 21, 28, 35, 42 days of age. In addition, carcass parts, and muscles as weights and as percentages of LBW6 for males and females of each line. All data were reported as least square means (LSM) ± standard errors (SE). Mean values were separated, when significance existed, using Duncan's multiple range test (Duncan's, 1955). Significance level was set at 5%. The following model was used:

$$Y_{ijk} = \mu + L_i + S_j + LS_{ij} + e_{ijk}$$

Where,

$Y_{ijk}$ : The  $K$ th observation of the  $j^{\text{th}}$  sex within the  $i^{\text{th}}$  lines.

$\mu$ : The overall mean.

$L_i$ : The effect of the  $i^{\text{th}}$  line.

$S_j$ : The effect of the  $j^{\text{th}}$  sex.

$LS_{ij}$ : The interaction between the  $i^{\text{th}}$  line and the  $j^{\text{th}}$  sex.

$e_{ijk}$ : Random error.

For HI titer results, data were analyzed as a one-way analysis of variance using the SAS software, general linear model (SAS Institute, 2009). The main effect was line. All data were reported as least square means (LSM) ± standard errors (SE). Mean values were separated, when significance existed, using Duncan's multiple range test (Duncan's, 1955). Significance level was set at 5%. The following model was used:  $Y_{ij} = \mu + L_i + e_{ij}$  Where,  $Y_{ij}$ : The  $j^{\text{th}}$  observation within the  $i^{\text{th}}$  line.  $\mu$ : The overall mean.  $L_i$ : The effect of the  $i^{\text{th}}$  line.  $e_{ijk}$ : Experimental error.

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On the other hand, genotypic frequencies were estimated by direct counting. Expected homozygosity, heterozygosity, and polymorphic information content (PIC) were computed using the Levene (1949) method and the Popgene version 1.32.0 software was used to estimate these parameters.

### **RESULTS AND DISCUSSION**

#### **Body weight from hatch to 42 days of age in Cairo B-2 and AA lines**

The current results indicated that the AA line had a significant higher LBW compared to Cairo B-2 line at hatch, 14, 21, 28, 35, 42 days of age (Table 1). The results indicated that AA line exhibited significantly higher LBW6 compared with Cairo B-2 line as shown in Table 1. Moreover, growth performance and LBW6 for each line were affected by the genotype component for each line. Furthermore, LBW from hatch until 42 days of age was influenced by sex. On the other hand, males from each line had significantly higher LBW from hatch until 42 days of age compared to females. The interaction between sex with genotype, genotype, and sex effects on LBW from hatch until 6 weeks of age for all lines is shown in Table 1.

There is a high level of genetic variation in LBW between different broiler populations occurred by the selection program for high LBW (Mebratie et al., 2017). As expected, AA line produced higher LBW6 than Cairo B-2 line because LBW at hatch significantly influenced broiler performance; thus, chickens with heavy initial weight produced higher LBW at market age (Mendes et al., 2011). On the other hand, many studies indicated that sex and genotype are primary factors affecting productive performance in meat type chicken (Siegel et al., 1984, Havenstein et al., 1994 and Nassar et al.,

2019). In broiler production cycle, the interaction between genotype and sex had critical effects on the broiler productive performance. Thus, an accurate assessment of broiler performance should be included these factors (Moreira et al., 2003 and Fernandes et al., 2013). Moreover, Benyi et al., (2015) study, the genotype with sex interaction in broiler production had significant effects on growth performance in broilers. The current study stated that the entity of the differences between lines is regard to the effects of sex with genotype interaction on LBW6. These results were in alignment with the results reported by many studies (Fernandes et al., 2013 and Gomaa et al., 2014).

#### **Carcass Traits and Meat yield at 42 days of age in Cairo B-2 and AA lines**

The results indicated that Cairo B-2 line had significantly lower wings with bones, abdominal fat pad, leg muscles, breast muscles, carcass weights compared with AA line (Table 2). Moreover, the AA line had significantly higher leg muscles, breast muscles, carcass, and abdominal fat pad percentages compared to Cairo B-2 line (Table 2). However, AA line had less wings with bone percentage compared with Cairo B-2 line (Table 2). The effect of genotype and sex interaction was observed with significant effect on the carcass traits at LBW6 for AA and Cairo B-2 lines as shown in Table 2.

