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Full Length Research Paper

Microsatellite markers associated with body and carcass weights in broiler breeders

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Microsatellite markers are presently used in selection to facilitate the genetic improvement of growth and carcass traits in chickens. The genetic improvement of six weeks live body and carcass weights of Cairo B-2 line, after six generation of selection, was compared with the control line (C line). Cairo B-2 line had higher body weight, breast meat, and carcass parts than the C line. Seven microsatellites, associated with body and carcass weights, were efficiently used to study the effect of selection on the microsatellite marker frequencies of Cairo B-2 line. The allelic number of the microsatellite markers ADL0328, MCW0097, and ROS0025 associated with live body, breast, carcass, and wings weights increased in the Cairo B-2 line than the C line. The association between these important economic characteristics and microsatellite loci will facilitate the selection process by applying marker assisted selection in future breeding programs.

Key words: Marker assisted selection, microsatellite, carcass, and breast meat.

INTRODUCTION

Mainline pedigree broiler populations, categorized into male and female lines, undergo continuous genetic selection to achieve higher improvements in the major economic traits. For the male lines, these traits include body weight, body conformation, growth rate, fitness, edible meat yield, and feed conversion ratio. For the female lines, these traits are the same as the male line with more stress on egg production, and hatchability traits. These major traits were improved by positive selection. That is regenerated from the best families while the minor traits, such as fertility, hatchability, and livability, are impacted by eliminating the few worst families (Emmerson, 1997; Pollock, 1999; Muir et al., 2008).

Body weight and carcass traits were under intensive selection for more than half a century, and are considered as the most important economic traits in broiler breeding programs. Progress in rapid growth has been accompanied by an increase in abdominal fat deposition in broilers (Liu et al., 2008; Uemoto et al., 2009; Howie et al., 2011).

Recently, a number of studies have reported associations between genetic markers and quantitative traits of economic importance (vanKaam et al., 1998, 1999a and b; Yonash et al., 1999, 2001). More than 800 highly polymorphic microsatellite markers are available in the chicken genome which allows scanning for markers linked to economic traits of interest by using a genome wide search (Groenen et al., 2000).

In the last few years microsatellites became one of the most popular molecular markers used in different fields. High polymorphism and the relative ease of scoring represent the two major features that make microsatellites of interest for many genetic studies (Zane et al., 2002). Also, microsatellite markers became a standard technique that can be used for molecular genetic evaluation and mapping of chicken chromosomes (Cheng, 1997; Gholizadeh and Mianji 2007). Maximum usefulness of microsatellite markers, as a tool for genetic analyses, is that it could be applied in populations other than the source of the primer sequence (Kaiser et al.,

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2000).

Genetic markers based selection and genomic selection are providing additional tools for more accurate selection that will result in even faster rates of genetic improvement in the near future. Improving the performance of broiler chickens is a continuous process involving the application of both old and new discoveries in poultry science. The recent growth of knowledge in molecular genetics, as applied to animal breeding, has opened new possibilities for improving broiler performance of specific broiler and breeder crosses (Toosi et al., 2010).

The objectives of the current study were to evaluate the body weights and carcass measurements of the Egyptian broiler breeder female line, Cairo B-2, after six generations of selection. Some of the microsatellite markers associated with these traits were investigated.

MATERIALS AND METHODS

History of Cairo B-2 line as a broiler breeder

A selection improvement program was started at the Poultry Farm, Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt, to develop the Cairo B-2 line as a local broiler female line. This selection program was started in 2003.A total of 10 rosters from Arbor Acres grandparent female line males were crossed with 150 females from the native Egyptian breed White Baladi chickens to produce the base generation of the Cairo B-2 line.

The produced cross were reared until maturity and housed in individual cages. A total of 20 males and 200 females were selected randomly from the first cross and were mated at a ratio of one male to every 10 females. This was done by artificial insemination to obtain pedigreed fertile eggs. Fertile eggs were collected for 15 days and hatched to produce the F_1 selected Cairo B-2 line. Also, fertile eggs were collected again for 15 days, from the same sires and dams, and hatched to produce the control line (C line). All produced chicks were wing banded to keep their pedigree. Both lines were mated using an out breeding program, with no full or half sibs mating allowed.

