Genetic analysis for association of some genetic markers and milk yield in dairy cattle

By

Mohamed Hamada Mohamed Abd Elhamid

B.Sc. Agricultural Science

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Minufiya University

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(Animal breeding) 2011

Mnufiya University

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Introduction

Random regression models (RRM) are currently used in the prediction of breeding values and in the estimating the variance components for milk production traits of dairy cattle in several countries. Direct modeling of test day (TD) records instead of 305-d yields allows the shape of the lactation curve to be modeled with subsequently more precise adjustment for temporary environmental effects, avoidance of extended records for culled cows or lactations in progress, and evaluation of lactation persistency (**Jamrozik and Schaeffer, 1997)**. In the basic structure of a RRM, the fixed part includes effects peculiar to all cows on the same test day and effects specific to cows on a given test day, such as pregnant or diseased, plus a factor accounting for the yield level on a specific day in milk **(Ptak and Schaeffer, 1993)** whereas individual lactation curves are fitted by random regression coefficients **(Schaeffer and Dekkers, 1994; Jamrozik and Schaeffer, 1997)**. This feature of RRM allows for the prediction of breeding values and estimating the (co)variance functions throughout the whole lactation. Mean lactation curves are usually estimated on a large number of records and are characterized by quite regular patterns. As stated by **(Schaeffer 2004)** the use of either mathematical functions or fixed intervals of days in milk will generally lead to the same results. In this study, the trend is investigated at the phenotypic level by a fixed regression analysis of individual deviations around the mean curves for milk yield of first lactation Canadian Holsteins using some of the functions proposed to fit random effects in RRM for milk production traits in cattle. The aim of the present study was to estimate variance components, heritability and genetic and phenotypic trend for Friesian cattle raised in Egypt.

In dairy cattle, selection programs through quantitative genetics are time consuming due to the long generation interval. Quantitative traits in dairy cattle are controlled by a large number of genes and are subjected to various environmental factors. Several genes that were selected based on their biological actions or that are located in genome regions of identified QTLs have been regarded as candidate genes affecting milk production traits in dairy cattle **(Meuwissen & Goddard, 1996**). Prolactin (PRL) and Lactoferrin (LF) genes play important regulatory functions in mammary gland development, milk secretion, and expression of milk protein genes (**Zhag et al, 2007)**. Lactoferrin gene is highly polymorphic and it has been shown that some of its variants are related to milk production traits and mastitis resistance in dairy cattle (**Kaminski et al., 2006; Wojdak-Maksymiec et al., 2006; Kaminski et al**). As stated by **Brym et al (2005),** the prolactin gene is a potential quantitative trait

locus and could be used as genetic marker of production traits in dairy cattle. Prolactin is known to have diverse biological functions such as water and electrolyte balance, growth and development, immune and reproductive function **(Gregerson, 2006)**. Bovine prolactin is located on chromosome 23, which is composed of five exons and four introns (**Dybus et al, 2005)**. Several polymorphic sites have been detected within PRL and LF genes **(Deepika and Salar, 2014)**. The prolactin gene is used as an informative molecular marker for milk yield and milk composition **(Collier et al., 1984)**, while lactoferrin gene is involved in many biological functions and is classified as acute phase protein **(Kanyshkova et al, 2001)**. The aims of the study were (1) to characterize prolactin and lactoferin genes in Friesian and local cattle in terms of Hardy Weinberg equilibrium, genotypic and allelic frequencies, observed and expected heterozygosity, polymorphism information content and inbreeding and (2) To investigate all possible associations among PRL and LF genes genotypes of three restriction enzymes (HinfI, Nael and Sm/l) and yields of milk, fat, protein and age at first calving.

2. REVIEW OF LITERATURE

2.1 Random Regression Model (RRM)

2.1.1 Test day and RRM advantages:-

The change in lactation traits with the age can be represented as a trajectory, that is, as a function of time. A number of regression coefficients are used to determine the number of covariance to be estimated for each source of variation in a RRM **(Kirkpatrick *et al*., 1994).** The estimates of variance components for test-day milk yields obtained by RRM were firstly published by **Jamrozik and Schaeffer (1997)**. **Meyer and Hill (1997) and Meyer (1998a)** demonstrated that the use of covariance functions is necessary for the model including the additive genetic and permanent environmental effects in the random regression of TD. The further development of the variance component estimation by RRM included modeling of permanent environmental effects by random regressions are cited by some investigators **(Van der Werf *et al*., 1998; Olori *et al*., 1999b; Rekaya *et al*., 1999; Strabel and Misztal, 1999)**. In addition, other authors modeled the heterogeneity of residual variance across the lactations **(Jamrozik and Schaeffer, 1997; Jamrozik *et al*., 1998; Brotherstone *et al.*, 2000; Jaffrezic *et al*., 2000).**

Covariance functions provide a method for analyzing independent components of variation that reveal specific patterns of change of the character over time. Therefore, covariance functions can be used for traits that: 1) are gradually changed over time, 2) are measured on trajectories, and 3) can be called 'infinite dimensional', e.g. growth, lactation traits. Covariance functions are applied because: 1) it allows accurate modeling of the variance-covariance structure of the traits described, 2) it is able to predict the covariance structures at any point along a continuous (time) scale, 3) it gives more flexibility in using measurements taken on any

moment along the trajectory without having to correct them to a certain time and 4) it provides a methodology to analyze the patterns of covariance that are associated with particular changes of the trait along the trajectory.

2.1.2 Variance components for test-day milk, fat and protein yields

The reviewed estimates of the variance components for milk, fat and protein yields in cattle are cited in ***Table1***. The variances for TD milk, fat and protein yields are high in magnitude with wide ranges for additive genetic, permanent and residual. Heterogeneity of variances seems to exist for all random effects in a random regression test-day model as reported by **Jamrozik *et al*. (1997a).** Also, the estimates of additive genetic variance and the permanent environmental and phenotypic variance for milk, fat and protein yields were relatively low at early lactation; increased gradually and then decreased except at the end where the estimates increased again suddenly. Residual variance for TD milk yield tended to be low at both edges as reported by **Jamrozik and Schaeffer (1997). Biassus et al (2011)** reported that, phenotypic, additive genetic, permanent environmental and residual variances were higher at the beginning of lactation, decreasing thereafter. In general, the variance estimates were higher at the beginning of the lactation and decreased thereafter, with the exception of genetic variance estimates which slightly increased at the end of lactation. **Mosharraf et al (2014)** found that residual variances for TD milk, fat and protein yields of Holstein dairy cows were considered homogeneous over the lactation period and were equal to 10.72, 0.04 and 0.01 for milk, fat and protein yields, respectively.

Table 1.The estimates of additive genetic (σ^2a), permanent environmental (σ^2pe), residual variances (σ^2e) and phenotypic variances (σ^2P) for test-day milk, fat and protein yields in cattle as cited in the literature.

Country	Test day (1)				Test day (2)				Test day (3)				Reference
	σ^2a	σ^2pe	σ^2e	σ^2P	σ^2a	σ^2pe	σ^2e	σ^2P	σ^2a	σ^2pe	σ^2e	σ^2P	
Test-day milk yield:													
Germany	1.97	NA	8.70	NA	2.79	NA	8.76	NA	3.14	NA	7.91	NA	Swalve, 1995a
Syria(first three lactation)	0.18	0.09	0.03	0.21	0.13	-0.03	0.14	0.30	0.10	-0.07	0.10	0.28	Alnajjar, 2001
	0.12	0.16	0.09	0.29	0.06	0.05	0.14	0.32	0.03	0.01	0.15	0.33	
	-0.06	0.28	0.07	0.26	-0.17	0.18	0.22	0.43	-0.19	0.13	0.17	0.40	
Brazil (two models)	21.08	NA	16.55	NA	9.70	NA	16.55	NA	11.81	NA	16.55	NA	Cobuci et al., 2005
	5.18	NA	30.11	NA	3.95	NA	20.92	NA	5.11	NA	21.81	NA	
Portugal	5.83	15.11	NA	30.09	4.57	11.55	NA	22.23	4.09	9.89	NA	19.86	Silvestre,et al., 2005
Egypt	0.01	0.17	NA	NA	0.01	0.15	NA	NA	0.02	0.13	NA	NA	Gamal et al., 2007
Iran	2.71	NA	22.54	NA	3.09	NA	23.43	NA	3.50	NA	22.28	NA	Shadparvar and Yazdanshenas, 2005
	25.07	NA	NA	43.79	17.63	NA	NA	36.35	13.18	NA	NA	0.19	Abdullahpour et al., 2010
	9.06	30.78	NA	70.40	2.36	27.26	NA	60.17	3.65	26.86	NA	61.08	Gharahveysi et al., 2012
Ethiopia	0.14	0.54	NA	0.82	0.18	0.54	NA	0.86	0.23	0.56	NA	0.93	Gebreyohannes et.al, 2016
Afghanistan	5.7 to18.2	18.7to27	17.2to31.4	45.8to64.8	5.7to18.2	18.7to27	17.2to31.4	45.8to64.8	5.7to-18.2	18.7to27	17.2to31.4	45.8to64.8	Fazel et al, 2018
Test-day fat yield:													
Germany	0.01	NA	0.03	NA	0.003	N	0.02	NA	0.003	NA	0.02	NA	Swalve, 1995a
Portugal	6.07	21.42	NA	52.06	4.77	15.57	NA	40.50	4.24	12.96	NA	37.19	Silvestre, et al., 2005
Test-day protein yield:													
Germany	0.001	NA	0.01	NA	0.002	NA	0.01	NA	0.002	NA	0.01	NA	Swalve, 1995a
Portugal	4.68	12.14	NA	27.02	3.49	9.18	NA	20.06	3.16	7.84	NA	18.72	Silvestre et al., 2005
Iran	3.97	19.7	21.3	44.9	3.76	16.7	23.1	43.5	4.5	17.3	20.4	42.2	Naserkheil et al, 2016

Table 1. Continue

Country	Test day (4)				Test day (5)				Test day (6)				Reference
	σ^2a	σ^2pe	σ^2e	σ^2P	σ^2a	σ^2pe	σ^2e	σ^2P	σ^2a	σ^2pe	σ^2e	σ^2P	
Test-day milk yield													
Germany	3.52	NA	7.22	NA	3.53	NA	7.09	NA	3.76	NA	6.65	NA	Swalve, 1995a
Syria(first three lactations)	0.09	-0.07	0.12	0.31	0.08	-0.05	0.11	0.34	0.08	-0.02	0.16	0.44	Alnajjar, 2001
	0.01	0.002	0.16	0.35	0.001	0.02	0.22	0.42	-0.01	0.04	0.12	0.36	
	-0.18	0.11	0.12	0.39	-0.16	0.09	0.15	0.46	-0.11	0.09	0.16	0.55	
Brazil (two models)	10.70	NA	16.55	NA	9.20	NA	16.55	NA	8.46	NA	16.55	NA	Cobuci et al., 2005
	5.41	NA	20.69	NA	5.59	NA	19.33	NA	5.90	NA	18.45	NA	
Portugal	4.08	9.43	NA	19.11	4.25	9.59	NA	18.69	4.41	9.91	NA	19.17	Silvestre et al, 2005
Egypt	0.04	0.12	NA	NA	0.05	0.12	NA	NA	0.05	0.12	NA	NA	Gamal et al., 2007
Iran	4.10	NA	21.53	NA	4.38	NA	21.36	NA	4.69	NA	21.16	NA	Shadparvar and Yazdanshenas, 2005
	11.19	NA	NA	0.19	10.04	NA	NA	0.21	10.02	NA	NA	0.21	Abdullahpour et al., 2010
	6.96	24.48	NA	62.00	9.98	20.59	NA	61.20	12.36	17.27	NA	60.19	Gharahveysi et al., 2012
Ethiopia	0.3	0.59	NA	1.03	0.38	0.65	NA	1.17	0.48	0.73	NA	1.53	Gebreyohannes et.al, 2016
Test-day fat yield													
Germany	0.003	NA	0.02	NA	0.004	NA	0.02	NA	0.004	NA	0.01	NA	Swalve, 1995a
Portugal	4.19	12.39	NA	34.59	4.39	12.78	NA	33.18	4.65	13.34	NA	33.52	Silvestre et al., 2005
Test-day protein yield													
Germany	0.002	NA	0.01	NA	0.002	NA	0.01	NA	0.002	NA	0.01	NA	Swalve, 1995a
Portugal	3.34	7.56	NA	18.25	3.71	7.84	NA	18.30	4.05	8.32	NA	19.14	Silvestre, et al., 2005
Iran	5.4	17.9	18.9	42.2	6.2	17.9	18.4	42.5	6.77	17.6	18.6	43.1	Naserkheil et al; 2016

