Egyptian J. Anim. Prod. (2012) 49(1):9-18

ASSOCIATION ANALYSIS BETWEEN MICROSATELLITE DNA MARKERS AND MILK YIELD AND ITS COMPONENTS IN EGYPTIAN BUFFALOES USING A RANDOM REGRESSION MODEL

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SUMMARY

Data on 813 lactating Egyptian buffaloes were used to test association between nine microsatellite DNA markers located on buffalo chromosome 7. Data were recorded from May 2009 until April 2010. For each buffalo cow the monthly test-day records of milk, fat, protein and lactose yields were available. A total of 5344 test-day records for each trait were used in the analysis, each cow had at least five test-day records. Number of genotyped animals ranged from 179 to 471. Single-trait random regression models were used to analyze daily milk, fat, protein and lactose yields. The Wilmink's function was used to fit the random genetic effects associated with marker genotypes and the permanent environmental effects of each cow. Most of the estimated heritabilities associated with the studied markers were close to zero during almost the whole lactation period. However, heritability estimates significantly different from zero were found for milk yield associated with markers BM143, BM1329 and ILSTS097 which may be considered as excellent candidates for QTL detection using daughter or grand-daughter designs. In general, the heritabilities associated with the studied markers increased with the increase of the days in milk for all studied traits. The models that allow for changing the genetic variances associated with markers over the lactation period seem to be more appropriate than the models that assume constant genetic variances during the lactation period.

Keywords: Buffalo, random regression, genetic parameters, microsatellite markers, milk production

INTRODUCTION

Buffalo is an important livestock species in Egypt. There are about four million heads and 46% of them are lactating animals which contribute 44.8% of the total milk production in Egypt (FAOSTAT, 2009). The use of molecular makers can enhance selection accuracy and the genetic gain of the breeding programs. Microsatellite markers are co-dominant and ideal for the association studies because they are highly polymorphic and found in large numbers distributed along the genome. Nevertheless most of the studies that used molecular markers in Egyptian buffaloes were concerned with genetic biodiversity (Moioli *et al*., 2001; Abdel-Rahman and ElSayed, 2007; Elbeltagy *et al*., 2008; Atta *et al*., 2009; Abdel-Aziem *et al*., 2010) while the studies on the association analysis that relate molecular markers to production in the Egyptian buffaloes are scarce.

Random regression models have been used in the analysis of test day records of the dairy cows (Jamrozik *et al*., 1997; Pool *et al*., 2000; Schaeffer *et al*., 2000) and more recently in the analysis of the test day records of buffaloes (Sesana *et al*., 2010). They allow for modeling the lactation curve of each cow with a limited number of parameters which permit a continuous changing of the (co)variances due to random effect(s) at different points of lactation (Van der Werf *et al*., 1998). Numerous linear and nonlinear functions have been used to model the lactation curve. Among them, functions with a minimum number of parameters and meaningful biological interpretation are the most desirable (Olori *et al*., 1999; Macciotta *et al*., 2005; Quinn *et al*., 2005). Most of the random regression models included the polygenic and environmental effects as time-dependent random effects; however these models could be extended to include time-dependent QTLs random effects which provide the pattern of the change of genetic variances associated with QTLs across time (Hadjipavlou and Bishop, 2009).

The objective of this study was to evaluate the association between the variation in nine microsatellite DNA markers located at chromosome seven with milk yield and its component content in the Egyptian buffaloes using a random regression model.

MATERIALS AND METHODS

Phenotypic Data:

The initial dataset included a total of 7819 monthly test-day records of 1205 buffaloes located in six different farms from six different governorates recorded by Cattle Information Systems/Egypt (CISE). Data were recorded

from May 2009 till April 2010. The cows having less than five test-day records were removed from the dataset. Moreover, the test-day records after 300 days in lactation were excluded from the analysis (less than 1% of the initial dataset). Also, the extreme phenotypic values of daily milk yield (less than 2 kg or more than 21 kg) were excluded. The final dataset included 5344 test-day records of 813 buffaloes located at six different farms. For each buffalo cow, test-day milk, fat, protein and lactose yields were available.