For all the selected generations of Cairo B-2 line, phenotypic selection was used to identify the best broiler breeders to produce the next generation. The highest six-week body weights males and females were selected as parents for the next generation. Independent culling level selection was also practiced to select only the female hens that produced more than 45 eggs during the first 36 weeks of age. Cairo B-2 and the C lines were not vaccinated against mark's disease. Mortality rate, due to mark's disease, during the six generation, was less than 0.01% from the total number of reared birds.

Cairo B-2 broiler breeder line is the first Egyptian female line specialized in meat production. The Cairo B-2 female line has a round, massive body shape. The tail feathers and saddle region in the males are rich. The females lay white to creamy shell color eggs. Both males and females have mostly white feathers, red single combs with long wattles, red earlobe color, yellow skin, and yellow shank colors.

Experimental populations and management

In this study, Cairo B-2 selected males and females, from the 6^{th} selected generation, were mated to produce the seventh generation. Also, males and females from the 6^{th} generation C line were mated to produce the control chicks. Cairo B-2 and the control pedigreed chicks' were sexed at hatch, using the vent method. All chicks were reared intermingled, 10 birds/m^2, in an open house, deep litter system, until six weeks of age. Birds were provided with a commercial broiler starter (23% CP and 3,050 kcal ME/kg) and a grower (21% CP and 3,100 kcal ME/kg) diets from one to 14 days and from 15 days to six weeks of age, respectively. Water and feed were provided *ad libitum*. Light was provided 24 h per day.

Chicks were vaccinated against Newcastle disease at seven days (eye drop, Hitchner, Nobilis®), 10 days (S/C injection by Newcastle inactivated vaccine, Nobilis®), and 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 D_{78} strain (Nobilis®). In February 2006, there was an outbreak of the virulent avian influenza virus (H_5N_1) in Egypt (Abdou et al., 2008). Thus, the baby chicks from that time on were vaccinated against avian influenza virus by using (S/C) injection of H_5N_2 inactivated vaccine at one week of age. The inactivated H_5N_2 vaccine was injected subcutaneously in the lower back of the neck region.

Trait measurements

At six weeks of age, 15 males and 15 females, from both the selected and the control groups, were chosen at random. Birds were weighed as live body weight and slaughtered after 8 h of fasting (Papa, 1991). Birds were slaughtered by slitting the throat, cutting the carotid arteries, jugular veins, esophagus and trachea without severing the head (Sams, 2001). After slaughtering each bird was hanged in a bleeding funnel for 3 min and weighed again. Birds were then scalded in a 68°C water bath for 30 s, and then the feathers were removed by an automatic circular feather plucker. The birds were then weighed again. Birds were then eviscerated, the head and shanks were removed and the carcasses were chelled.

Each chelled carcass was weighed to obtain the carcass weight. Dressing percentages were expressed as the percentage of dressed weight to live weight. The wings with bones were then removed from the front parts and weighed. Also, the skinless pictoralis 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. All previous muscles and organs were also calculated as percentages of live body weight.

Microsatellite markers associated with carcass measurements

The experiment reported here was carried out at the Molecular Biology Laboratory of the Genetic Engineering Research Center (GERC), Faculty of Agriculture, Cairo University, Egypt.

Individual genomic DNA was isolated from venous blood collected in ethylenediaminetetraacetic acid (EDTA) from 15 males and 15 females from each line. A polymerase chain reaction (PCR) was carried out with 50 ng genomic DNA from the two lines (30 males and 30 females) to determine polymorphism. Seven microsatellite markers were chosen from a total of 12 microsatellite markers that are related to chicken body weight and carcass composition were used from a public chicken genome database (GenBank®, http://www.ncbi.nlm.nih.gov/genbank/). The sequence of the microsatellite markers used in this study are presented in (Table 1).The reaction mixture (20 µl) contained 50 ng DNA, 200 µM dNTPs, 1 µM from each primers, 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,

Table 1. Microsatellite primer codes and sequences and their distribution in chicken chromosomes.