Genetic selections for increasing productive performance in broilers have been highly successful in improving LBW and carcass traits at market age (Mason et al., 2020). Thus, the differences observed between Cairo B-2 and AA lines in yields percentages may be associated with the various genetics origins of different chicken breeds or lines (Goliomytis et al., 2003 and Nassar

et al., 2017). Moreover, intense selection for rapid growth in the AA line induces increased fat deposition compared to Cairo B-2 line with low productive performance (Rance et al., 2002 and Schmidt et al., 2009). In addition, Boschiero et al. (2009) reported that, broiler strain had significant effects on carcass and abdominal fat as percentage of LBW. Moreover, the highest relative breast meat yield in AA line explains by the highest LBW6 of this line because birds with higher LBW produced the greatest breast portions as reported by Schmidt et al. (2009). In the current study, the AA line had less wings with bone percentage compared with the Cairo B-2 line. This may be occurred by increasing in breast meat portion that caused decreases in the other carcass parts on relative basis. (Fletcher and Carpenter, 1993 and Nassar et al., 2019).

On the other hand, the effect of sex and genotype on carcass parts was shown in Table 2. As expected, male's birds, in AA line, had significantly higher abdominal fat pad, wings with bones, leg muscles, breast muscles, and carcass weights and percentages compared to females from the same line and females and males from Cairo B-2 line. The differences in productive performance at market age will be higher in males than females in modern broiler as reported previously by Vargas et al., (2020). Moreover, a higher significant interaction of genotype and sex was observed for carcass yield in AA line compared with Cairo B-2 line as shown in table 2. In addition, carcasses yield are mainly influenced by breed and sex. These results are in alignment with the results conducted by (Fernandes et al., 2013). Moreover, Males from Cairo B-2 and AA line had higher meat yield weights and proportions than female's

birds from the same line, which indicates the sexual dimorphism presence between males and females in AA and Cairo B-2 lines. These results are similar to the results reported by (Nassar et al., 2012 and Zhao et al., 2015).

#### **Internal and lymphoid organs at 42 days of age in Cairo B-2 and AA line**

The results indicated that AA line had significantly higher heart, gizzard, liver, and spleen weights compared to Cairo B-2 line (Table 3). However, Cairo B-2 line had significantly higher heart, spleen liver, and gizzard percentages compared to AA line (Table 3). In addition, sex and genotype interaction was observed for LBW6 with significant effects on lymphoid and internal organs as shown in Table 3. Moreover, males from AA line had significant higher heart, spleen, liver, and gizzard weights compared to females and males from the Cairo B-2 line.

Females from Cairo B-2 line had significantly lower heart, spleen, gizzard, and liver percentages (Table 3). In general, there are negative correlation between LBW6 with internal and lymphoid organs percentage that explain the decreases in in the percentages of the internal and lymphoid organs in AA line compared to Cairo B-2 line. These results are reported privously by (Gaya et al., 2006 and Schmidt et al., 2009). Moreover, the sexual dimorphism presence in each line explain the highest lymphoid and internal organs in males compared to females in the two lines as reported previously by Mignon-Grasteau et al. (2000) and Zhao et al., (2015).

#### **Humoral Immune response against NDV in Cairo B-2 and AA line**

The NDV caused the agglutinating of the red blood cells of chicken that inhibited by ND immune serum. Thus, the hemagglutination inhibition (HI) titers are

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suitable and easier method used for evaluate the immune response (Alexander, 2003). Moreover, HI titer works as specific humoral immunity indicator. Thus, it's a great magnitude to enhance the HI titer (Swayne and Halvorson, 2003).