Microsatellite DNA markers data

A volume of 10 ml peripheral blood was collected from the jugular vein in Falcon tubes supplied with proper amount of EDTA. Blood samples were placed on cooling gel in ice box immediately after their collection and brought to the Animal Biotechnology Lab and stored frozen temporarily at -20°C before DNA extraction.

DNA extraction

DNA was isolated from the peripheral leukocytes using Fermentas® kits, cat. no. k0512, Fermentas Life Science, EU, according to Sambrook and Russel (2000).

The yield, concentration and purity of DNA of the samples were done using ScanDrop® 200, Anyltikajena. The whole genome of each sample was run in 0.8% agarose gel through a horizontal gel electrophoresis system (mini gel, Biometra® EU). Standard DNA/DNA ladder was used and all samples were brought at the same concentration level (50 ng/ µl).

A total of nine Microsatellite DNA markers located on buffalo chromosome 7 were utilized (Table 1). These markers on chromosome 6 in cattle found to be associated with milk production traits (Georges *et al*., 1995; Rodriguez-Zas *et al*., 2002; Weller *et al*., 2003; Reinecke *et al*., 2005). Chromosome seven in Buffalo is homologous to chromosome six in cattle (Goldammer *et al*., 2007).

Amplification of microsatellites markers and genotyping:

The PCR was carried out on 50 ng of genomic DNA in a 20 µl reaction volume of 50 mM KCL, 10 mM Tris-Hcl (pH 8.8), 200 µM dNTP, 1.5 mM $MgCl_2$, 5 Pmol of each primer and 1.0 U Taq DNA polymerase. The amplification was realized using thermal cycler (G-Storm®, GeneTechnologies, UK) machine. The primary denaturation was done at 95°C for 3 minutes followed by 10 cycles of denaturation at 94°C for 30 sec., annealing at 58.5- 59.5°C (decrease in temperature set 1°C after each cycle) for 30 sec. and extension at 72°C for 45 sec. Following these cycles with variant annealing temperature, 30 cycles with constant annealing temperature at 54°C were performed and reaction ended with final extension at 72°C for 5 minutes and final storage temperature of 4 °C.

Statistical model:

The preliminary analysis of the data included many functions as Wood (1967), Wilmink (1987), Guo and Swalve (1995) and Legendre polynomial functions with two and three coefficients (Pool *et al*., 2000). The analysis indicated that Wilmink's function performed better in the fitting of the milk production curve and its components of the Egyptian buffalo.

The Wilmink's function is:

$$Y_t = a + be^{-kt} + ct$$

Where, Y_t is the yield (milk, fat, protein or lactose) in day t of the lactation. According to Wilmink (1987), the parameters a, b, and c are associated with the level of production, the increasing phase of lactation before the peak, and the declining phase of lactation, respectively. Parameter k is related to the time of peak lactation and it is usually assumed as a fixed value, derived from a preliminary analysis made on the average of production. This implies that the model converted to linear and has only three parameters to be estimated. In the preliminary analysis, k was estimated at 0.09.

Single-trait random regression models were used to analyze daily milk, fat, protein and lactose yields. The model included fixed regression coefficients of Wilmink's function that accounts for the average changes of the general lactation curve of all animals. The fixed effects included in the analysis were farm, year-season of calving, number of milking events per day (two or three times) and parity. The parities ranged from 1 to more than 10 parities, where the parity number higher than 10 were grouped together with the tenth parity as these parities had a few number of observations. Wilmink's function was used to fit the random genetic effects associated of each marker genotypes and the permanent environmental effects of each cow. The random regression was used to account for the change of the genetic variance associated with each marker's genotypes over the lactation period. Parity was considered as a fixed effect due to the low number of observations within parities. Consequently, the estimates of the heritabilities associated with the genetic markers were free of the parity effect. Although, it should be probably better to treat the production of each parity as a different trait, the limited number of observations was

an obstacle to assume this approach. For simplicity, the effects associated with the genetic markers were assumed to be uncorrelated. The pedigree information was not available on the studied animals, thus animals were assumed to be unrelated. Analyses were done by the program REMLF90 (Misztal *et al*., 2002).

The genetic variance associated with each maker was calculated as follows:

$$\sigma^2_{g_{jk}} = t_j G_k t'_j$$

where σ^2_{gjk} is the genetic variance associated with maker k at time j ; G_k is a matrix of the coefficients that defines the covariance function of the genetic effect associated with marker k and t is a vector of the elements [1, exp(-0.09*j), j] associated with Wilmink's function coefficients at time j.