***Table*1.** Continue

Country	Test day (7)				Test day (8)				Test day (9)				Reference
	σ^2a	σ^2pe	σ^2e	σ^2P	σ^2a	σ^2pe	σ^2e	σ^2P	σ^2a	σ^2pe	σ^2e	σ^2P	
Test-day milk yield:													
Germany	3.15	NA	7.12	NA	2.81	NA	8.09	NA	NA	NA	NA	NA	Swalve, 1995a
Syria, three lactation	0.08	0.02	0.09	0.47	0.09	0.07	0.12	0.60	0.09	0.12	0.06	0.69	Alnajjar, 2001
	-0.01	0.07	0.17	0.46	-0.01	0.11	0.16	0.55	-0.01	0.15	0.10	0.60	
	-0.06	0.09	0.20	0.69	0.00	0.09	0.19	0.83	0.07	0.11	0.00	0.83	
Brazil Two model	8.75	NA	16.55	NA	10.10	NA	16.55	NA	12.53	NA	16.55	NA	Cobuci et al, 2005
	6.41	NA	18.19	NA	7.13	NA	18.61	NA	8.07	NA	19.70	NA	
Portugal	4.46	10.18	NA	19.04	4.38	10.39	NA	19.63	4.30	10.78	NA	19.99	Silvestre, et al, 2005
Egypt	0.04	0.14	NA	NA	0.02	0.18	NA	NA	0.01	0.26	NA	NA	Gamal et al, 2007
Iran	4.45	NA	21.32	NA	4.86	NA	20.12	NA	4.55	NA	20.08	NA	Shadparvar and Yazdanshenas, 2005
	10.00	NA	NA	0.21	10.58	NA	NA	0.20	12.37	NA	NA	0.19	Abdullahpour et al, 2010
	14.46	15.75	NA	60.77	16.52	15.68	NA	62.76	18.21	16.14	NA	64.90	Gharahveysi et al, 2012
Ethiopia	0.59	0.82	NA	1.55	0.71	0.94	NA	1.97	0.85	1.08	NA	2.06	Gebreyohannes et.al, 2016
Test-day fat yield:													
Germany	0.004	NA	0.01	NA	0.004	NA	0.02	NA	NA	NA	NA	NA	Swalve, 1995a
Portugal	4.88	13.67	NA	32.59	5.08	13.76	NA	33.31	5.34	14.03	NA	33.16	Silvestre et al, 2005
Test-day protein yield:													
Germany	0.002	NA	0.01	NA	0.002	NA	0.01	NA	NA	NA	NA	NA	Swalve, 1995a
Portugal	4.23	8.80	NA	19.29	4.26	9.28	NA	20.26	4.25	9.95	NA	21.34	Silvestre, et al, 2005
Iran	7.17	18.1	18.3	43.2	7.5	18.6	16.7	42.4	7.9	18.5	16.1	42.6	Naserkheil et al, 2016

2.1.3 Heritability estimates for test-day milk, fat and protein yields

The estimates of h^2 for milk, fat and protein yields are reviewed and presented in Tables 3. Heritability in most estimates were low at the beginning of the lactation, gradually increased reaching the highest value, and decreased gradually until reached the lowest value. Most heritability estimates obtained by RRM for dairy cows were high at the edges **Van der Werf *et al* (1998)**. **Mosharraf et al (2014)** revealed that estimates of heritability for milk, fat and protein yields ranged from 0.18 to 0.26, 0.06 to 0.11 and 0.09 to 0.22, respectively.

The studies on genetic parameters for fat and protein yields on test-day in tropical countries are scarce **(Jamrozik and Schaeffer, 1997; Lidauer and Mäntysaari, 1999 ; Lidauer et al., 2003)**.The estimates for test-day fat yield were within the range found in these studies in temperate climate, ranging from 0.06 to 0.68 and showing a large discrepancy among results. The changes in heritability estimates for test-day protein yield were also similar to those estimated for test day fat yield, generating different values between models, especially in initial and final periods of lactation. **Jamrozik and Schaeffer (1997), Lidauer and Mäntysaari (1999) and Lidauer et al. (2003),** reported that the heritability estimates ranged from 0.10 to 0.69 for protein yield over lactation period

Table2. Estimates of heritability for test-day milk, fat and protein yields as cited in the literature in dairy cattle.

Country	Test day										Reference
	1	2	3	4	5	6	7	8	9	10	
Test-day milk yield:											
Germany	0.18	0.24	0.28	0.33	0.33	0.36	0.31	0.26	NA	NA	Swalve, 1995a
Brazil	0.20	0.20	0.20	0.20	0.32	0.20	0.24	0.16	0.12	0.04	Machado et al., 1999
Syria	0.44	0.17	0.14	0.11	0.13	0.13	0.18	0.19	0.24	0.24	Alnajjar, 2001
For first three lactations	0.14	0.05	0.03	0.04	0.05	0.08	0.09	0.10	0.11	0.10	
	0.03	0.14	0.21	0.21	0.15	0.10	0.07	0.07	0.09	0.08	
Portugal	0.19	0.21	0.21	0.21	0.23	0.23	0.23	0.22	0.22	0.21	Silvestre, et al., 2005
Egypt	0.04	0.03	0.08	0.12	0.11	0.14	0.1	0.07	0.03	0.04	Gamal et al., 2007
Turkey (for two models)	0.21	0.28	0.32	0.32	0.3	0.27	0.23	0.19	0.14	0.1	Takma and Akbas, 2007
	0.08	0.17	0.23	0.26	0.27	0.26	0.22	0.19	0.21	0.28	Çankaya et al., 2014
Iran (for many models)	0.11	0.12	0.13	0.16	0.17	0.18	0.17	0.19	0.19	0.17	Shadparvar and Yazdanshenas, 2005
	0.14	0.16	0.19	0.19	0.21	0.21	0.21	0.2	0.19	0.18	Abdullahpour et al., 2010
	0.13	0.04	0.06	0.11	0.16	0.21	0.24	0.26	0.28	0.28	Gharahveysi et al., 2012
Brazil (for many models)	0.20	0.20	0.20	0.20	0.32	0.20	0.24	0.16	0.12	0.04	Machado et al., 1999
	0.56	0.37	0.41	0.39	0.36	0.34	0.34	0.38	0.43	0.49	Cobuci et al., 2005
	0.15	0.16	0.19	0.21	0.22	0.24	0.26	0.28	0.29	0.3	
	0.28	0.32	0.33	0.4	0.4	0.4	0.3	0.3	0.27	0.32	Costa et al., 2008
Ethiopia	0.17	0.21	0.25	0.29	0.33	0.36	0.38	0.4	0.41	0.42	Gebreyohannes et.al, 2016
Iran	From 0.17 to 0.28										Torshizi, 2017
Afghanistan	From 0.1 to 0.32										Fazel et al, 2018
Test-day fat yield:											
Germany	0.16	0.12	0.14	16	0.18	0.23	0.21	0.2	NA	NA	Swalve, 1995a
Canada	0.42	0.40	0.37	0.34	0.35	0.36	0.40	0.41	0.47	NA	Jamrozik and Schaeffer, 1997
Finland	0.16	0.07	0.09	NA	0.12	0.12	NA	0.09	NA	0.06	Lidauer and Mäntysaari, 1999
Portugal	0.12	0.12	0.11	0.12	0.13	0.14	0.15	0.15	0.16	0.17	Silvestre, et al., 2005
Iran	From 0.04 to 0.13										Mohammadi et al, 2014
Test-day protein yield:											
Germany	0.12	0.17	0.16	0.19	0.21	0.22	0.22	0.17	NA	NA	Swalve, 1995a
Canada	0.34	0.34	0.35	0.39	0.42	0.44	0.46	0.47	0.52	NA	Jamrozik and Schaeffer, 1997
Finland	0.10	0.10	0.13	NA	0.14	0.13	NA	0.12	NA	0.11	Lidauer and Mäntysaari, 1999
Portugal	0.17	0.17	0.17	0.18	0.2	0.21	0.22	0.21	0.2	0.19	Silvestre, et al., 2005
Iran	From 0.07 to 0.24										Mohammadi et al, 2014
Iran	0.09	0.09	0.1	0.13	0.15	0.16	0.17	0.18	0.19	0.19	Naserkheil et al, 2016
Iran	0.1	0.1	0.2	0.3	0.32	0.33	0.33	0.33	0.33	0.34	Fazel et al, 2018

2.2 Reviewed genetic and phenotypic trends for TD milk, fat and protein yields

Estimates of genetic and phenotypic trends for milk, fat and protein yields are reviewed and clarified in Tables 3. When the control population is not available, the animal model methodology can be useful for estimating genetic and environmental trends of a selection process **(Henderson, 1984)**. In development and evaluation of breeding programs, both genetic parameters (e.g. heritability and predicted breeding values) and genetic trends are needed to be evaluated accurately.

In Egypt few estimates have been reported for the Friesian cattle in terms of genetic and environmental trends**.** The general genetic and phenotypic trends for the three traits of milk, fat and protein yields were increased or decreased together.

Table 4. Estimates of genetic and phenotypic trends for test-day milk, fat and protein yields in cattle as cited in the literature.

Country	Trend		Reference
	Genetic	Phenotypic	
Test-day milk yield:			
Zimbabwe	Increased	Increased	Kunaka and Makuza, 2005
Ireland	Increased	Decreased	Evans et al., 2006
Egypt	Increased	Increased	Abdelharith, 2008
Egypt	Increased	Decreased	Abou-Bakr, 2009
Egypt	Increased	NA	Osman et al., 2013
Egypt	Decreased	Decreased	El-Bramony, 2014
Pakistan	Increased	Decreased	Javed et al., 2007
Pakistan	Increased	Increased	Hussain et al., 2014
Brazil	Increased	Increased	Peixoto et al., 2006
Brazil	Increased	Increased	Bruneli et al., 2014
Brazil	Increased	Decreased	Chaudhari et al., 2014
Iran	Increased	Increased	Muller and Botha, 2003
	Increased	Increased	Razmkabir et al., 2006
	Increased	Increased	Bakir and Kaygisiz, 2009
	Increased	Increased	Bakir et al., 2009
	Decreased	Increased	Ulutaş et al., 2010
	Decreased	Increased	Kaygisiz, 2010
	Increased	Decreased	Abdullahpour et al., 2010
	Increased	Increased	Hossein-Zadeh, 2011
	Increased	Decreased	Katok and Yanar, 2012
	Increased	Decreased	Chegini et al., 2013
Costa Rica	Increased	Increased	Vargas and Gamboa, 2008
Cuba	Decreased	Increased	Hernández et al., 2011
Poland	Increased	Increased	Rozanska-Zawieja et al., 2013
Test-day fat yield:			
Brazil	Decreased	Decreased	Freitas et al., 1995
Kenya	Increased	NA	Musani and Mayer, 1997
South Africa	Increased	Increased	Hallowell, 1998
America	Increased	Increased	Roman et al., 1999
	Increased	NA	Abdallah and McDaniel, 2000
South Africa	Increased	Increased	Muller and Botha, 2003
Thailand	Increased	Increased	Sanpote and Buaban, 2003
Zimbabwe	Increased	Increased	Kunaka and Makuza, 2005
Iran	Increased	Increased	Razmkabir et al., 2006
Brazil	Increased	Increased	Costa et al., 2009
Iran	Decreased	NA	Abdullahpour et al., 2010
Iran	Increased	Increased	Hossein-Zadeh, 2011
Iran	Increased	NA	Yaeghoobi et al., 2011
Turkey	Increased	Decreased	Katok and Yanar, 2012
Iran	Decreased	NA	Abdullahpour et al., 2013
Iran	Decreased	Decreased	Khanzadeh et al., 2013
Egypt	Increased	NA	Osman et al., 2013
Poland	Increased	Increased	Rozanska-Zawieja et al., 2013
Brazil	Increased	Increased	Bruneli et al., 2014
Test-day protein yield:			
Brazil	Decreased	Decreased	Freitas et al., 1995
South Africa	Increased	Increased	Hallowell, 1998
Florida	Increased	Increased	Roman et al., 1999
South Africa	Increased	Increased	Muller and Botha, 2003
Thailand	Increased	Increased	Sanpote and Buaban, 2003
Zimbabwe	Increased	Increased	Kunaka and Makuza, 2005

Iran	Increased	Increased	Razmkabir et al., 2006
Brazil	Increased	Increased	Costa et al., 2009
Iran	Decreased	NA	Abdullahpour et al., 2010
Iran	Increased	Increased	Hossein-Zadeh, 2011
Egypt	Increased	NA	Osman et al., 2013
Brazil	Increased	Increased	Bruneli et al., 2014
Iran	Decreased	NA	Abdullahpour et al., 2013
Iran	Decreased	Decreased	Khanzadeh et al., 2013
Poland	Increased	Increased	Rozanska-Zawieja et al., 2013

NA= Not available.