Results demonstrated that the Cairo B-2 line had significantly higher HI titer against NDV than AA line at 28, 35, 42 days of age (Table 4) that is due to the impact of lighter LBW of the Cairo B-2 line. Moreover, continuous selection for high LBW might have changed the genetic component of commercial meat type chicken since a negative correlation between high LBW and immune response exists (Qureshi and Havenstein, 1994 and Shapiro et al., 1998). In addition, selection for improving broiler productive performance caused reduces in different lymphoid organs growth, such as spleen, on relative basis (Cheema et al., 2003). Thus, birds with higher LBW6 that occurred in the AA line caused negative effects on lymphoid organs weights in relative basis that also caused general decreases in the antibody response against NDV compared to Cairo B-2 line that had lighter LBW6. In addition, evaluation of the impact of selection for high LBW on lymphoid organs function and genetic resistance to different diseases will required continues assessment for many generations in broiler breeds/lines as reported previously by (Qureshi and Havenstein, 1994).

### **LEI0258 microsatellite markers associated with productive performance in Cairo B-2 and AA lines.**

Seven differently sized alleles of LEI0258 marker (231-577 bp) were identified for Cairo B-2 line and AA line, (Table 5; Figure 1). Allele 473bp only was shared by two lines, whereas alleles

231, 261, 295, 305, 357, and 577 bp were represented by single line only (Table 5; Figure 1). Our results indicated that allele 473bp was only shared by two lines with higher allelic frequency (57.14%) in Cairo B-2 than allelic frequency (37.50%) in AA line as shown in table 3, which suggested that there was a positive association between this 473 allele with LBW6 and carcass traits in two lines. Moreover, the effects of different alleles on different productive economic traits should be studied breed/line-wise that is associated with in local chicken breeds particularly based on the predominance of high diversity at MHC as reported privously by Ewald et al., (2007).

The correlation between LEI0258 alleles and productive performance including LBW were found to be breed-specific. As mentioned in many studies, alleles of LEI0258 had a significant positive association with LBW of Tanzanian native chickens (Lwelamira et al., 2008). In addition, a positive correlation was observed between LEI0258 alleles and LBW recorded at 4 and 8 weeks of age in Indian breeds (Haunshi et al., 2020). However, a negative correlation with LBW at 8 and 12 weeks of age was stated in Iranian chickens (Nikbakht and Esmailnejad, 2015), and with LBW at 7 weeks of age (Ewald et al., 2007).

Associations between LEI0258 alleles and immune traits was observed in the current study that caused by the direct effects of LEI0258 alleles. However, the indirect association caused by continuous selection for fast growth which may cause reduced in lymphoid organs percentages that led to decrease in immune function in Cairo B-2 and AA line. Our results confirm the results previously reported by Van der Most et al., (2011).

**LEI0258 microsatellite markers associated with immune response against viral diseases in Cairo B-2 and AA lines.**

The use of DNA markers is currently one of the most practical methods for the determination of MHC-B variabilities in chicken populations than traditional serological methods (Fulton, 2020). The current results indicated that the LEI0258 allele 357bp was present with high content in Cairo B-2 line only (Table 5; Figure 1). Many studies indicated that allele 357bp is strongly associated with MD resistance in some chicken populations (Briles et al., 1977, Bacon et al., 2001, Fulton et al., 2006 and Kannaki et al., 2017); However, it may be absent from some other populations. This allele was absent in Chinese, Tanzanian, Vietnamese, Brazilian, Indian chicken populations (Lwelamira et al., 2008, Lima-Rosa et al., 2005, Schou et al., 2007 and Kannaki et al., 2017). The high content of allele 357bp at the LEI0258 locus and consequently the B21 haplotype of the MHC linked with it in Cairo B-2 line chickens suggests that there is a genetically-determined resistance of Cairo B-2 line chickens to Marek's disease than AA line. Our results are in alignment with the results previously conducted by (Fulton et al., 2006 and Kannaki et al., 2017).

The LEI0258 allele 295 bp was present with high content in Cairo B-2 line only (allele frequency= 31.25%), while the allele 261 bp was present with high content in AA line only (allele frequency= 7.14%) as shown in Table 5 and Figure 1. Thus, increases in humoral immune response against ND vaccination occurred in Cairo B-2 line than AA line (Table 3). In addition, the 261 bp and 295 bp alleles were significantly positively

dealing with HI titers to NDV vaccine which indicated the critical importance of MHC in vaccine response to the NDV antigen. Our results are congruent with the results conducted by (Lwelamira et al., 2008, Esmailnejad et al., 2017 and Haunshi et al., 2020).