The permanent environmental variance was calculated as follows:

$$\sigma^2_{pe_j} = t_j G t'_j$$

where σ^2_{pej} is the permanent environmental variance at time j; G is a matrix of coefficients that defines the covariance function of permanent environmental effect and t is a vector of the elements [1, exp(-0.09*j), j] associated with Wilmink's function coefficients at time j.

Heritability estimate associated with maker k at time j (h^2_{jk}) was calculated as following:

$$h^2_{jk} = \frac{\sigma^2_{g_{jk}}}{\sigma^2_{g_j} + \sigma^2_{pe_j} + \sigma^2_e}$$

where σ^2_{gjk} and σ^2_{pej} are descried previously; σ^2_{gj} is the overall genetic variance associated with all makers at time j and σ^2_e is the residual environmental effect. Ratios of permanent environmental variances to phenotypic variances were expressed in similar pattern.

RESULTS AND DISCUSSION

Table 1 shows the number of genotyped animals (NGA), number of alleles (NA), number of genotypes (NG), observed heterozygosity (HO) and the range of the allele size for each marker. We intended to genotype all animals for the studied 9 microsatellite markers but due to technical reasons the number of genotyped animals ranged from 179 to 471. The number of alleles per marker ranged from 17 for RM28 and BM415 markers to 29 for BMC4203 marker. BMC4203 marker has the highest number of genotypes (138) while the lowest (48) was shown by BM415 marker. The studied markers had observed heterozygosity higher than 0.85 except ILSTS097 marker which had the lower heterozygosity (0.52).

Milk yield:

The estimates of heritability of the daily milk yield associated with the genetic markers are shown in Figure 1. The heritabilities associated with markers RM28, BM415, AFR227, ILSTS93 and BMS483 were very close to zero along the whole lactation period where the heritabilities associated with markers BM143, BM1329 and BMC4203 were close to zero at the beginning of lactation and increased gradually up to 0.02 at the end of lactation. Marker ILSTS097 had heritability 0.01 at the beginning of lactation, decreased instantly after few days of lactation and finally increased up to 0.03 by the end of lactation. The congregated heritability of all makers range from 0.02 at the beginning of lactation up to 0.10 by the end of lactation.

Fat and protein yields:

Heritability estimates of markers associated to fat and protein yields had similar patterns to milk yield (Figures 2 and 3). Heritability estimates of most markers were very close to zero during the whole lactation period. Heritabilities associated with markers BM143 and BMC4203 were close to zero at the beginning of lactation then increased slightly up to approximately 0.01 by the end of lactation for both traits. Heritability associated with marker BM1329 was close to zero at the beginning of lactation then increased slightly up to 0.01 by the end of lactation for fat yield. The overall heritabilities of the genetic markers associated with fat and protein yields had pattern similar to milk yield and ranged from 0.00 at the beginning of lactation to 0.03 by the end of lactation for both traits.

Lactose :

According to figure 4 the markers can be grouped into three categories: 1) Markers RM28, BM415, BMC4203 and BMS483 where their effects were close to zero during the whole lactation period; 2) Markers AFR227 and ILSTS93 which had a relatively higher heritabilities at the beginning of lactation then dropped rapidly to zero after few days until the end of lactation; and 3) Markers RM143, BM1329 and ILSTS97 with heritabilities close to zero at the beginning of lactation then increased slightly up to 0.01 by

the end of lactation. The overall heritability was 0.04 at the beginning of lactation, decreased rapidly to zero at 26th day of lactation and finally increased gradually up to 0.04 by the end of lactation.

Permanent environmental effect

Figure (5) shows the evolution of the permanent environmental variances of the studied traits as proportion of the total variance of each trait. All traits had the similar form of curves during lactation where the proportions of the environmental variance were small at the beginning of lactation, decreased slightly to zero after few days for all traits and increase up to 0.18, 0.15, 0.21 and 0.26 for milk, fat, protein and lactose yields, respectively, by the end of lactation.