2-3 Molecular analysis of milk, fat and protein yields:-

2-3-1. Lactoferrin (LF) and prolactin (PRL) as major genes

The study of candidate genes is one of the primary methods to determine the specific genes related to the economic traits in farm animals. In marker-assisted selection of dairy cattle, some genes are proposed as potential candidates associated with dairy performance traits. Several genes that were selected based on their biological actions or that are located in genome regions containing previously identified QTLs have been regarded as candidate genes affecting milk production traits in dairy cattle **(Meuwissen and Goddard, 1996; Ruane and Colleau, 1996).**

Lactoferrin gene (LF) plays an important regulatory function in mammary gland development and milk secretion, and expression of milk protein genes **(Zhang J L et al 2007)**. LF gene is highly polymorphic and it has been shown that some of its variants are related to milk production traits and mastitis resistance in cattle (**Kaminski et al., 2006; Maksymiec et al 2006**).

In marker-assisted selection of dairy cattle, some genes are proposed as potential candidates associated with

dairy performance traits. Among the various candidates, the prolactin gene seems to be promising, because it plays a crucial role in mammary gland development and in the initiation and maintenance of lactation and expression of milk protein genes. Allelic variation in the structural or regulatory sequences of the prolactin gene would be of interest because of the possible direct or indirect effect on milk production. It may also influence the chemical composition of milk or at least be an effective DNA marker in dairy cattle selection. **Othman et al (2011)** cited that prolactin gene is 10-kb long and is composed of five exons and four introns, and this gene was mapped to chromosome 23 in bovine. Prolactin is one of the most multifunctional hormones in the body.

Prolactin's biological activity consists of various roles in the reproduction, lactation and a number of homeostatic biological functions including immune functions. Prolactin is a polypeptide hormone with multiple functions, secreted mainly by the anterior pituitary gland. Gene disruption experiments have proved their mandatory role for mammary gland development, lacto genesis and expression of milk protein genes. Therefore, the bovine prolactin gene seems to

be an excellent candidate for linkage analysis of quantitative trait loci (QTL) affecting milk production trait.

2-3-2 Single nucleotide polymorphism (SNP) and molecular weights of lactoferrin and prolactin genes:

2-3-2-1. SNP for lactoferrine gene:

The restricted enzymes, primers and molecular weights of lactoferrin gene reviewed from literature are presented in **Table 5**. **Daly et al., (2006**) **and Halloran et al. (2009)** found that, 3 combined genotypes within 379 bp region in the 5'- flanking region of bovine *Lf* gene were identified in Holstein cattle at Esfahan province of Iran, three SNPs have been previously identified in this region (-602 T>G, -600 A>G and -586 T>C). In addition, it has been proved that some of the SNPs of the *Lf* 5'- flanking region occur only in few dairy cattle breeds. For example, polymorphisms in positions - 926 and - 915 have been found only in Holstein Friesian (HF), New Zealand HF and Montbeliarde cattle (**Daly et al 2006)**. **Zaho et al (2009)** reported that, different fragments were separated by PCR products in Lf gene cows, 2 allele genes and 3 genotypes were detected in each group. Allele gene (638bp) was

defined as A, whilst allele gene (462bp) were found as B. Other related genotypes were defined as A/A, A/B and B/B, respectively. **Dolatabady and Shafaeipour (2014)** identified three distinct haplotypes in the amplified fragment of 5'-flanking region at bovine Lf gene. **Nanaei et al (2012)** reported that the A and B alleles of the LTF gene were identified based on amplification of specific primer (LFTR and LFTF), followed by digestion with the restriction enzyme EcoRI. The frequency of A allele ranged from 0.775 to 0.831 and the frequency of B allele ranged from 0.169 to 0.225 and the two genotypes AA and AB of the LF gene frequencies were 0.606 and 0.394, respectively, while BB genotype was not detected.

Table 5 Restriction enzymes, primers and molecular weights for lactoferrin gene as cited in literature

Reference	Breed	Primer	Restriction enzyme	Molecular weight
K.Wojdak Makcymiec et al 2006	Polish black and White cows	F: 5'-GCC TCA TGA CAA CTC CCA CAC-3 R 5'-CAG GTT GAC ACA TCG GTT GAC-3'	EcoRI	100:201:30 1bp
K.Wojdak et al 2009	Polish black and White cows	F 5'-GCC TCA TGA CAA CTC CCA CAC-3' R 5'-CAG GTT GAC ACA TCG GTT GAC-3'	EcoRI	100:201:30 1bp
Zhao et al 2009	Chinese cows	F 5'-CACATTACAAGCAGGATCTTTTGCTG-3' R 5'-CTGGCCAATGAGCCCTATATGTGT-3'	HinfI	462:638bp
Nanaei et al 2012	Holstein cattle	F 5' - CAG GTT GAC ACA TCG GTT GAC - 3' R 5' - GCC TCA TGA CAA CTC CCA CAC - 3'	EcoRI	100:201:30 1bp
Feraye ESEN Gursel et al 2013	Red and East Anatolian cattle	F: 5'- GCC TCA TGA CAA CTC CCA CAC -3' R 5'- CAG GTT GAC ACA TCG GTT GAC -3'	EcoRI	100:201:30 1bp
Alka chopra et al 2013	Sahiwal cattle	F 5'- GATAAAGGGACGCAGAACGAGC-3 R 5'- ATACCTGCACTCACCAACGGCTCC -3	StyI	125 bp
Alka chopra et al 2013	Friese karan cattle		StyI	
T.Zabolewic et al 2014	Polish Holstein	Not. Available	Sequencing	N. AV
M.M.Dolatabadi and Aria 2014	Holstein cattle	F 5-TGTTGTGCTTCAAGGGAGTG-3 R 5-CCAGCCTGGAGTTTTGAAAG-3	seqencing	N. AV
A.I Ateya et al 2016	Holstein cattle	F: 5'-GCC TCA TGA CAA CTC CCA CAC-3' R 5'-CAG GTT GAC ACA TCG GTT GAC -3'	Sequencing	301bp
Nanaei et al 2016	Iranian Holstein cattle	F 5'- GCC TCA TGA CAA CTC CCA CAC -3' R 5'- CAG GTT GAC ACA TCG GTT GAC -3'	EcoRI	100:201:30 1bp

N.AV=Not available

2.3.2.2 SNP for Prolactin gene

The restricted enzymes primers and molecular weights of lactoferrin gene reviewed from literature are presented in Table 6. **He et al (2006)** identified SNPs within bovine prolactin using PCR-SSCP analysis and four SNPs, namely CHBP1, CHBP2, CHBP3 and CHBP4, were

identified, which was located at positions of -767 (A/C), -485 (G/T), -247 (C/A), 427 (C/T) of bovine prolactin gene, respectively. Three genotypes were found for each SNP and named as AA, AB, and BB, respectively. **Dybus et al (2005)** found that the following DNA restriction fragments were obtained for the **PRL-RsaI** polymorphism: 82 and 74 bp for the BB genotype, 156, 82 and 74 bp for the AB and 156 bp (no digestion) for the AA genotype.

Zhao et el (2009) showed that, 2 allele genes and 3 genotypes were revealed in each group. Allele gene (638bp) was defined as A, whilst allele gene (462bp) as B. Other related genotypes were defined as A/A, A/B and B/B, respectively. **Othman, et al (2011)** reported that, at PRLR locus, polymorphism occurring at position 486 in Exon 10 was targeted which was a single nucleotide change (SNP), A/C (transition) which was recognized by PCR-RFLP with ***Nae*I**. The PCR amplified DNA fragment of 232 bp was digested with ***NaeI*** restriction enzyme that yielded banding pattern corresponding to three different genotypes *viz*., AA genotype with one bands (232 bp), AC genotypes with three bands (232, 125 + 107 bps) and CC genotype with two band (125 + 107 bps).

Deepika and Salar (2014), found that the PCR amplified DNA fragment of 168 bp was digested with ***SmlI*** restriction enzyme and three genotypes GG, GT and TT were observed. ***SmlI*** restriction analysis of the PCR product yielded banding pattern corresponding to three different genotypes viz., GG genotype with one bands (168 bp), GT genotypes with three band (168, 123 and 45 bps) and TT genotype with two bands (123 and 45 bps).

Table 6.primers and molecular weight for prolactin gene:

Reference	Breed	Restriction enzyme	Primer	Molecular weight

He et al 2006	Holstien Friesian cattle	RsaI	F 5-TTCCCCAGTATGAACTCCC-3 R 5CGTCCCCAGATTGAGAAAC3 F 5ATCCCACTGTTTCTCAATC-3 R 5-CTGGCTGAAGTCAAGATTT-3 F 5-AATCTTGACTTCAGCCAG-3 R 5GAGAGATAATGATGGTGAG-3 F 5-CTCACCATCATTATCTCTC-3 R 5AAGAAACACACTTCACCAG-3 F 5-GTACAGCAGCTTGTGGAGT-3 R 5-TATCTCTGTTCTCTACCCC-3 F 5GGGGTAGAGAACAGAGATA3 R 5-AGACTACTCTGCTGTCACT-3	209 bp 248 bp 261 bp 280 bp 279 bp 281 bp
Alipanah et al 2007	Russian Red Pied cattle	RsaI	R 5′-CGAGTCCTTATGAGCTTGATTCTT-3 R 5′-GCCTTCCAGAAGTCGTTTGTTTTC-3′	156 bp 82 bp 74 bp
Alipanah et al 2008	Russian Black Pied and Russian Red Pied cows	Rsa I	F 5'-CGAGTCCTTATGAGCTTGATTCTT-3' R 5'-GCCTTCCAGAAGTC GTTTGTTTTC-3'	NF
Nasrin Ghasemi et al 2009	Montebeliard cows	*RsaI*	F 5'-CGAGTCCTTATGAGCTTGATTCTT-3' R 5'- GCCTTCCAGAAGTCGTTTGTTTTC -3'	156 bp 82 bp 74 bp
Othman et al 2011	Egyptian buffalo	RsaI	F CCA AAT CCA CTG AAT TAT GCT T R ACA GAA ATC ACC TCT CTC ATT CA	294 bp
Bukhari et al 2013	Frieswal Cow	Rsa I	F 5′CGAGTCCTTATGAGCTTGATTCTT3′ R 5′GCCTTCCAGAAGTCGTTTGTTTTC3′	156 bp 82 bp 74 bp
Singh et al 2014	Indian Frieswal (HF 3 Sahiwal) cows	RsaI	F 5-CGAGTCCTTATGAGCTTGATTCTT-3 R 5- GCCTTCCAGAAGTCGTTTGTTTTC-3	398-294-104 bp
Deepika1 and Salar 2014	Indigenous Grey Cattle breeds of India	*NaeI*	F 5'-CCTATTTTCTGGCCAATGGA-3' R 5'-TCTGACTCCCTCTGCTTGGT-3'	232 bp
		SmlI	F 5'-AGATGGAGTGCTGGCTCTGT-3' R 5'-GCCTTCTTGGCTGGTTCTTC-3'	168 bp
Abukhaizran and al razem 2014	Holstien Friesian cattle	RsaI	F -CCAAATCCACTGAATTATGCTT R –ACAGAAATCACCTCTCTCATTCA	294 bp