Results from the current study indicated that observed mean number of alleles ( $N_a$ ) for marker LEI0258 in Cairo B-2 and AA lines was 6 vs. 4, respectively, whereas the effective mean number of alleles ( $N_e$ ) was 3.67 in Cairo B-2 line vs. 2.37 in AA line (Table 6). Moreover, LEI0258 alleles are correlated with MHC haplotypes and the alleles may differently associated serologically with the defined MHC haplotype in the different genetic lines or breeds (Izadi et al., 2011) which allocate resistance to Marek disease, (Kaufman, 2000) and to ND (Lwelamira et al., 2008). High diversity of allelic numbers, and consequently higher polymorphism in LEI00285 marker evaluated in Cairo B-2 line compared to the AA line (Figure 1) makes Cairo B-2 line have higher immune response than AA line, as shown in table 4, and protect Cairo B-2 line from infection with MD at the same time.

Moreover, single MHC markers LEI0258 allele or band can cause phenotypic variation associated with antibody response to NDV vaccination in broilers (Younash et al., 2000 and Lwelamira et al., 2008). These results indicate that the strong pressure of artificial selection that occurred at the Cairo B-2 line for twelve generations had positive effects on the assessment of LEI0258 markers polymorphism in Cairo B-2 line genome which is associated with increased immune response against MD and increased humoral immune response against ND vaccination, as mentioned by

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(Fonteque et al., 2014 and Ngenoet et al., 2014). In addition, the genetic diversity of breeds or lines can be evaluated by the proportion of polymorphic loci, genotype and allelic frequencies, and by observed and expected heterozygosities (Fonteque et al., 2014). High allelic number of LEI0258 with higher frequency of heterozygosities suggests a higher antigen diversity being presented to T cells to mediate disease tolerance in Cairo B-2 line than AA line. Our results concur with the results stated by (Chazara et al., 2013).

The current results indicated that the observed heterozygosities (Ob-Het) were 0.87 and 0.75 in Cairo B-2 and AA lines, respectively (Table 6). Also, the expected heterozygosities (Exp-Het) were 0.78 and 0.62 in Cairo B-2 and AA lines, respectively (Table 6). In Fonteque et al., (2014) study, the high genetic diversity of markers occurred when the heterozygosity values were above 0.5. Thus, the Cairo B-2 line had high genetic diversity of LEI0258 than AA line, which may associate our breeding program. Our results are in alignment with the results demonstrated by (Fonteque et al., 2014). Increased MHC heterozygosity caused improvements in the diversity of antigens that improve the disease tolerance in Cairo B-2 line than AA line. Our results are congruent with the results reported by (Wegner et al., 2004 and Chazara et al., 2013). In addition, the polymorphic information content (PIC) used as an indicator of the suitability of the marker to be used. Also, PIC value more than 50% is considered as quite informative (Fonteque et al., 2014). PIC values were 0.78 and 0.64 in Cairo B-2 and AA lines, respectively (Table 6).

Finally, associations between alleles of LEI0258 and immune response observed in the current study could be resulted from the direct effects of LEI0258 alleles. However, the indirect association caused by continuous selection for fast growth

may change the result of reduced immune function with genetic make-up in Cairo B-2 and AA line. Our results are in alignments with the results mentioned by Van der Most et al. (2011).

### **CONCLUSION**

The balance between improving growth performance and immune response faces critical challenges in poultry breeding programs. Evaluation of the impact of selection for high LBW on lymphoid organs function and genetic resistance to different diseases will required continues assessment for many generations in broiler breeds/lines. Moreover, the genes of MHC region is very strong association with genetic disease resistance in chickens. The current study indicated that there were high genetic diversity and heterozygosity observed in the MHC region in Cairo B-2 line than AA line. In addition, specific allele 357 and 261bp at the LEI0258 locus was present with high content only in Cairo B-2 line, which caused the high immune resistance against NDV and MDV. Thus, positive evolution had occurred in MHC markers LEI0258 polymorphism within Cairo B-2 line genome, which caused increased immune response against MD, and increased humoral immune response against ND vaccination due to the natural selection against MD with artificial selection for high BW6 occurred at the Cairo B-2 line for twelve generations. In addition, the current study indicated the direct effects of LEI0258 locus and consequently the B21 haplotype of the MHC on disease resistance in chickens. The use of DNA markers is currently one of the most practical methods for identification of MHC variation associated with diseases such as MD and ND in chicken populations than traditional serological methods. Moreover, different genes in this critical region of Cairo B-2 genome associated with resistance to many diseases will be focused and merged into our breeding programs in the future.