Literature estimates on the effects of the studied markers on the milk yield traits in buffalo were not available; however there are a plethora of studies on QTL detection using these markers in dairy cattle. Rodriguez-Zas *et al*. (2002) found a significant variation of protein pattern in two of the studied three U.S. Holstein families associated with marker BM143. Also, significant QTL for milk yield trait between markers BM1329 and TGLA37 have been detected in U.S. Holsteins (Georges *et al*., 1995). The ILSTS039 marker had a significant effect on milk production traits in an Israeli Holstein population (Weller *et al*., 2003). Moreover, Ron *et al*. (2001) reported a significant association between marker BM143 with milk, protein yields and fat and protein percentages in Israeli Holstein but this association was non-significant with fat yield. Reinecke *et al*. (2005) classified the lactation period into three sections (1–100 days), (100–200 days) and (200–300 days) and they indicated that marker ILSTS97 had a highly significant effect on milk yield during the second period of lactation and marker BM143 had highly significant effect on milk yield during the last lactation period. However, in our study, the effects of these markers on milk production were non-significant.

In the current study most of the estimated heritabilities associated with the studied markers were close to zero during the lactation period. Nevertheless, significant heritabilities for milk yield found to be associated with markers BM143, BM1329 and ILSTS097 which could be considered as good candidates for QTL detection using daughter or grand-daughter designs. In general, the heritabilities associated with the studied markers increased with the days in milk for all studied traits. The genetic variances associated with these markers consequently increased with the days in milk because the residual variances of the studied traits were assumed to be homogenous across the lactation period and the permanent environmental variances increased with the days in milk. However, it is clear that a model that allow changing the genetic variances associated with markers over the lactation period seems to be more appropriate than the model that assumed constant genetic variances during the lactation period.

On the other hand, the heritabilities associated with the studied markers could be underestimated because of the expected high level of linkage equilibrium within the studied population (unrelated animals); with the availability of pedigree information linkage disequilibrium can be exploited within families for QTL detection using daughter or grand-daughter designs (Weller *et al*.,1990).

Moreover, the missing of pedigree information could inflate the estimate of the permanent environmental variances of the studied traits because the statistical confusion between additive genetic effects and permanent environmental effects of the studied animals.

Only nine microsatellite markers were studied and each of them associated with small proportion of the genetic variations of the studied traits but the overall heritabilities associated with all markers reached to 0.10, 0.03, 0.03 and 0.04 by the end of lactation for milk, fat, protein and lactose yields, respectively. Application of molecular technology in buffalo breeding seems to be a powerful tool for the identification of the regions of the DNA affecting the milk production traits. However, a denser marker map is recommended to capture as high as possible of the genetic variation of these traits.

ACKNOWLEDGEMENT

This project was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No 218 titled "Microsatellite markers: as a tool for genetic improvement of milk yield and its contents in Egyptian buffaloes".

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Table 1. Genetic characterization of the microsatellite DNA markers under investigation

Marker	NGA	NA	NG	Ho	Allele size (bp)
RM28	471	17	59	0.87	94-126
BM415	436	17	48	0.98	129-161
BM143	373	19	96	0.90	100-136
BM1329	323	24	98	0.99	123-169
AFR227	449	21	104	0.91	90-130
BMC4203	446	29	138	0.95	136-192
ILSTS093	179	24	88	0.90	183-229
ILSTS097	203	24	136	0.52	220-266
BMS483	393	23	77	0.97	100-144

NGA is number of genotyped animals; NA is the number of alleles; NG is the number of genotypes; Ho is observed heterozygozity (HO).