2-4) Genotypic and allelic frequencies for lactoferrin and prolactin genes:

2-4-1 Genotyping for lactoferrin gene:

Wojdak et al (2006) using 124 Polish Black-and-White dairy cows of various share of the Holstein-Friesian (HF) breed showed that Lactoferrin (LF) gene polymorphism was obtained with PCR-RFLP method using ***EcoRI*** **enzyme.**Two alleles of LTF, A and B, were found in the studied population and their frequencies were 0.68 and 0.33, respectively. The alleles controlled the occurrence of three genotypes: AA, BB and AB, of frequencies equal to 0.38, 0.2 and 0.60, respectively. **Chopra et al (2013)** reported that lactoferrin gene revealed three different genotypes namely, AA, AB and BB in Karan Fries cattle whereas the BB genotype was absent in Sahiwal cattle. In Karan Fries cattle AA, AB and BB genotypes were having frequencies of 0.19, 0.77 and 0.5, respectively**.**

Nanaei et al (2012) found that the frequency of A, allele ranged from 0.775 to 0.831 and the frequency of B allele was 0.169 to 0.225and two genotypes AA and AB of the LF gene were observed with their frequencies of 0.606 and 0.394, respectively, while the BB genotype was not detected. Genotype AA was characterized by single 301-bp fragment and AB genotype was determined by the presence of three restriction fragments of 301, 201 and 100-bp.The

frequency of the AA genotype in the all herds was higher than AB genotype, while the frequency of A and B allele in Karan Fries cattle was 0.57 and 0.43, respectively.

2-4-2 Genotyping for prolactin gene:

Othman et al (2011) found frequencies of genotypes were 0.81, 0.15 and 0.04 for A/A, A/B and B/B, respectively. The frequency of PRL A allele is 0.89. Also found that out of 455 animals screened, 0.76 animals were homozygous AA, 0.3 of animal were homozygous CC and 0.21 animals were heterozygous (AC). The frequency of C allele was significantly lower than the A allele in all the cattle breeds. From the allelic pattern, it was observed that A allele was predominantly present than the C allele in all the ten indigenous cattle. The frequency distribution of A allele varied from 0.787 (Kangayam) to 0.958.

He et al (2006) reported that the prolactin gene and genotypic frequencies were listed. From the whole, the frequency of AA for each of four SNPs, 0.3, 0.10, 0.6 and 0.1, was very low, respectively. A relatively high frequency of the genotype BB was 0.82, 0.51, 0.78 and 0.85, respectively. **Dybus et al (2005)** revealed that in Black-and-

White cattle the *AA* genotype was the most frequent in the studied herds (0.7222 and 0.6983), followed by the heterozygotic *AB* (0.2778 and 0.2931), whereas the *BB* was the least frequent (0 and 0.0086). In contrary, in Jersey cows the *BB* genotype was the most frequent. The influence of the ***PRL-Rsa*I** polymorphism on milk production traits in Jersey cows.

Miceikienė et al (2006) found that two different alleles of the polypeptide hormone PRL were detected in four Lithuanian cattle breeds. The PRL gene A allele was most frequent (0.97) in Lithuanian White Back cattle breed and the least frequent (0.77) in Lithuanian Red cattle breed. A allele frequency was very high – 0.87 in all population, whereas that of B allele was only 0.13. 87% individuals of the population had A allele and 13% had B allele. The frequencies of PRL alleles in Lithuanian cattle breeds are shown. Three genotypes were identified for the polypeptide hormone PRL (AA, AB and BB) in Lithuanian cattle breeds. The prolactin gene AA genotype was the most frequent in all investigated breeds (0.62– 0.94), followed by AB (0.06–0.37). The BB genotype was the least frequent (0.00–0.08). The BB genotype was not found in Lithuanian

White Back and Lithuanian Light Grey cattle breeds. 75% of all investigated population had AA genotype, 18% had AB genotype and 7% BB genotype.

2-5) Heterozygosis and inbreeding for lactoferrin and prolactin genes:

Sonmez and Ozdemir (2017) found that, heterozygosity was found at a medium rate as 0.338 for PRL-RsaI polymorphism and the population was in H-W equilibrium. Heterozygosity was 0.186 in EAR (Akyuz et al., 2012), 0.038 in Holstein, and 0.33 in Jersey cattle **Brym et al (2005). Dybus et al. (2005)** found 0.28 in Black and White and 0.43 in Jersey. The FIS value for the region was 0.072 in EAR population. According to this value, despite the small number of the EAR population as a genetic resource, homozygosity was at a low rate.

2.6) Associations among milk yield traits and age at first calving and lactoferrin and prolactin genes:

2.6.1 Lactoferrin gene:

Daly et al. (2006)., **Halloran et al., (2009)** found that combined genotypes of three SNPs in 5'-flanking region of *Lf* gene were associated with fat percentage and tended to associate with protein percentage and milk yield. There were no significant associations between studied haplotypes and SCS. The associations of combined genotypes with milk production traits could be explained by the mapping quantitative trait loci (QTL) for these traits on bovine chromosome 22. **Kaminski et al (2006)** found that SNP at position -32 (G/C) relative to transcription start codon site of bovine *Lf* gene was significantly associated with milk protein content, but is not related to SCC, in Polish Holstein-Friesian cows. According to the author mentioned, a G allele reduces the *Lf* gene expression, and encourages a lower somatic cell count in milk. Lactoferrin polymorphism at position 216 related to the higher milk protein yields.

Wojdak et al (2006) reported that, Significantly more heterozygous AB genotypes were found in relation to the expected rate of heterozygotes, whereas there were significantly fewer BB homozygotes in comparison. **Steciwko et al (2014)** reported that, positive interaction between AA genotype in lactoferrin gene and fat content in

two Holstein herds, in milk cows with AB genotype an opposite tendency was observed. Results presented by other authors were showed that the level of fat decrease. **Cheng et al. (2008)** showed that there is no significant relation between lactoferrin level and fat content in milk.

2.6.2 Prolactin gene:

Zhao et el (2009) showed that the highest milk, fat, and protein yields were obtained by cows with the BB genotype for **PRL-*RsaI***, different results for milk and fat yields were reported by **Dybus (2005)** who found that cows with the AA and AB PRL genotypes yielded fat more than BB animals. **Othman et al (2011)** found that, the associations were analyzed between polymorphisms of the prolactin gene **(PRL-*RsaI*)** and milk production traits of Montebeliard cows. The results showed that AA genotype cow's yielded high milk in compared of other groups.

Brym et al (2005) used the same primer and restriction enzyme and found assessing allele frequencies in Black-and-White cows (0.113 and 0.887 for A and G, respectively) and in Jersey cows (0.706 and 0.294 for A and

G, respectively). Black and White cows with genotype AG showed the highest milk yield, while cows with genotype GG showed the highest fat content. The high frequencies of allele G in different cattle breeds were reported previously and ranging from 0.61 in Brown Swiss breed to 0.95 in Holstein breed.

He et al (2006) found that associations between SNPs with milk performance traits the association analysis of the 4 SNPs within bovine prolactin gene with the milk performance traits was carried out using least square estimation. Statistical results indicated that, among the 4 SNPs, only CHBP2 in the promoter was significantly associated with milk yield, fat yield, protein yield, and protein percentage. Further, multiple comparisons analysis showed that cows with genotype BB had significantly higher milk yield, fat yield, and protein yield than those of genotype AA, while cows with genotype AA had significant higher protein percentage than cows with genotypes BB and AB. Other three SNPs showed no association with milk performance traits.

Instead of using ***HaeIII*** restriction enzyme, the authors of cattle studies primarily used ***RsaI*** to study

polymorphisms in the prolactin gene. In Polish Friesian cows, the ***RsaI*** polymorphism was studied in three different lactations for its effect on milk production traits, and in the first lactation both AA and AB cows had higher milk yields while in the seconds lactation, AB cows produced more milk than AA cows **(Dybus et al (2002)**; in the third lactation there was not a significant difference in milk yields amongst the genotypes. In 98 Holstein dairy cows, an ***RsaI*** polymorphism also revealed that cows with AA genotype had greater milk yield than BB genotype **Chung et al (1996)**.

Dybus et al (2005) revealed that, the influence of the *PRL-**Rsa***I polymorphism on milk production traits in the analyzed Jersey cows. The statistically significant differences between individuals of different *PRL* genotypes were found in milk fat yield, milk fat content and and sum of fat and protein content. In the first 305-day lactation, the cows of the *AA* genotype produced less milk fat (-15.9 and –14.6 kg) than the *AB* and *BB* individuals ($P \leq 0.01$). In the case of milk fat content, statistically significant differences between individuals of different *PRL* genotypes were found in the first lactation ($P \leq 0.01$). The cows with the *AA*

genotype produced milk with lower fat content (-0.19 and -0.18%) than *AB* and *BB* individuals, respectively.

In Brown Swiss cows, no statistically significant differences between genotypes on milk yield could be determined **Chrenek et al (1999)**. In fact, in Russian Red Pied cattle, the ***RsaI*** prolactin polymorphism indicated that the BB genotype had a higher milk yield than AA or AB genotypes **Alipanah et al (2007)**. **Khatami et al (2005)** found that a negative association with fat content and milk production was observed for the BB genotype in Yaroslavl cattle; the proportion of cows producing milk with less than 4.5% percent fat was 19-20% higher in BB genotypes than AA and AB genotypes, and the proportion of cows that were high milk producers (more than 6000 kg) was 9-10% lower in BB genotypes than AA and AB genotypes.

3. Material and methods

3.1 Random regression model and genetic trends

3.1.1 Management and data structure

A total of 5237 test-day milk, fat and protein yield records from 2000 to 2016 were used in this study. Cows were fed, from May to November, daily in ration consisting cotton seed cakes, barley wheat and rice bran, cows fed mainly on berseem and rice straw. In addition concentrates feed mixture from December to April. Mineral mixture bricks were offered adlibitum as soled minerals mixture in front animals, and on a balancehd ration of a concentrates according to their production and weight. Limited amount of clover hay was used when available. Animals housed under open sheds covered with 3.5-4 meters high roofs. Heifers were bred for the first time when they reached 18-22 months or 350 kg body weight. Cows were artificially inseminated not before 60 days of calving using frozen semen from U.S.A and Canada. Cows milked two times a day by milking machine.

Data collected at monthly intervals in three experimental herds (Sakha and El karaka in Kafrelshikh governorate and El Serw in Damietta governorate) belonging to Animal Production Research Institute (APRI), Ministry of Agriculture, Egypt. Test-day milk yield (TDMY) records were measured based on an alternative am-pm monthly recording scheme. Milking was practiced twice a day at 7 am and 4 pm throughout the lactation period. In general, using TD models could have advantages over a 305-day model **(Wilmink, 1987; Danell, 1982;**

Keown and VanVLeck, 1971). Cows with less than four TD records per lactation were excluded from the data set, while the maximum number of test day records per lactation was 10 records. Moreover, up normal phenotypic values of daily milk yield, fat and protein yield were removed from the dataset. All the known relationships among the individuals were considered in the animal model employed in analysis. The structure of the data analyzed is shown in Table7.