**Table (1):** Live Body weight from hatch to 42 days in Cairo B-2 and AA lines

Lines	Age						
	Hatch	7 days	14 days	21 days	28 days	35 days	42 days
AA	45.8 <sup>a</sup>	171 <sup>a</sup>	377 <sup>a</sup>	717 <sup>a</sup>	1197 <sup>a</sup>	1707 <sup>a</sup>	2120 <sup>a</sup>
Cairo B-2	41.2 <sup>b</sup>	141 <sup>b</sup>	301 <sup>b</sup>	473 <sup>b</sup>	683 <sup>b</sup>	937 <sup>b</sup>	1207 <sup>b</sup>
SEM	0.09	1.45	1.85	2.99	3.65	3.89	5.11
<b>Sex</b>							
Male	44.2 <sup>a</sup>	166 <sup>a</sup>	368 <sup>a</sup>	652 <sup>a</sup>	1038 <sup>a</sup>	1432 <sup>a</sup>	1869.5 <sup>a</sup>
Female	42.7 <sup>b</sup>	145 <sup>b</sup>	310 <sup>b</sup>	538 <sup>b</sup>	841 <sup>b</sup>	1212 <sup>b</sup>	1457.5 <sup>b</sup>
SEM	0.10	1.11	1.75	2.96	4.56	4.89	7.21
<b>Line*sex</b>							
AA ♂	46.5 <sup>a</sup>	185 <sup>a</sup>	409 <sup>a</sup>	786 <sup>a</sup>	1316 <sup>a</sup>	1832 <sup>a</sup>	2337 <sup>a</sup>
AA ♀	45.1 <sup>b</sup>	156 <sup>b</sup>	345 <sup>b</sup>	648 <sup>b</sup>	1079 <sup>b</sup>	1582 <sup>b</sup>	1903 <sup>b</sup>
Cairo B-2 ♂	41.9 <sup>c</sup>	147 <sup>c</sup>	327 <sup>c</sup>	518 <sup>c</sup>	760 <sup>c</sup>	1032 <sup>c</sup>	1402 <sup>c</sup>
Cairo B-2 ♀	40.5 <sup>d</sup>	134 <sup>d</sup>	275 <sup>d</sup>	428 <sup>d</sup>	603 <sup>d</sup>	842 <sup>d</sup>	1012 <sup>d</sup>
SEM	0.12	1.98	2.09	2.56	3.58	3.89	4.32
<b>Probability</b>							
Line	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Sex	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Line*Sex	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

\*AA line =Arbor Acers ♂ × Arbor Acers ♀, Cairo B-2 line = Cairo B-2 ♂× Cairo B-2 ♀

<sup>a-b</sup>Means between line or sex or the interaction, within age, followed by different superscripts, differ significantly (p≤ 0.05).

**Table (2):** Means and SEM of Carcass, Breast muscles, leg muscles, wings with bones, and abdominal fat bad weights and percentages in Cairo B-2 and AA lines at 42 days of age.