Chicken chromosome No.	Primer code	Sequence (5′ → 3′)	Expected fragment size (bp)	Reference
1	ADL0328	F-CACCCATAGCTGTGACTTTG R-AAAACCGGAATGTGTAACTG	109-120	Liu et al., 2008
1	ROS0025	F- AGATTGCTGGGGGAAAAAGT R- ACTGAAAACCTGAACAGAAGGC	205-219	Liu et al., 2008
1	MCW0010	F- TCTGTAGAATTACAGAAATACA R- TAGTACAAGAATCTAGTGTTAAAA	93-109	Croojimans et al., 1996
1	MCW0018	F- GGAATTTGAACACCTGAGATTTCC R- CACTATATGTTTATGGCAAACTCCTG	199-221	Croojimans et al., 1996
3	c3-46151949	F- AAAGCTCACCACTTCAGCAG R- TGAACACTTAATTCCATGCCATA	231	Uemoto et al.,2009
5	c5-4999025	F- CCATCACGGTTTCTTCAAGG R- GCTGCTGACAGACAGTTCTCC	245	Uemoto et al.,2009
11	MCW0097	F- AGGAGAGCACATCTGCCTTCCTAG R- TGGTCTTCCAGTCTATGGTAG	263-309	Croojimans et al., 1996

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 2.0% agarose gel and visualized by staining with ethidum bromide.

Statistical analysis

Data were analyzed as a two-way analysis of variance using the SAS software, general linear model (SAS Institute, 1999). The main effects were line and sex. Traits analyzed were: six-week live body weights and carcass parts, and muscles as weights and as percentages of live body weight for both males and females Cairo B-2 and the C lines. 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 Kth observation of the jth sex within the ith line.
μ: The overall mean.
L_i: The effect of the ith line.
S_j: The effect of the j th sex
LS_{ij}: The interaction between the ith line and the jth sex
E_{ijk}: Random error.
Correlations between the numbers of microsatellite alleles and six-week body weight and carcass part weights were calculated. (SAS Institute, 1999).

RESULTS AND DISCUSSION

Genetic improvement of body and carcass weights by conventional breeding program

Genetic variability, within the more common commercial broiler breeds, has been greatly decreased by intense selective breeding programmes. Conservation of livestock genetic variability is thus important, especially when considering possible future changes in production environments (Boettcher et al., 2010). Also, the increase in sexual dimorphism, in body weight could be reduced by selecting animals based on body weight, as is usually done in commercial broiler lines (Mignon-Grasteau et al., 2000).

In the present study the live body weight and the carcass measurements of Cairo B-2 line, which have been subjected to intensive selection for six generations, were compared with the C line at the age of six weeks. The results indicate that, the Cairo B-2 line males exhibited higher live body weight (average = 1135 g) compared with the males from the C line (average = 7812

Table 2. Least Square Means ± SE from the ANOVA of live body weight and carcass parts weights (g) of six-week old Cairo B-2 and C lines.

Trait / Source of variation	Live body weight	Carcass	Breast meat	Leg meat	Wings with bones	Abdominal fat
Strain						
Cairo B-2	1039[a]	702[a]	116[a]	154[a]	82[a]	19.6[a]
Control	750[b]	483[b]	74[b]	95[b]	64[b]	12.0[b]
SE	21	15	3.0	3.6	1.9	0.39
Sex						
Male	958[a]	640[a]	104[a]	133[a]	77[a]	16.8[a]
Female	830[b]	545[b]	87[b]	117[b]	69[b]	14.8[b]
SE	32	24	4.6	3.0	2.4	0.79
Strain*Sex						
Cairo B-2 Male	1135[a]	769[a]	130[a]	164	89[a]	21.1[a]
Cairo B-2 Female	943[b]	634[b]	104[b]	144	76[b]	18.1[b]
Control Male	781[c]	510[c]	79[c]	101	65[c]	12.5[c]
Control Female	718[c]	455[d]	69[d]	89	62[c]	11.5[c]
SE	24	17	3.3	4.6	2.4	0.47
Probability						
Strain	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
Strain*Sex	0.0097	0.0246	0.0271	0.3909	0.0465	0.0388

g). A similar trend was observed in the Cairo B-2 line females (average = 943 g) compared with the females of the C line (average = 718 g). The differences between six-week body weight of males and females of Cairo B-line were statistically significant. However the differences between the males and females of the C line at six weeks of age were not statistically significant (Table 2).

These results also indicate that, the live body weight of Cairo B-2 line at six weeks of age, com-pared with the C line, was significantly improved (45.3% for males and 31.3% for females) due to the intensive selection that had been done for six generations. These results are in agreement with the results previously reported by (Kestin et al. 1999). The higher percentage increase in six-week body weight of males than females (14%) might be due to the higher selection intensities imposed on the males than females throughout the selection process (4% *vs.* 40%).