Table7. Structure of test day data analyzed for cattle's

Item	**Data**
No. of sires	**208**
No. of dams	**944**
No. of base animals	**953**
No of non-base animals	**1560**
Total number of animals	**2513**
Total number of lactation records	**5237**

3-1-2 Measuring fat and protein percentages in milk.

Fat and protein percentages were measured by the automated method of infrared absorption spectrophotometry (Milk-o-Scan; Foss Electric, Hillerφd, Denmark) at the

Dairy Services Unit, Animal Production Research Institute, Sakha, and Kafr El-Sheikh Governorate. The device needs a set of solutions: The first solution is used to wash the device after the analysis of the samples and before turning it off the name of this solution none foaming Stella 0.5% (Foss electric company, Denmark). The second solution is used to reset the device which gives the readings 0.000 so it is ready to read the new samples and its name is Triton x-100 and we use only 1 cm/liter of distilled water, and finally we have to give the device the order Prog. 2 then Prog. 3 then Prog. 4, and then the device is programmed to read the cow milk samples .After that the percentages of fat and protein had been converted to yields in grams.

3-1-3 Statistical analyses

3-1-3-1 Estimating variance (co) components using random regression model

All the individuals with known relationships were considered in the analyses using the animal model. The monthly test-day milk yield (TDMY) were measured between 4 and 304 days in milk (DIM), divided into 10 classes. The first class included milk yield between 4 and 30 DIM, the second included milk yield between 31 and 60

DIM, and so on until the last class, which included milk yield between 270 and 304 DIM. The orthogonal polynomials of standardized units of time have been recommended as covariables **(Kirkpatrick *et al*., 1990; Meyer, 1998b)**. Orthogonal polynomials have computational advantages; the primary general advantage is the reduced correlations among the estimated coefficients.

The standardized unit of time, w, ranges from ⁻1 to +1, was derived as:

$$t^* = \frac{2(t - t_{min})}{t_{max} - t_{min}} - 1$$

where t_{min} is the earliest date (or the youngest age) and t_{max} is the latest date (or oldest age) represented in the data. The first six Legendre polynomial functions of standardized units of time are given in Table 2. Thus, if w = - 0.2, then the covariables that would go into the model (for order equal to 5) are shown in the last column of Table 2. Covariables based upon orthogonal polynomials are small numbers that reduce problems with rounding errors, and they provide relatively small correlations between the estimated regression coefficients.

Table 8 The first six Legendre polynomial functions of standardized units of time.

Order	**For w =**

		−0.2
0	$0.7071w^0$	0.7071
1	$1.2247w^1$	−0.2449
2	$-0.7906w^0+2.3717w^2$	−0.6957
3	$-2.8062w^1+4.6771w^3$	0.5238
4	$0.7955w^0-7.9550w^2+9.2808w^4$	0.4921
5	$4.3973w^1-20.5206w^3+18.4685w^5$	−0.7212

And so on, the first six Legendre polynomial functions can be put into a matrix of polynomial coefficients (Λ) as:

$$\Lambda' = \begin{pmatrix} 0.7071 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1.2247 & 0 & 0 & 0 & 0 \\ -0.7906 & 0 & 2.3717 & 0 & 0 & 0 \\ 0 & -2.8062 & 0 & 4.6771 & 0 & 0 \\ 0.7955 & 0 & -7.9550 & 0 & 9.2808 & 0 \\ 0 & 4.3973 & 0 & -20.5206 & 0 & 18.44685 \end{pmatrix}$$

Now we can define another matrix, M, as a matrix containing the polynomials of standardized time values. Legendre polynomials are defined within the range of values from -1 to +1. Thus, ages or time periods have to be standardized (converted) to the interval between -1 to +1.

The formula is M=$t^* = \frac{2(t-t_{min})}{t_{max}-t_{min}} - 1$ this gives Φ = M Λ. Orthogonal polynomials tend to reduce the correlations between estimated regression coefficients. This is advantageous, because the estimates would converge faster to the maximum or appropriate posterior distribution. There are other kinds of orthogonal polynomials, but Legendre polynomials are probably the easiest to calculate and utilize. Covariables based upon orthogonal polynomials are small numbers that reduce problems with rounding errors, and they provide relatively small correlations between the estimated regression coefficients.

Variance-covariance components were estimated by REML using the computer package VCE6 **(Groeneveld *et al*., 2010).** The animal model was:

$$Y_{ijkl} = HYS_i + \sum_{m=1}^{4} \beta_{km} Z_{jlm} + \sum_{m=1}^{4} \alpha_{jm} Z_{jlm} + Pe_j + e_{ijkl}$$

where: Y_{ijkl} is the record *l* on trait within lactation made on herd-year-season (HYS) subclass i for the j^{th} cow belonging to k^{th} subclass (k ranged from 1 to 10 starting with k=1 and increased by 1 every 30 days thereafter along the trajectory from 4 to 304-d); HYS_i is the fixed effect of herd-year-season, Pe_j = random effect of permanent environment associated with all TD yields of the j^{th} cow; β_{km} and α_{jm} = fixed and random regression coefficient, and e_{ijkl} = random residual effect associated with Y_{ijkl}.

The VCE6 program applying the Random Regression Model (RRM) was used to analyze the data using the Legendre polynomials method **(Kirkpatrick *et al.*, 1990)**. The general RRM can be represented in matrix notation as:

$$Y = Xb + Za + Wp + e,$$

Where, Y = vector of observations on animal; b = vector of the fixed effects (**HYS** is the fixed effect of herd-year-season combination and **P** is the fixed effect of the l^{th} parity (l = 12 levels for all parities); a = vector of solutions for additive genetic random coefficients; p = vector of solutions for permanent environmental random coefficients; e = vector of N different residuals; X, Z, and W = incidence matrices for fixed and random genetic and permanent environmental random effects, respectively. The assumptions with respect to the components of the model were **(Jamrozik and Schaeffer, 1997; Schaeffer, 2004)**:

$$\begin{pmatrix} a \\ p \\ e \end{pmatrix} \sim N\left(0, V\right) \quad where, \quad V = Var \begin{pmatrix} a \\ p \\ e \end{pmatrix} = \begin{pmatrix} G \otimes A & 0 & 0 \\ 0 & I\sigma_p^2 & 0 \\ 0 & 0 & R \end{pmatrix}$$

where G and P are (co)variance matrix of additive genetic and permanent environment random regression coefficients, respectively; A is an additive genetic relationship matrix among the cows; $\otimes$ is a Kronecker product function; I is identity matrix and R is the diagonal matrix of temporary

environmental variances. The mixed model equations for this model would be:

$$V = \begin{pmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + G^{-1} \otimes A^{-1} & Z'W \\ W'X & W'Z & W'W + I \otimes P^{-1} \end{pmatrix} \begin{pmatrix} b \\ a \\ p \end{pmatrix} = \begin{pmatrix} X'Y \\ Z'Y \\ W'Y \end{pmatrix}$$

$$E \begin{bmatrix} y \\ a \\ p \\ e \end{bmatrix} = \begin{bmatrix} Xb \\ 0 \\ 0 \\ 0 \end{bmatrix}; \text{ and } \begin{matrix} V(a) = K_a \otimes A; \\ V(p) = K_p \otimes I; \\ V(e) = R, \end{matrix}$$

Where k_a and k_p are the genetic and permanent environmental covariance matrices between random regression coefficients, respectively. A is the additive genetic relationship matrix; I is an identity matrix, and R represents a diagonal matrix containing the residual variances.

The genetic (G) and permanent environmental (P) covariance between test-days were estimated using:

$$G = (1 \; ti \; ti^2 \; ...) Ka \begin{bmatrix} 1 \\ tj \\ tj^2 \\ ..... \end{bmatrix} \quad and \quad P = (1 \; ti \; ti^2 \; ...) Kp \begin{bmatrix} 1 \\ tj \\ tj^2 \\ ..... \end{bmatrix}$$

Heritabilities (h^2) are computed using the package of VCE6 as **(Groeneveld *et al*., 2010)**:

$$h^2 = \frac{\sigma^2_{gi}}{\sigma^2_{gi} + \sigma^2_{pei} + \sigma^2_{ei}}$$

Where: σ^2_{gi} is the additive genetic variance of the i^{th} TD; σ^2_{pi} is the permanent environmental variance and σ^2_{ei} is the residual variance.

3-1-3-2 predicting the breeding values of the genetic trend

Predicted breeding values (PBVs) for cows were estimated using the computer package of **PEST** program **(Groeneveld *et al.*, 2001)** for test day milk, fat and protein yields and age at first calving according to the following model:

$y = Xb + Za\ a + Zc\ c + e$

where: y = Vector of observations, X = Incidence matrix relating fixed effects to y, b = Vector of an overall mean and fixed effects, (**HYS** is the fixed effect of herd-year-season combination and **P** is the fixed effect of the l^{th} parity (l = 12 levels for all parities), Za = Incidence matrix relating direct additive genetic effects to y, a = Vector of random effect (direct additive genetic associated with the incidence matrix Za), Zc = Incidence matrix for permanent environmental effect, c = Vector of permanent environmental effect

associated with the incidence matrix Zc and e = Vector of random residual effects N (0, $I\sigma^2_e$); I is an identity matrix.

Solutions for the equations of animals were computed from the pedigree file, one animal at a time for animals with records and animals without records (sires and dams). A diagonal element (d_t) and an adjusted right-hand side ($^{y^*}_t$) were accumulated with each pedigree file record for the t^{th} animal. For the animals with and without records, the formula used to estimate the PBV was that of (**Kennedy 1989)**:

PBV = [$^y_t/d_t$]

The predicted error variances (PEV) of predicted breeding values (PBVp) were estimated for each individual as: $PEV_p = d_j\sigma^2_e$ **(Korsgaard *et al.*, 2002)**; where d_j is the j^{th} diagonal element of inverse of the appropriate block coefficient matrix and σ^2_e is the residual variance. The accuracy of PBV for each individual was estimated according to **Henderson (1975)** as:

$$r_{\hat{A}} = \sqrt{1+F_j - d_j\alpha_a}$$

Where

$r^{\wedge}_A$ = the accuracy of prediction of the i^{th} animal's breeding

value; F_j=inbreeding coefficient of animals (assumed equal to be zero); d_j was defined before; and $\alpha_a = \sigma^2_e/\sigma^2_a$.

3-1-3-3 plotting the genetic and phenotypic trends

The phenotypic trend was measured as the regression of least squares means on years. As stated before, the breeding values of the animals with records and without records were estimated using the **PEST** program **(Groeneveld *et al*., 2001)**. Accordingly, the genetic trend was measured by regressing the breeding values on years.

3.2 Lactoferrin and prolactin genes studies:

3-2-1 Experimental work and DNA extraction:

A total of 494 lactation records for 180 cows in three herds (62 Friesian cows from Sakha herd, 80 Friesian cows from Elkarada herd and 38 local cows from Elserw herd) were collected on the herds of monthly intervals over four years period from 2013 through 2016.The three experimental herds are belonging to the Animal Production Research Institute (APRI), Ministry of Agriculture and, Egypt. Friesian cows were raised in Sakha and Elkarada herds, while the local cows were raised in El serw herd.

Test day (TD) records for milk, fat and protein yields were measured following an alternative am-pm monthly recording scheme. Milking was practiced twice a day at 7 am and 4 pm throughout the lactation period. Fat and protein yields were measured by the automated method of infrared absorption spectrophotometry (Milk-o-Scan; Foss Electric, Hillerφd, Denmark) at the Dairy Services Unit, Animal Production Research Institute, Sakha, Kafr El-Sheikh Governorate. Cows with less than four TD records per lactation were excluded from the data set, while the maximum number of test day records per lactation was 10 records. The up normal values of daily milk, fat and protein yields were removed from the dataset. TD records were transformed to 305 days milk yield using Fleishman equation **(Tangorra et al., 2008).** The structure of data analyzed is shown in Table1.