Lines*	Traits										
	LBW (gm)	Carcass		Breast Muscles		Leg Muscles		Wings with Bones		Abdominal Fat Pad	
		Weight (gm)	%LBW	Weight (gm)	%LBW	Weight (gm)	%LBW	Weight (gm)	%LBW	Weight (gm)	%LBW
AA	2270 <sup>a</sup>	1653.24 <sup>a</sup>	72.83 <sup>a</sup>	456.61 <sup>a</sup>	20.12 <sup>a</sup>	367.17 <sup>a</sup>	16.18 <sup>a</sup>	173.31 <sup>a</sup>	7.63 <sup>b</sup>	58.91 <sup>a</sup>	2.60 <sup>a</sup>
Cairo B-2	1310 <sup>b</sup>	909.01 <sup>b</sup>	69.39 <sup>b</sup>	237.83 <sup>b</sup>	18.16 <sup>b</sup>	200.89 <sup>b</sup>	15.33 <sup>b</sup>	105.46 <sup>b</sup>	8.05 <sup>a</sup>	26.59 <sup>b</sup>	2.03 <sup>b</sup>
SEM	11.63	9.46	0.58	4.56	0.19	4.12	0.21	1.89	0.11	1.56	0.10
<b>Sex</b>											
Male	1927 <sup>a</sup>	1388.65 <sup>a</sup>	71.63 <sup>a</sup>	381.07 <sup>a</sup>	19.57 <sup>a</sup>	312.27 <sup>a</sup>	16.06 <sup>a</sup>	149.48 <sup>a</sup>	7.80 <sup>b</sup>	47.38 <sup>a</sup>	2.39 <sup>a</sup>
Female	1653 <sup>b</sup>	1174.98 <sup>b</sup>	70.59 <sup>b</sup>	314.59 <sup>b</sup>	18.71 <sup>b</sup>	256.54 <sup>b</sup>	15.45 <sup>b</sup>	129.16 <sup>b</sup>	7.89 <sup>a</sup>	38.33 <sup>b</sup>	2.24 <sup>b</sup>
SEM	18.23	17.56	0.56	4.56	0.13	5.32	0.13	1.46	0.02	0.63	0.11
<b>Line*sex</b>											
AA ♂	2385 <sup>a</sup>	1751.78 <sup>a</sup>	73.45 <sup>a</sup>	487.73 <sup>a</sup>	20.45 <sup>a</sup>	397.58 <sup>a</sup>	16.67 <sup>a</sup>	181.74 <sup>a</sup>	7.62 <sup>c</sup>	63.92 <sup>a</sup>	2.68 <sup>a</sup>
AA ♀	2155 <sup>b</sup>	1556.12 <sup>b</sup>	72.21 <sup>b</sup>	426.26 <sup>b</sup>	19.78 <sup>b</sup>	337.90 <sup>b</sup>	15.68 <sup>b</sup>	164.85 <sup>b</sup>	7.65 <sup>c</sup>	54.09 <sup>b</sup>	2.51 <sup>b</sup>
Cairo B-2 ♂	1469 <sup>c</sup>	1025.50 <sup>c</sup>	69.81 <sup>c</sup>	274.41 <sup>c</sup>	18.68 <sup>c</sup>	226.96 <sup>c</sup>	15.45 <sup>c</sup>	117.23 <sup>c</sup>	7.98 <sup>b</sup>	30.85 <sup>c</sup>	2.10 <sup>c</sup>
Cairo B-2 ♀	1151 <sup>d</sup>	793.84 <sup>d</sup>	68.97 <sup>d</sup>	202.92 <sup>d</sup>	17.63 <sup>d</sup>	175.182 <sup>d</sup>	15.22 <sup>d</sup>	93.46 <sup>d</sup>	8.12 <sup>a</sup>	22.56 <sup>d</sup>	1.96 <sup>d</sup>
SEM	10.11	6.78	0.46	2.13	0.13	3.14	0.10	1.85	0.05	1.16	0.02
<b>Probability</b>											
Line	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Sex	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Line*Sex	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

\* AA line =Arbor Acers ♂ × Arbor Acers ♀, Cairo B-2 line = Cairo B-2 ♂× Cairo B-2 ♀ LBW= Live body weight.

<sup>a-b</sup>Means between line or sex or the interaction, within trait, followed by different superscripts, differ significantly (p≤ 0.05).

**Table (3):** Means and SEM of heart, Gizzard, liver, and spleen weights and percentage in Cairo B-2 and AA lines at 42 days of age.