On the other hand, the correlated responses of the genetic selection for increased body weight on carcass indicated that Cairo B-2 line had significantly higher carcass weights, breast meat, leg meat, and wings with bone weights than the C line (Table 2). Males from Cairo B-2 line had significantly higher live body, carcass, breast meat and wings with bones weights than females from the same line, and also in comparison with males and females from the C line (Table 2).

The increase in broiler performance has been

Table 3. Total allelic number for the microsatellite markers of the Cairo B-2 and C lines.

Marker name	Located chromosome	Male		Female	
		Cairo B-2	Control	Cairo B-2	Control
ADL0328	1	6	5	6	5
ROS0025	1	6	3	7	6
MCW0010	1	8	7	8	7
MCW0018	1	6	3	7	6
c3-46151949	3	6	2	5	4
c5-4999025	5	6	4	7	6
MCW0097	11	10	10	11	7

[a-d]Means, within source of variation and trait, with different superscripts are significantly different (P≤ 0.05).
N= 15 male and 15 females from each line.

tremendous in recent years, as measured by growth rate and carcass yield (Siegel et al., 2011). Variation in the yield of chickens, as reported by several workers, is affected by strain and sex (Broadbent et al., 1981; Orr et al., 1984; Bilgili, 2002). In general, for any breeding program to succeed, careful selection to maximize the yield of sealable products from each bird should be followed. Positive correlations between carcass yield and body weight increases were evident in the present study. These results are in agreement with previously reported results (Brake et al., 1993).

The major effect of Cairo B-2 line selection has been the increase in overall muscle mass of the chicken. This is particularly evident in the breast and leg muscles. The heavier body weight of Cairo B-2 line caused higher relative yield of breast and leg meat. Heavier birds produced greater breast portions. These results are in agreement with the results previously reported by Goliomytis et al. (2003), Schmidt et al. (2009), and Sandercock et al. (2009). For most studied traits, males from the Cairo B-2 line had higher body, carcass, wings with bones, and abdominal fat pad weights than females of the same line indicating the presence of sexual dimorphism. These results are in agreement with the results previously reported by Mignon-Grasteau et al. (2000).

Microsatellite associated with body and carcass weights

Chicken body weight is under complex genetic control. Uncovering the molecular mechanism of growth will contribute to more efficient selection for growth in broiler chickens (Deeb and Lamont, 2002; Sewalem et al., 2002; Sazanov et al., 2010). There are many studies indicating that the microsatellite markers ADL0328 (van Kaam et al., 2002; Lagarrigue et al., 2006; Liu et al., 2007; Liu et al., 2008; Zhang et al., 2008), ROS0025 (Sewalem et al., 2002; Nones et al., 2006; Liu et al., 2007; Liu et al., 2008; Zhang et al., 2008), MCW0010 (Sewalem et al., 2002; Navarro et al., 2005; Liu et al., 2007; Zhang et al., 2008), MCW0018 (Sewalem et al., 2002; Navarro et al., 2005), MCW0097 (Navarro et al., 2005), c3-46151949 and c5-4999025 (Uemoto et al., 2009) are associated with chicken body weights at six weeks of age.

The 6th selected generations of Cairo B-2 line and the C line were subjected to PCR analysis using seven specific microsatellite markers associated with body weight and carcass characteristics. Our results indicate that, the allelic frequencies of the simple sequence repeats (SSR) loci ADL0328 were higher (six alleles) in the Cairo B-2 line, males and females, while the C line shows only five alleles (Table 3). A similar trend was observed in which the selected males and females of Cairo B-2 line showed higher allelic frequencies for the ROS0025, MCW0010, MCW0018, c3-46151949, c5-4999025, and MCW0097 compared to the corresponding males and females from the C line (Table 3).

There are increases in allelic numbers and increasing polymorphism of all the microsatellite tested in Cairo B-2 line were in comparison to the C line (Figure 1). These results indicate that the genetic selection had an effect on the evolution of markers polymorphism within the genome of Cairo B-2 line which led to increase live body weight at six weeks of age. These results are in agreement with the results previously reported by Loywyck et al. (2008).