Whole peripheral blood samples for 180 cows of the three herds were collected from the jugular vein into test tubes containing an anticoagulant (K3EDTA). Genomic DNA extraction was performed according to salting out method (Miller et al., 1988). After salting out, RFLP

method was used to restrict the DNA product by using three restriction enzymes.

Table 9 The number of cows in each herd and number of lactation records in all data set.

Item	**Data**
No. of Friesian cows in Sakha herd	62
No. of Friesian cows in Elkarada herd	80
No of local cows in Elserw herd	38
Total No. of lactating cows	180
Total number of 305-day lactation records	494

3-2-2 Primers design and (PCR) performed for prolactin and lactoferrin genes:

For PCR of **lactoferrin** gene, the primers were designed on the basis of DNA sequence of LF gene promoter accepted by the Gene bank (Accession: AY319306) using the oligonucleotide design tool Primer 5.0 software (Table 10).The primer sequences used for the 1143pb fragment containing a polymorphic *Hinfi* restriction site at the position were 462 to 638bp. The Forward and reverse primers were 5'-CACATTACAAGCAGGATCTTTTGCTG-3' and 5'-

CTGGCCAATGAGCCCTATATGTGT-3', respectively (Matrix Scientific Trade CO., Egypt). The PCR reactions were performed in a 25 µl mixture containing 0.7µl forward primers, 0.7µl reverse primer, 1.5µl of 2.5mM dNTP (deoxyribonucleotide triphosphate), 2.5 µl 10×reaction buffer, 1.5µl of 2.5mM MgCl2, 2µl 1 unit of Taq-DNA polymerase, and 2.5µl of 50 ng/µl genomic DNA as template. The PCR was performed under the following conditions: 94°C for 4 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 61°Cfor 45 s, extension at 72°C for 45s; a final extension at 72°C for 10 min. PCR products were electrophoretic ally separated on 0.7% agarosegel at a constant 75 V, stained with ethidium bromide and excised for sequencing.

For PCR of **prolactin** gene, the primers were designed for the region of interest. The primer sequences used for the two pairs of oligonucleotide primers were synthesized to amplify two different fragments of 232 and 168 bp in length, based on the available sequences of the bovine PRL gene exon 10 (Gene Bank accession number: FJ901285, SNP location 486bp and 828bp) and using primer-3 software (Table 10). The primer sequences used

for the 232-bp fragment containing a polymorphic ***Nael*** restriction site at the 125 position were: the Forward Primer was CCTATTTTCTGGCCAATGGA and the Reverse Primer was TCTGACTCCCTCTGCTTGGT. The 168-bp fragment containing a polymorphic ***Sm/l*** restriction site at the 123 position were: the Forward Primer was AGATGGAGTGCTGGCTCTGT and the Reverse Primer was GCCTTCTTGGCTGGTTCTTC. The PCR was

Table 10. The lactoferin and prolactin primers sequences.

Gene	Restriction enzyme	Sequences of Forward and Reverse primers respectively	Time and temperatures	Amplification (Pb)	Fragmentation site
Lactoferrin	HinfI	**5'-CACATTACAAGCAGGATCTTTTGCTG-3'** **5'-CTGGCCAATGAGCCCTATATGTGT-3'**	37^0c for 3 hour	1143	462 638
Prolactin	Nael	**5'-CCTATTTTCTGGCCAATGGA-3'** **5'-TCTGACTCCCTCTGCTTGGT-3'**	37°C overnight	232	125
Prolactin	Sm/l	**5'-AGATGGAGTGCTGGCTCTGT-3'** **5'-GCCTTCTTGGCTGGTTCTTC-3'**	55°C overnight	168	123

performed in a volume of 25µl using 1.5 mM MgCl2, 200 µM dNTPs, 5 pmol of each primer, 50-100 ng of genomic DNA and 0.***5*** Units of Taq DNA polymerase (Matrix Scientific Trade CO., Egypt). Amplification reactions were carried with a program as 5 min denaturation at 94°C

followed by 35 cycles of 94°C for 45 s, annealing at 60°C for 45 s and extension at 72°C for 1 min, with a final extension of 10 min at 72°C. PCR products were separated by using electrophoresis unit on 0.3.5% agarose gel at a constant 75 V, stained with ethidium bromide and excised for digestion by specific enzymes.

3-2-3 Digestion of PCR products using restriction enzymes:

Digestion using ***HinfI and Nael*** restriction enzymes was performed in 10µl mixture containing 8.8µl PCR products, 0.2µl *Hinfe* or *Nael* 1µl Buffer. The reaction system was performed under the conditions of 37°C for 3h and the resulting fragments were separated on 3% agarose gel and detected by electrophoreses unit and U.V. unit to detect the number of bands. Software unit was used to detect the genetic molecular weights for each genotype. Digestion by ***Sm/I***, restriction enzyme was performed in a 10µl mixture containing 4.9µl PCR products + 0.1µl ***Sm/l*** + 5µl Buffer. The reaction system was performed under the conditions of 55°C overnight and the product of fragments were separated on 3% agarose gel and detected by

electrophoreses unit and U.V. unit to detect the number of bands for each genotype. All the molecular investigations were carried out in laboratories of molecular genetics, Faculty of Agriculture, Benha University, Egypt.

3-2-4 Statistical analyses:

Hardy Weinberg equilibrium (HWE) within each population was estimated by using POPGENE program **(Oner and Elmaci, 2006)** and applying Chi-square test **(Yeh and Yang, 1999)** Genotypic and allelic frequencies of the PRL and LF genes were estimated by **Campell et al (1996).** Also, POPGENE program was used to calculate the observed (H_O) and expected (H_E) heterozygosity, polymorphism information content (PIC) and fixation index (F_{IS}),

$$Ne = \frac{1}{\sum_{i=1}^{n} P_i^2} \quad ; \quad \mathbf{HO} = \frac{\text{No. of heterozygosity}}{n}$$

$$\mathbf{He = 1 - \sum_{i=1}^{n} P_i^2}$$

Where Pi was the frequency of the i^{th} alelele, pj was the frequency of the j^{th} allele and n was the number of alleles **Peakall and Smouse (2012)**.

$$PIC = 1 - \sum_{i=1}^{n} P^2_i - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P^2_i 2P^2_j$$

Kalinowski et al (2007)

$$F_{IS} = 1 - \frac{H_O}{H_e}$$

The association between the lactoferrin and prolactin genes genotypes from three restriction enzymes and 305 days yields of milk, fat, and protein and age at first calving were analyzed by procedure Mixed, of SAS, 9.1 package **(SAS, 2002)** according to the following linear mixed model or (matrix notations) :

$$\mathbf{y = Xb + Za\ a + Zc\ c + e}$$

Where: y = The vector of lactation observations, X = The incidence matrix relating the fixed effects to y, b = the vector of an overall mean and the fixed effects of year-season of calving, parity and restriction enzymes***(HinfI*** for Lactoferrin gene, ***Nael*** and ***Sm/l*** for prolactin gene; three levels for each enzyme, Za = the incidence matrix relating the direct additive genetic cow effects to y, a = the vector of

the random direct additive genetic effect associated with the incidence matrix Za, Zc = the incidence matrix relating the permanent environmental effects, c = the vector of permanent environmental effects associated with the incidence matrix Zc and e = the vector of random residual effects N, (0, $I\sigma^2 e$) where I is an identity matrix.

4. Results and dissection

4.1 Random regression model

4.1.1 Means and variations:

Number of observations, means and their standard deviations and coefficients of variation (CV) for the traits studied are shown in Table 11.

Table11. Number of observations, means, and standard deviations (SD) and coefficients of variation (CV) for test day (TD) milk, fat and protein yields and age at first calving (AFC).

TD NO	**N. of observations for all milk traits**	TDMY			TDFY			TDPY			AFC			
		Mean kg	SD kg	Cv %	**Mean** Kg	SD kg	Cv %	Mean kg	SD kg	Cv %	**N .of observations**	Mean Mo.	SD Mo.	Cv %
1	809	10.4	5.6	46	0.43	0.2	55	0.32	0.2	51	533	32.2	5.8	15
2	861	11.3	6.6	45	0.43	0.2	50	0.31	0.2	51	566	32.3	5.7	15
3	870	12.9	5.2	44	0.45	0.3	56	0.31	0.2	50	573	32.2	5.8	15
4	866	11.4	5.1	45	0.39	0.2	50	0.29	0.1	47	566	32.2	5.8	15
5	621	11.1	5.8	43	0.40	0.2	51	0.28	0.1	46	384	32.2	4.3	13
6	457	10.4	5.6	44	0.35	0.2	53	0.27	0.1	48	268	32.4	4.4	14
7	331	10.2	5.6	45	0.36	0.2	55	0.26	0.1	48	194	32.2	5.5	14
8	227	10.6	5.6	48	0.33	0.2	54	0.24	0.1	48	123	32.2	5.6	14
9	135	9.2	5.6	50	0.33	0.2	53	0.23	0.1	48	73	32.2	5.8	15
10	59	9.4	5.9	53	0.32	0.2	58	0.25	0.1	57	32	32.3	5.7	15

TD = Test-day, TDMY = Test-day milk yield, TDFY = Test-day fat yield, TDPY = Test-day protein yield and AFC = Age at first calving.

Means and standard deviations of test-day milk yield increased from the first test day (TDMY1) to the peak in the third test day (TDMY3) which was 12.9±5.2 kg. TDM decreased from the fourth and fifth TDMY which were11.1±5.8 kg and fixed from the sixth TDMY to the eighth test day which were 10.6±5.6 kg and in the last two test-day were 9.4±5.9 kg. Means and standard deviations of test day fat yield were fixed from the first (TDFY1) to the seventh test day fat (TDFY7) which was 0.36±0.2 g. TDFY decreased from the eighth TDFY8 to the last TDFY10 which were 0.32 g±0.2 g. (Table3). For protein- test day yield, the means were fixed from the first (TDPY1) to the fifth test day protein (TDPY5) which was 0.28±0.1 g. TDPY decreased from the sixth TDPY6 to the last TDPY10 which were 0.25 ±0.1 g. For age at first calving (AFC) the mean was nearly constant to be 32±5 month. Coefficients of variation ranged from 43% to 53% for TDMY, from 50% to 58% for TDFY, from 46% to 57% for TDPY and from 0.13 to 0.15 for age at first calving.

4-1-2 Variances

Estimates of additive genetic **(V_A)**, permanent environmental **(V_{Pe})**, phenotypic **(V_P)** and residual **(V_E)** variances are presented in Figures (1), (2), (3) and (4) for test-day milk, fat, protein yields and age at first calving(AFC). The additive genetic variance (V_A) estimates at first test day were 4.7 kg, 13.3 g, 5.3 g and 2.8 month then it increased at the fourth test day to be 7.6 kg, 38.7 g, 14.3 g and 3.5 month and decreased thereafter, reaching the lowest value at the tenth test day for milk yield (3.4 kg) and at the tenth test day for fat, protein yield and age at first calving 13 g, 7.7 g and 5.7 month. Similar results have been reported by **(Biassus et al. 2011)** and (**El Faro 2012)** reported that the genetic variance values ranged from 2.3 to 5kg, from 1 to 7.5g and from 1 to 5g for TDMY, TDFY and TDPY, respectively.

Permanent variance estimates (**Vpe**) were ranged from 4.6 to 12.7 kg, 30.9 to 69g, 10.6 to 23.9 g and 2.6 mo to 8.6 mo, for TDMY, TDFY, TDPY and AFC respectively. Similar trends were also reported by **(Jamrozik and Schaeffer 1997)** and (**Jensen et al. 2001)** in cattle. The residual variance estimates **(Ve)** for all traits ranged from 8kg to 11kg, 0 to 8g, 1g to 4g and from 16 to 23mo for milk, fat, protien and AFC traits, respectively.