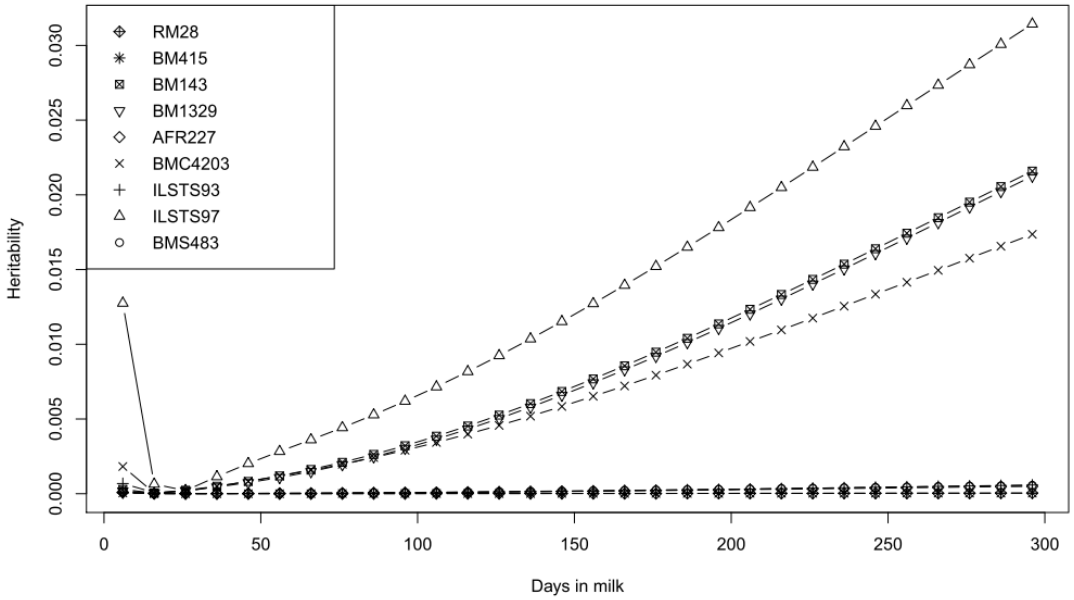

Fig. 1. Heritability estimates (h^2) associated with each genetic marker for daily milk yield

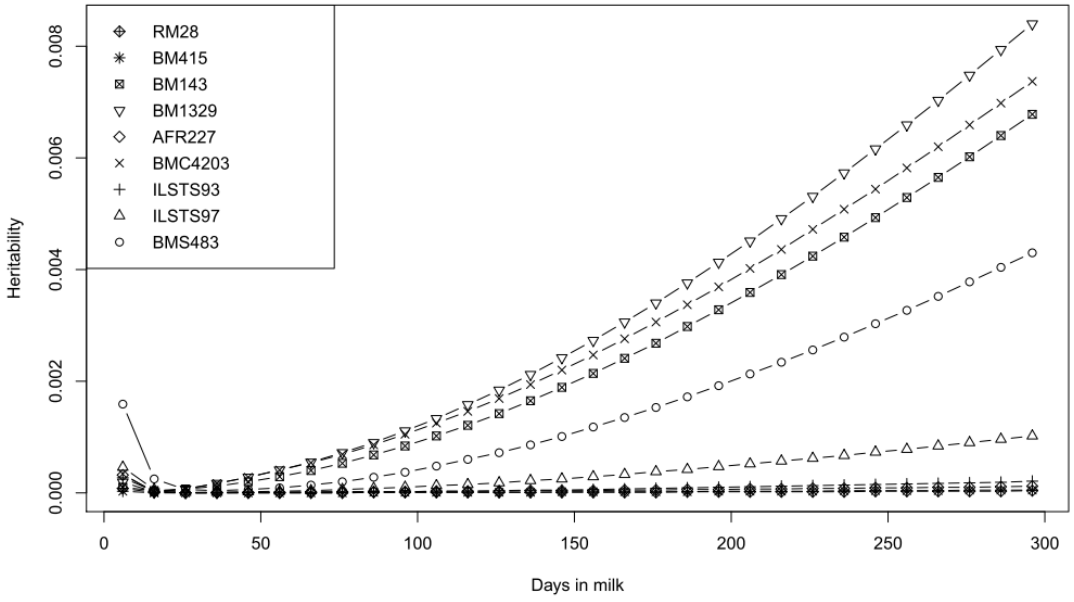

Fig. 2. Heritability estimates (h^2) associated with each genetic marker for daily fat yield