The microsatellite marker ROS0025 is associated with wing weight (Ikeobi et al., 2004).The microsatellite marker MCW0018 is related to carcass parts weight (Nones et al., 2006). Also, the microsatellite marker ADL0328 is associated with breast meat and abdominal fat weight in chickens. The high association between the weights of body parts and whole body weight could confound the identity of genes controlling variability in body weight with those involved in carcass composition variability (Lagarrigue et al., 2006). On the other hand, ADL0328 is a microsatellite marker associated with carcass percentage and is located on chromosome 1 (van Kaam et al., 2002). It is also associated with breast

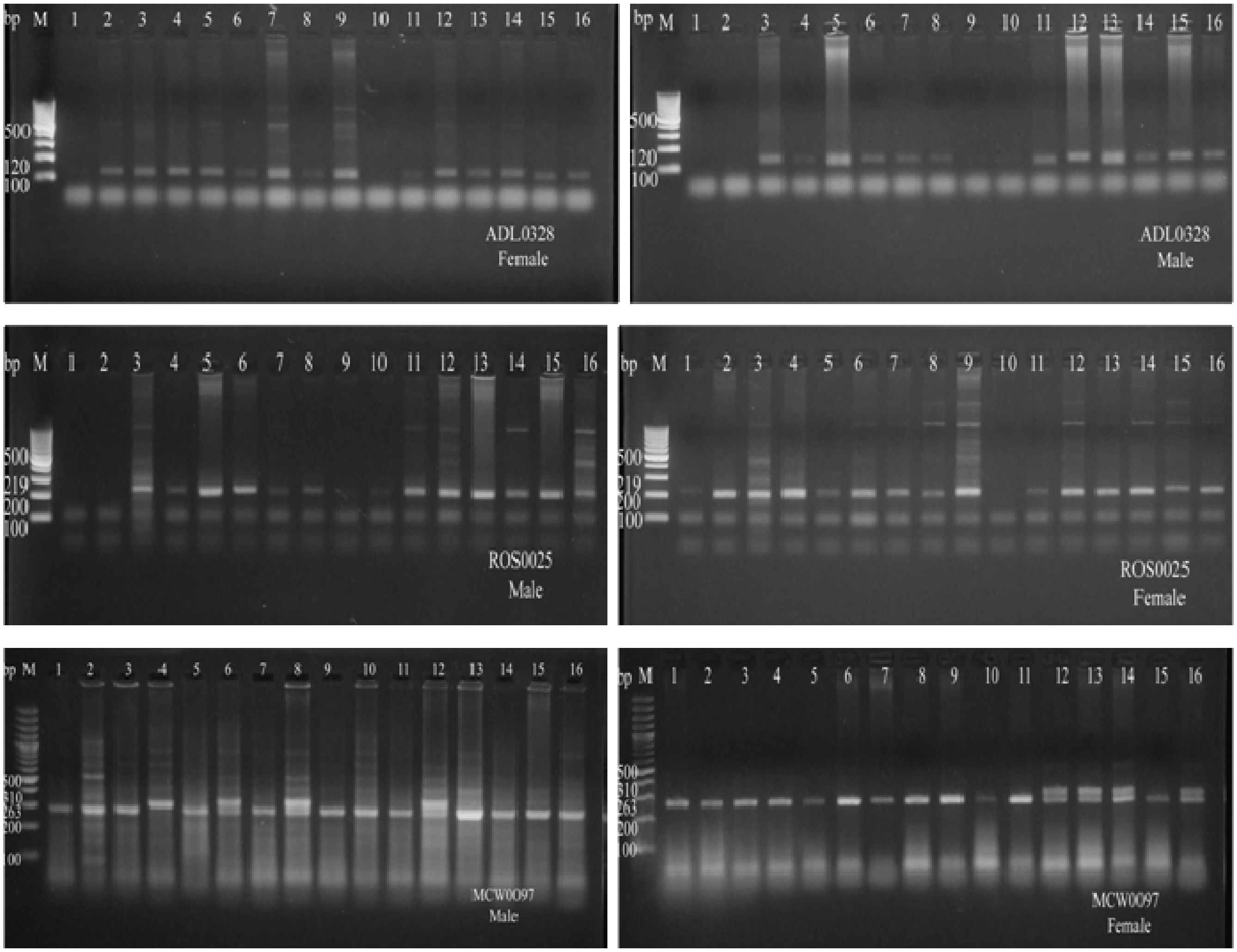

Figure 1. Males and females PCR products and allelic numbers from both Cairo B-2 and C lines for ADL0328, ROS0025, and MCW0097. 1 to 8, C line samples; 9 to 16, Cairo B-2 line samples. M, 100 bp DNA ladder (Fermentas Life Science, UK). PCR, Polymerase chain reaction.

Table 4. Correlations between the allelic number of the different markers and six-week body weight and carcass parts weights for both the Cairo B-2 and C lines.