The phenotypic variance ($\mathbf{V_P}$) estimates for milk, fat, protein and age at first calving traits ranged from 19.2 to 26.3 kg, 45.6 to 113g, 17.9 g to 41.5g and 24.7 mo to 34.8 mo, respectively. The phenotypic variance for the milk test-day decreased until TD6 which it was 19.6 kg then increased until the TD10 which it was 23.9 kg. The fat test-day increased until TD4, it was 113.1 g then it decreased until TD7, it was 99,9g then decreased until TD10, it was 55.2 g. The same trend was obtained for protein trait. For AFC trait $\mathbf{V_A}$ decreased until TD6, it was 21.27 mo then it increased until TD7 to be 24.12 mo, then increased until TD10 which reached 24.7 mo. These results were agreeing with **(Biassus et al. 2011)** who showed that the values of phenotypic variances ranged from 20 to 27 kg, 25 to 35 g and 20 to 35 g for TDMY,TDFY and TDPY respectively i.e. **(El Faro et al 2012)** revealed that the phenotypic, permanent and genetic variances ranged from 2 to 7 kg, 1.8 to 5 and 0.2 to 1.2 kg for TDMY. These results were nearly from **(cobuci et al 2005)** permanent and genetic variances values ranged from 19 to 29 kg and 8 to 20 kg for TDMY respectively.

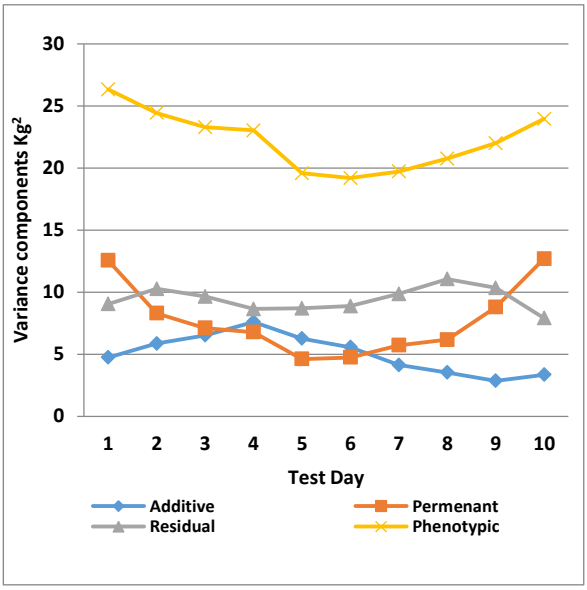


Figure 1. Estimates of additive genetic (v_A), permanent environmental (V_{Pe}), residual variances (V_E) and phenotypic variances (V_P) for test day milk yield (*kg*).

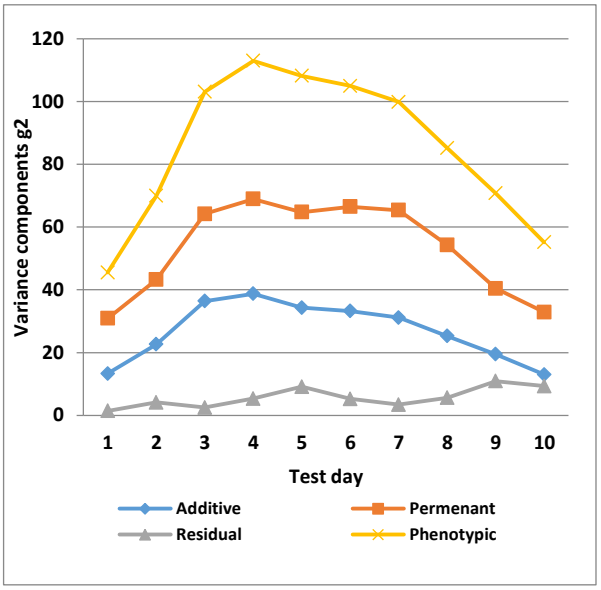


Figure 2. Estimates of additive genetic (v_A), permanent environmental (V_{Pe}), residual variances (V_E) and phenotypic variances (V_P) for test day fat yield (*g*).

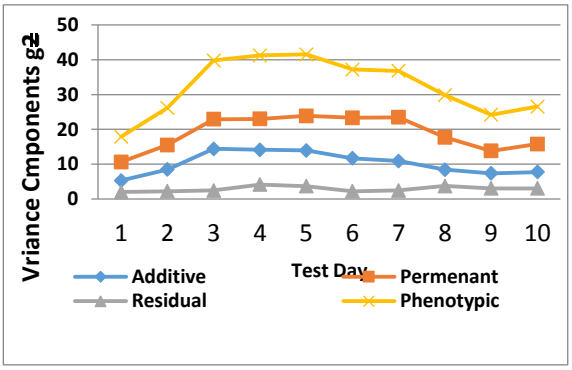


Figure 3. Estimates of additive genetic (V_A), permanent environmental (V_{Pe}), residual variances (V_E) and phenotypic variances (V_P) for test day protein yield (*g*).

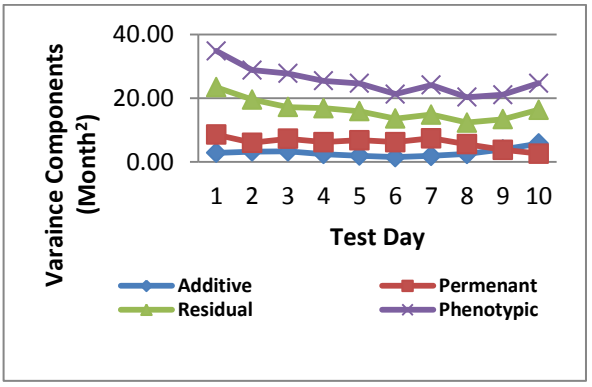


Figure 4. Estimates of additive genetic (V_A), permanent environmental (V_{Pe}), residual variances (V_E) and phenotypic variances (V_P) for age at first calving (AFC).

4-1-3 Heritability values

Heritability estimates for TDMY at selected TD are shown graphically in (Figure 5). Estimates were low at the beginning of the test day (0.17), and gradually increased reaching the highest value at the fourth test day (0.33) and decreased gradually until reached the lowest value at the tenth test day of lactation (0.14). The heritability estimates for fat and protein yields showed the same trend where the estimates were 0.29 and 0.3 on the first test day and reached 0.35 and 0.36 at the fourth test day, and finally decreased at the ninth test day in fat (0.24) and at the tenth test day in protein yield (0.29). Similar trends were reported by Rosati and **(Van Vleck 2002)** for milking cows. Heritability estimates for AFC were low at the beginning of the test day (0.07), and gradually increased, reaching the highest value at the third test day (0.12) and decreased in the sixth test day (0.07), and then it increased until the tenth test day which it was 0.24.

In general, heritability estimates for traits had wide ranges and tended to increase toward the edges of the defined lactation trajectory. Most heritability estimates obtained by RRM were high at the edges as stated by **(Jamrozik and Schaeffer, 1997)** in dairy cows. Difficulties in the model in getting acceptable variances at the extremes of the lactation can be explained, in part, by the biological

processes that occur at the beginning of lactation and the smaller number of records at the end. (**Jamrozik and Schaeffer 1997)** and **(El- Saied 2004)** pointed out that these parametric functions tend to overestimate the genetic variances and underestimate the genetic correlations among milk yield at the beginning and the end of lactations.

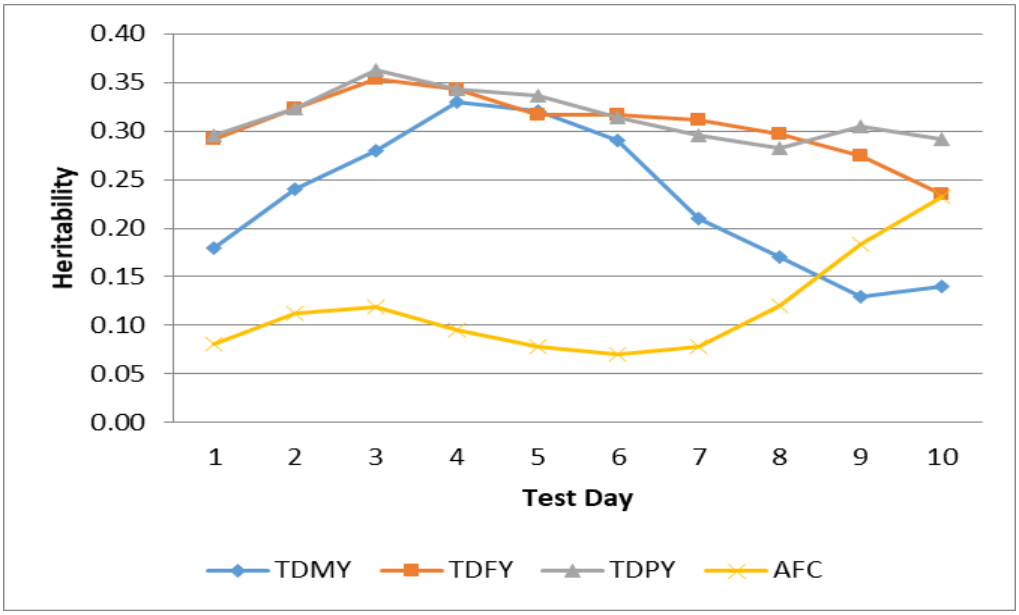


Figure5. Estimates of heritability of test-day milk (***TDMY***), fat (***TDFY***) and protein (***TDPY***) yields and age at first calving (***AFC***)

4.1.4 Predicted breeding value (PBV)

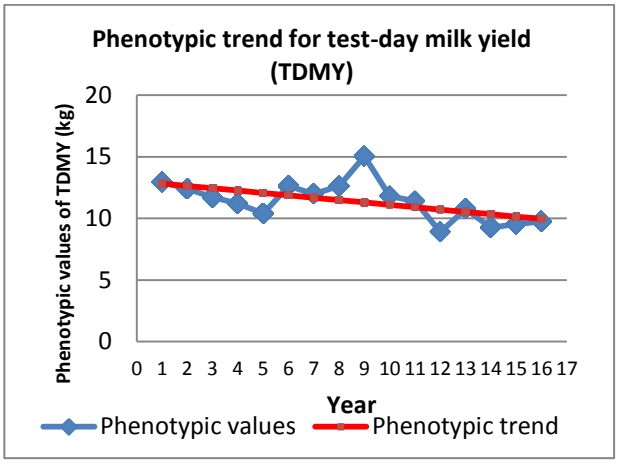
The minimum and maximum predicted breeding values (PBV) and their accuracies for milk, fat and protein yields and age at first calving are given in **Table 12**. The PBV for milk, fat and protein yields and age at first calving ranged from -1297 to 1896 kg, -5.9 to 6.2 *k*g and -7.1 to 7.5*k*g and -2.6 to 2.4 month, respectively. Using TD animal model methodology in cattle, **(Zutere, 2008)** found that the estimated breeding values for milk, fat and protein ranged from -1014 to 1966 kg, from -40.75 to 93.59 kg and from -37.33 to 59.86 kg, respectively. The accuracies ($^{rA\wedge}$) of minimum and maximum estimates of PBV were high in all traits (Table 4). This may be due to that estimate of heritability were highly associated with more available pedigree information for all individuals **(Korhonen, 1996; Korsgaard *et al.*, 2002).**

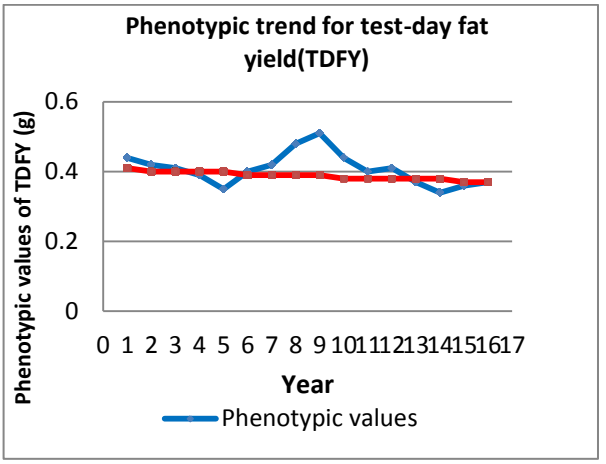
Table12. Minimum, maximum and ranges of predicted breeding values (PBV), predicted error variance (PEV) and accuracy of prediction ($^{rA\hat{}}$) for TD milk, fat and protein yields in Friesian cattle raise in Egypt.