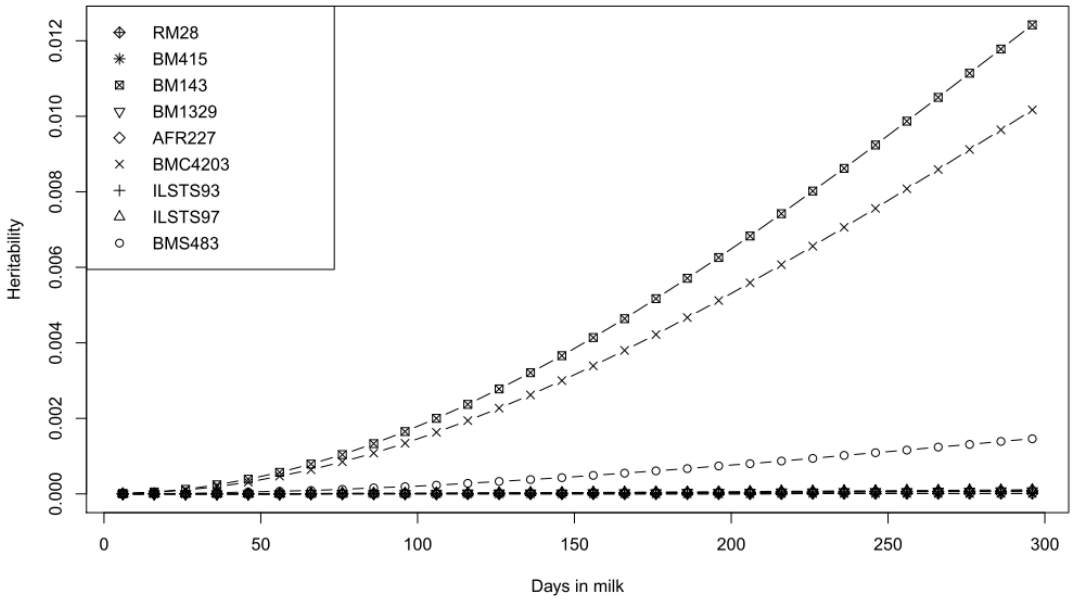

Fig. 3. Heritability estimates (h^2) associated with each genetic marker for daily protein yield

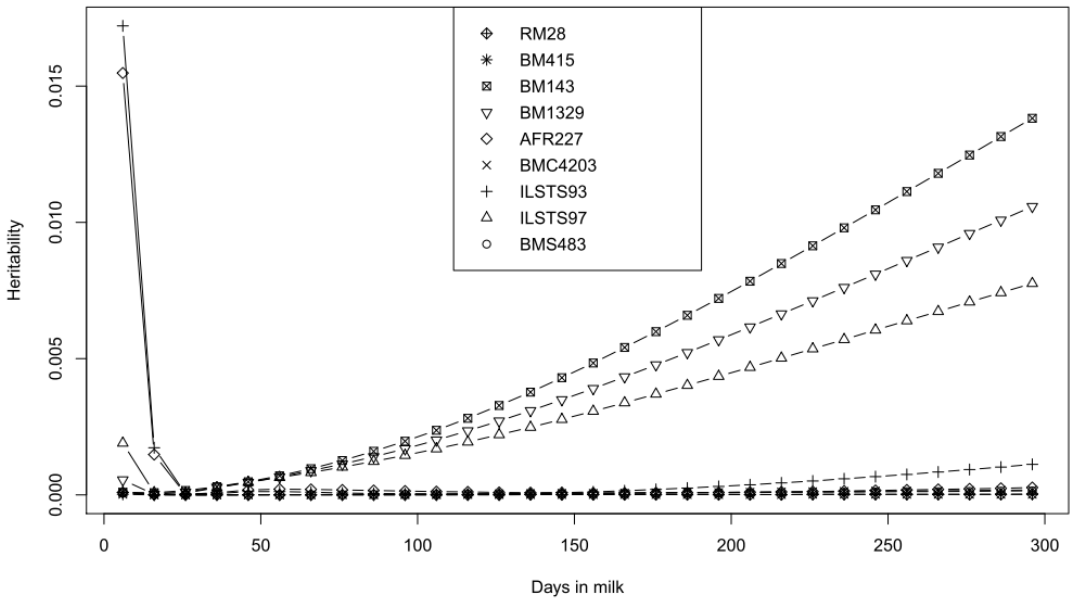

Fig. 4. Heritability estimates (h^2) associated with each genetic marker for daily lactose yield