Marker name	Body weight	Carcass weight	Breast meat	Leg meat	Wings	Abdominal fat
ADL0328	0.57**	0.58**	0.57**	-0.20^{NS}	0.62**	0.49**
ROS0025	0.69**	0.66**	0.64**	-0.13^{NS}	0.68**	0.58**
MCW0097	0.45**	0.50**	0.50**	0.08^{NS}	0.44**	0.53**
MCW0010	0.07^{NS}	0.05^{NS}	-0.01^{NS}	0.13^{NS}	0.07^{NS}	0.11^{NS}
MCW0018	0.62**	0.63**	0.62**	-0.14^{NS}	0.61**	0.58**
c3-46151949	0.61**	0.58**	0.59**	0.21^{NS}	0.58**	0.52**
c5-4999025	0.34**	0.35**	0.35**	0.28*	0.35**	0.37**

Significant at 5% level. ** Significant at 1% level. ^{Ns}not significant. N= 60 per microsatellite.

meat and breast muscle weight adjusted for live body weight in chicken (Lagarrigue et al., 2006).

The results also indicate that Cairo B-2 line had more alleles than the C line for the microsatellite markers: MCW0018, ADL0328 and ROS0025 (Table 3). These high allelic numbers makes Cairo B-2 line more efficient as a local broiler breed to be used in our breeding program. Also, since there are high genetic associations between chicken body weights with carcass traits, direct selection for live body weight at six weeks of age could produce indirect genetic gains for carcass, breast muscles and leg muscles weights (Gaya et al., 2006; Sandercock et al., 2009).

Body fat should be limited in order to enhance production efficiency and product quality. Also, abdominal fat characters are complex and economically important in the poultry industry. Selection against abdominal fat may

benefit from the implementation of marker-assisted selection. The ROS0025, MCW0010, and ADL0328 markers are suggestively associated with deposition of abdominal fat in broiler chickens (Liu et al., 2007, Liu et al., 2008; Zhang et al., 2008). The microsatellite marker ADL0328 is associated with abdominal fat weight (Lagarrigue et al., 2006).

Our results also indicate that, the correlation coefficient between the allelic numbers for most of these micro-satellite markers are related to six-week body weight, measured as combined sex in both lines. This means that the heavier birds have more alleles for the marker, than the lighter birds; the reason behind that could be that some of these alleles might have more beneficial effect on the studied traits (Table 4).

The microsatellite marker MCW0010 is supposed to be associated with breast muscles, leg muscles and carcass weight (Nones et al., 2006; Ikeobi et al., 2004). In our study, no significant correlations were present between the allelic numbers for that microsatellite (MCW0010) and any of the studied traits (Table 4). Thus it was recommended not to use this marker in selecting our lines in the future. The correlations coefficient between the allelic numbers for the markers ADL0328, ROS0025, MCW0018, MCW0097, c3-46151949, c5-4999025 and six-week body weight, carcass, breast meat, wings, and fat weights, measured as combined sex in both lines, were mostly highly significantly ($P \leq 0.01$) correlated. However, the correlation coefficient between the allelic numbers of the microsatellite marker c5-4999025 and leg meat was also significant ($P \leq 0.05$). These markers are going to be used in our future selection program for this line (Table 4).

Conclusion

The main objectives of broiler breeding programs are complex. Generally it involves selection for increase muscle percentage, and decrease offal yields produced from broilers in slaughter houses. Cairo B-2 line was superior in body weight, breast meat, and different carcass parts in comparison to the C line. The results also indicated improvements in body weight of the Cairo B-2 line of 38.5% after six generations of selection (48 grams per generation). If these improvements, in body weight, of the Cairo B-2 line will continue at the same rate, we can expect that after several generations of selection, Cairo B-2 line will be a local female broiler line with very good commercial performance. Also, our biotech-nology studies results will be applied to its breeding program to accelerate and enhance its productivity.

The present study also indicates the possibility of using microsatellite markers, related to body and carcass weights, in poultry breeding programs. These genetic markers can be incorporated in breeding programs aimed to improve chicken productivity. Therefore, marker assisted selection (MAS) is needed. Also, to accomplish MAS, it is essential to detect linkages between Microsatellite markers and Quantitative Trait Loci (QTL) associated with the traditional selection traits such as growth and carcass traits. These findings support the idea of improving the productivity of Cairo B-2 line as a local commercial broiler breed through MAS in the future.

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