Lines	Traits							
	Heart		Gizzard		Liver		Spleen	
	Weight (gm)	%LBW	Weight (gm)	%LBW	Weight (gm)	%LBW	Weight (gm)	%LBW
AA	13.06 <sup>a</sup>	0.58 <sup>b</sup>	35.88 <sup>a</sup>	1.58 <sup>b</sup>	44.96 <sup>a</sup>	1.98 <sup>b</sup>	4.99 <sup>a</sup>	0.22 <sup>b</sup>
Cairo B-2	8.92 <sup>b</sup>	0.68 <sup>a</sup>	24.59 <sup>b</sup>	1.88 <sup>b</sup>	30.21 <sup>b</sup>	2.33 <sup>a</sup>	3.07 <sup>b</sup>	0.23 <sup>a</sup>
SEM	0.18	0.03	0.18	0.01	1.96	0.06	0.02	0.01
<b>Sex</b>								
Male	11.98	0.64	32.84	1.74	39.38	2.06	4.31 <sup>a</sup>	0.22 <sup>b</sup>
Female	10.00	0.62	27.62	1.72	35.79	1.99	3.75 <sup>b</sup>	0.23 <sup>a</sup>
SEM	0.13	0.01	1.01	0.02	1.02	0.02	0.04	0.01
<b>Line*sex</b>								
AA ♂	13.83	0.58	37.92	1.59	47.46	1.99	5.25 <sup>a</sup>	0.22 <sup>c</sup>
AA ♀	12.28	0.57	33.83	1.57	42.45	1.97	4.74 <sup>b</sup>	0.22 <sup>c</sup>
Cairo B-2 ♂	10.14	0.69	27.76	1.89	31.29	2.13	3.38 <sup>c</sup>	0.23 <sup>b</sup>
Cairo B-2 ♀	7.71	0.67	21.41	1.86	29.12	2.02	2.76 <sup>d</sup>	0.24 <sup>a</sup>
SEM	0.31	0.01	0.45	0.01	0.01	0.01	0.11	0.01
<b>Probability</b>								
Line	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Sex	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Line*Sex	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

\*AA line =Arbor Acers ♂ × Arbor Acers ♀, Cairo B-2 line = Cairo B-2 ♂× Cairo B-2 ♀, LBW= Live body weight.

<sup>a-b</sup>Means between lines or sex or the interaction, within traits, followed by different superscripts, differ significantly (p≤ 0.05).

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**Table (4):** Effect of selection on antibody titers against Newcastle disease virus (NDV) of Cairo B-2 line and AA line at 28, 35, and 42 days of age.

Lines	HI titers		
	28 days	35 days	42 days
Cairo B-2	6.89 <sup>a</sup>	7.65 <sup>a</sup>	7.60 <sup>a</sup>
AA	5.75 <sup>b</sup>	5.70 <sup>b</sup>	5.25 <sup>b</sup>
SEM	0.17	0.15	0.18
Probability	0.0001	0.0001	0.0001

\*AA line =Arbor Acers ♂ × Arbor Acers ♀, Cairo B-2 line = Cairo B-2 ♂× Cairo B-2 ♀, HI titer= hemagglutination inhibition (HI) titers

<sup>a-b</sup>Means between lines or sex or the interaction, within days, followed by different superscripts, differ significantly (p≤ 0.05) for each other.

**Table (5):** Allele frequencies of LEI0258 microsatellite marker in Cairo B-2 and AA line.

Allele (bp)*	Cairo B-2 line	AA Line
231	2	0
261	0	1
295	5	0
309	1	0
357	2	0
473	6	8
577	0	5

\* AA line =Arbor Acers ♂ × Arbor Acers ♀, Cairo B-2 line = Cairo B-2 ♂× Cairo B-2 ♀, Allele (bp) = Allele (base pair).

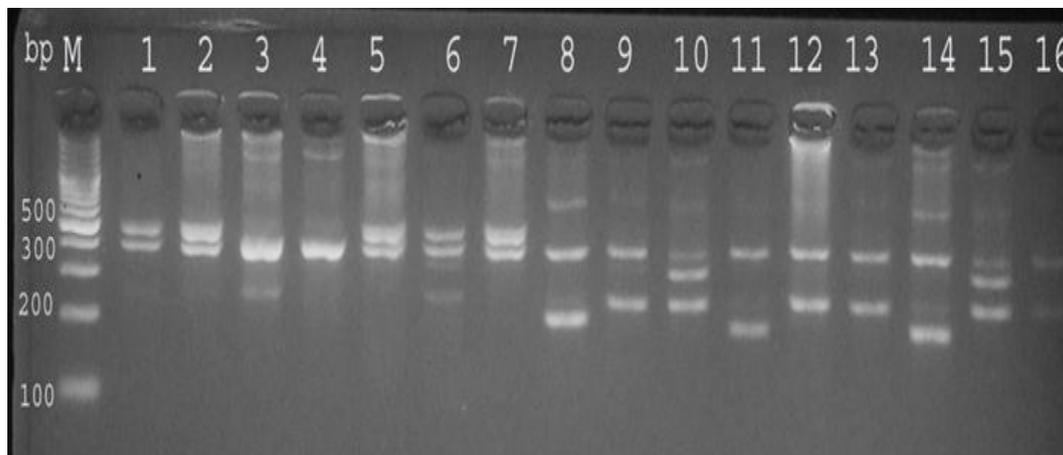
**Table (6):** Observed and expected heterozygosity and homozygosity in Cairo B-2 line and AA line.