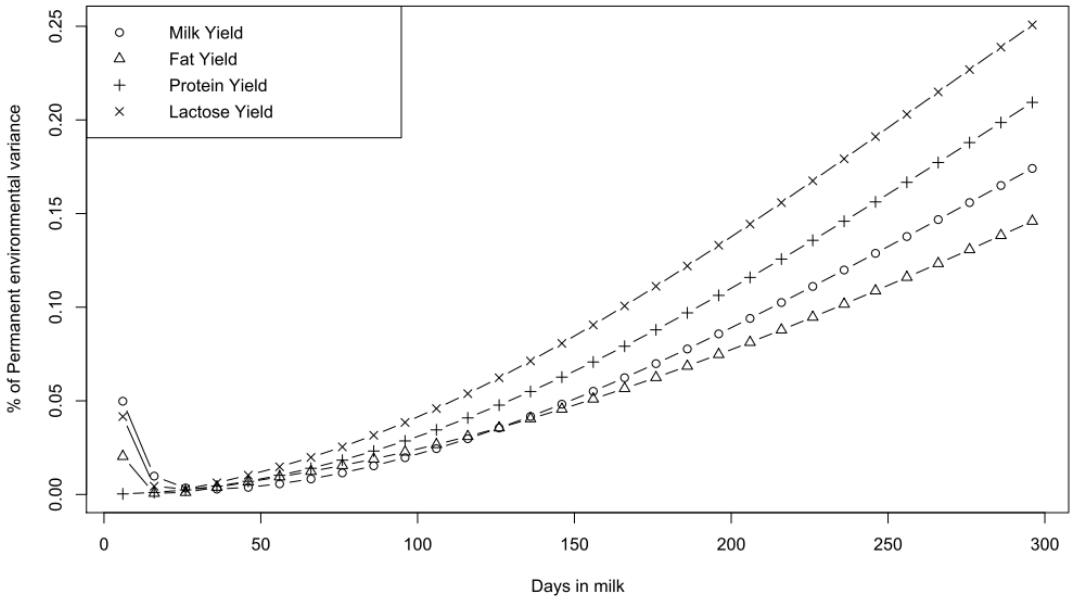

Fig. 5. The proportions of the phenotypic variances corresponding to permanent environmental variances of the studied traits

تحليل التلأزم بين الواسمات الوراثية الدقيقة وإنتاج اللبن ومحتوياته في الجاموس المصري بإستخدام نموذج الإنحدار العشوائي

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إستخدمت بيانات ٨١٣ جاموسة حلابة لإختبار التلازم بين تسعة واسمات وراثية دقيقة موجودة على الكروموسوم ٧. سجلت البيانات من مايو ٢٠٠٩ حتى أبريل ٢٠١٠. تم تسجيل إنتاج اللبن، والدهن والبروتين واللاكتوز اليومى مره كل شهر لكل جاموسة. أستخدم ٥٣٤٤ سجلاً لكل صفة في التحليل، كان لكل جاموسة على الأقل خمسة سجلات. تراوح عدد الحيوانات التى تم الكشف على الواسمات الوراثية فى الحامض النووى لها بين ١٧٩-٤٧١. واستخدم نموذج الانحدار العشوائي لتحليل إنتاج اللبن، والدهن والبروتين واللاكتوزاليومى. وقد استخدمت دالة Wilmink لمؤائمة التاثيرات الوراثية العشوائية المتلازمة مع الواسمات الوراثية وكذلك الآثار البيئية الدائمة.

وأشارت النتائج أن معظم قيم المكافئات الوراثية المقدرة قريبة من الصفر خلال موسم الحليب. ومع ذلك، وجدت بعض قيم المكافئات الوراثية مختلفه معنويا عن الصفر بالنسبة لصفة إنتاج اللبن وذلك بالنسبة للواسمات BM143 ، BM1329 ILSTS097، والتى يمكن اعتبارها مرشحا ممتازا للكشف عن المواقع الوراثية للصفات الكمية (QTL) بإستخدام تصميم الأبنة أو الحفيدة. بوجه عام، وجد أن قيم المكافئات الوراثية المتلازمة مع الواسمات الوراثية تزيد مع زيادة أيام الحلب لجميع الصفات المدروسة. وكذلك وجد أن النماذج الأحصائية التي تسمح بتغيير التباينات الوراثية المرتبطة بالواسمات الوراثية على مدى موسم الحلب أكثر ملاءمة من النماذج التي تفترض ثبات التباينات الوراثية خلال موسم الحلب.