Line	Na	Ne	Ob-Het	Ob-Homo	Exp-Het	Exp-homo	PIC
Cairo B-2	6	3.76	0.87	0.13	0.78	0.22	0.78
AA	4	2.37	0.75	0.25	0.62	0.38	0.64

\* AA line =Arbor Acers ♂ × Arbor Acers ♀, Cairo B-2 line = Cairo B-2 ♂× Cairo B-2 ♀.

Na= observed mean number of alleles, Ne= effective mean number of alleles, Ob-Het= observed heterozygosities, Ob-Homo=observed Homozygosities, Exp-Het= expected heterozygosities, Exp-Homo= expected Homozygosities, PIC= polymorphic information content.

**Figure (1):** Females PCR products and allelic numbers from both Cairo B-2 and AA lines for LEI0258. 1 to 8, AA line samples; 9 to 16, Cairo B-2 line samples. M, 100 bp DNA ladder (Fermentas Life Science, UK). PCR, Polymerase chain reaction.



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## المخلص العربي

المرتبط LEI0258 التنوع الوراثي للنسيج التوافقي المركب باستخدام مرقم المايكروستاليت مع الكفاءة الإنتاجية والأمراض الفيروسية في أمهات دجاج إنتاج اللحم

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يرتبط النسيج التوافقي المركب في الدجاج بشكل كبير مع المناعة ضد العديد من الامراض الفيروسية بالإضافة لذلك فإن عملية اختيار الأفراد المقاومة وراثيا للأمراض من أهم أهداف برامج التربية لإنتاج اللحم والتي تحتاج عند إجرائها إلى وجود توازن مع باقي الصفات الإنتاجية الأخرى مثل وزن الجسم الحي وإنتاج اللحم أيضا. تم استخدام مرقم المايكروستاليت LE0258 في تقييم كفاءة الانتخاب لوزن الجسم العالي عند 6 أسابيع وكذلك الانتخاب الطبيعي ضد مرض الماريك في التأثير الوراثي على مقاومة مرض الماريك وكذلك الاستجابة المناعية عند التحصين ضد مرض النيوكاسيل في سلالة الأمهات المحلية Cairo B-2 line وسلالة الأمهات الأجنبية AA كسلالة مقارنة. أظهرت النتائج أن Cairo B-2 line كان لها وزن جسم منخفض مع وزن أعضاء داخلية مرتفعة عند المقارنة بسلالة الأمهات التجارية. بالإضافة لذلك، كانت اليلات ذات الوزن الجزيئي 357 bp و 295bp موجودة بتكرارات عالية في سلالة الأمهات Cairo B-2 Line فقط مع ملاحظة زيادة مقاومتها وراثيا ضد مرض الماريك بالإضافة إلى الاستجابة المناعية العالية ضد مرض النيوكاسيل مقارنة بسلالة الأمهات الأجنبية المستخدمة في المقارنة. إن عملية الارتباط بين الصفات الاقتصادية الهامة مثل المقاومة للأمراض وزيادة كفاءة إنتاج اللحم المرتبطة مع مرقم المايكروستاليت LEI0258 سوف يتم استخدامه مستقبلا في عمليات الانتخاب في سلالة الأمهات المحلية في المستقبل القريب.

الكلمات المفتاحية: دجاج التسمين، أمهات التسمين، وزن الجسم، الاستجابة المناعية، مرض الماريك، الانتخاب.