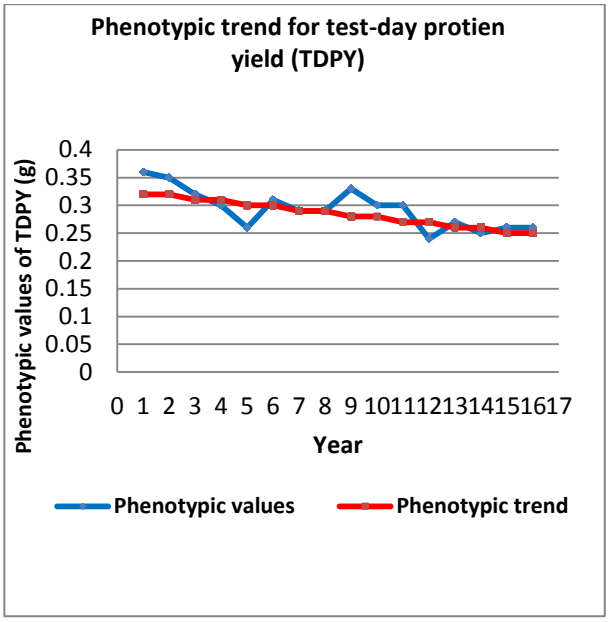
Trait	Minimum			Maximum			Range in PBV
	PBV	PEV	$A\hat{}$	PBV	PEV	$rA\hat{}$	
TDMY, kg	-1296.5	324.9	0.99	1895.6	634.0	1.00	3192.1
Fat yield, kg	-5.9	4.1	0.89	6.2	5.2	0.92	12.1
Protein yield, kg	-7.1	4.6	0.91	7.5	5.8	0.93	14.6
Age at first calving, mo.	-2.6	1.2	0.65	2.4	1.7	0.78	5.0

Phenotypic trend:

Phenotypic trend for TDMY, TDFY and TDPY and age at first calving are shown in Figure 6. Phenotypic trend for TDMY, TDFY, TDPY and AFC were decreased from TD1 to TD10. The decrease in phenotypic trend in all traits may be attributed to low nutritional level applied and management practices in different herds.







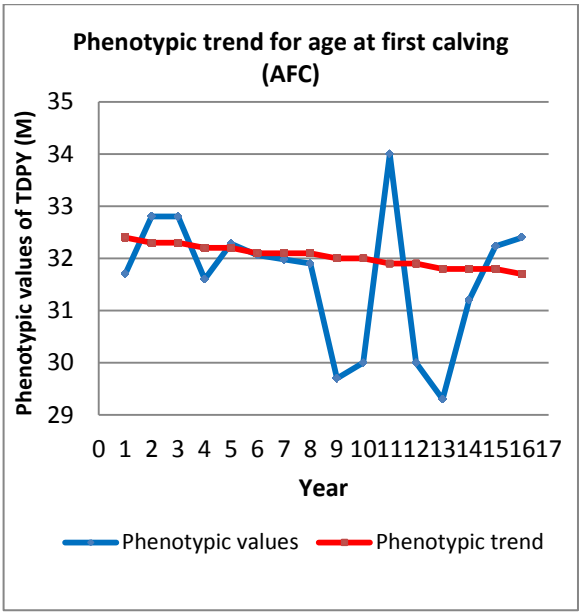
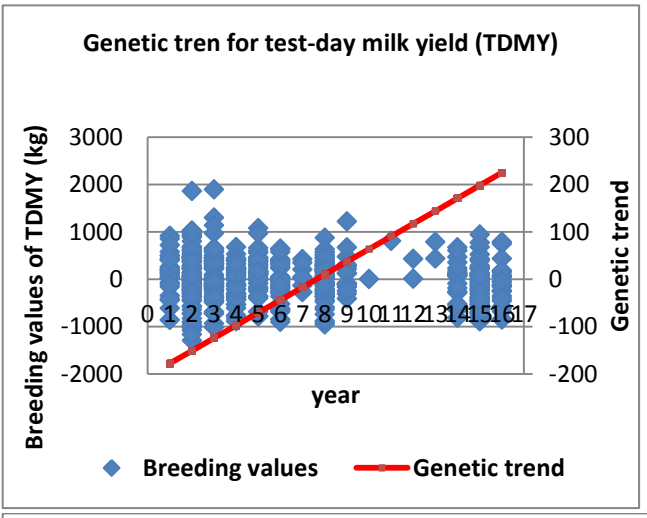
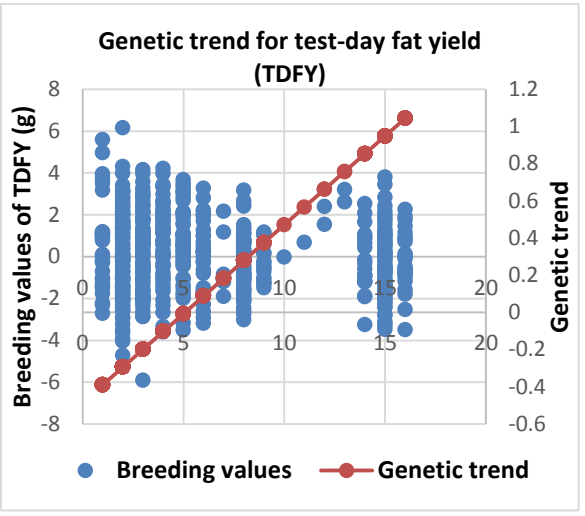


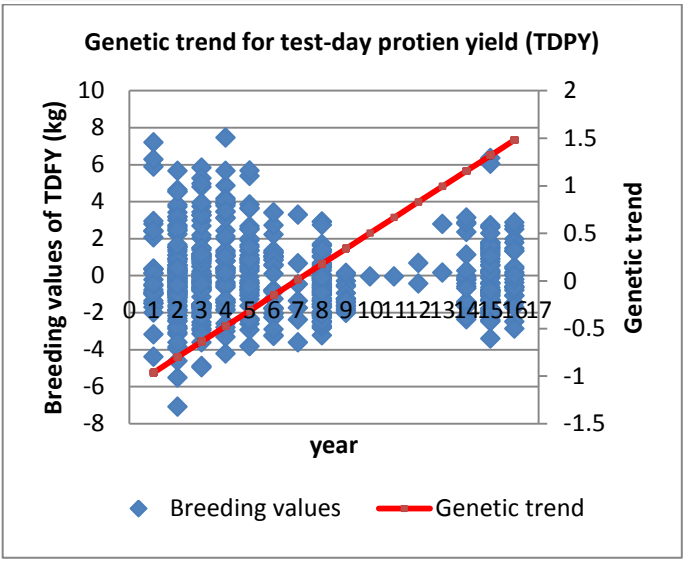
Figure6. Phenotypic trend for test-day milk, fat, protein yields and age at first calving in Friesian cattle raised in Egypt.

Genetic trend:

Genetic trend for TDMY, TDFY, TDPY and age at first calving are shown in Figure7, the values of genetic trend for TDMY, TDY, TDPY, and AFC traits increased from TD1 to TD10. These results indicated that improvement program of selection was practiced in the farms. Similar trends were also reported by some investigators e.g. **(Yaeghoobi *et al* 2011)** and (**Katok and Yanar 2012)**, (**Muller and Botha, 2003)** for TDMY, **(Jamrozik** and **Schaeffer, 1997)**.







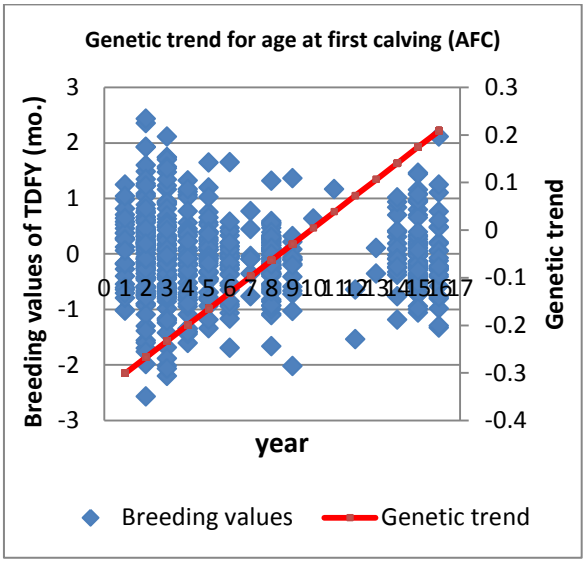


Figure7. Genetic trend for test-day milk, fat, protein yields and age at first calving in Friesian cattle raise in Egypt.

4.2. Lactoferrin and prolactin genes

Results obtained in the present study are divided into three sections according to the three restriction enzymes used (***HinfI*** for lactoferrin gene and ***Nael*** and ***Sm/l*** for prolactin gene).

4-2-1 Molecular analysis for using *HinfI* restriction enzyme genotypes polymorphism of lactoferrin gene:

4-2-1-1 Molecular weights:

The PCR amplified DNA fragment of 800 bp was digested with ***HinfI*** restriction enzyme and the various variants observed are shown in Fig 1. Three genotypes AA, AB and BB were observed. ***HinfI*** restriction analysis of the PCR product yielded banding pattern corresponding to three different genotypes viz., AA genotype with one band (800 bp), AB genotypes with three bands (800, 650 and 150 bps) and BB genotype with two bands (650 and 150 bps). These results agree with **Zhao et.al (2009)** who showed that two allele genes and three genotypes for ***HinfI*** restriction enzyme in 120 dairy cows where allele gene with 638bp was defined as A, whilst allele gene with 462bp as B and

the other related genotypes were defined as A/A, A/B and B/B, respectively.

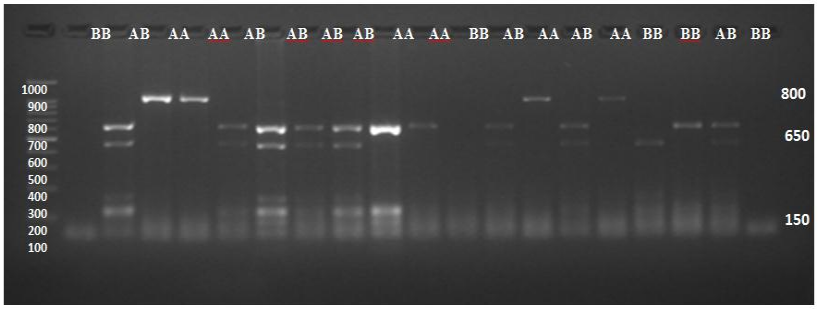


Figure8. The representive restriction pattern at Lactoferrin / ***HinfI*** locus on 3.0% agarose gel.

4-2-1-2 Genotypic and allelic frequencies:

Chi square values for ***HinfI*** genotypes of Friesian in El karada, Sakha and local cattle in Elserw herd were 6.9,and 5.1, respectively 0< 0.05; (Table 3). The genotypes frequencies from ***HinfI*** restriction enzyme of AA, AB and BB were 0.15, 0.48 and 0.37 for Friesian cows in Elkarada herd, respectively (Table 3), i.e. AB genotype was the highest frequency value. In Sakha Friesian herd, frequencies of genotypes were 0.26, 0.50 and 0.24, respectively, i.e. AB genotype was also the highest frequency. In Elserw local herd, frequencies of AA, AB, and BB genotypes were 0.05,

0.33 and 0.62, respectively with BB genotype was the highest frequency value. Accordingly, AB genotype was the highest frequency in Frisian cattle but BB genotype was the highest in local cattle. These results agree with **Chopra et al (2013)** who revealed that LF gene had three different genotypes namely AA, AB and BB in Karan Fries cattle and these genotypes having frequencies of 0.18, 0.77 and 0.05 respectively and the frequency of A and B allele was found to be 0.57 and 0.43 respectively, so the AB genotypic was the highest frequency. **Maksymiec et al (2006)** found that two alleles of A and B for lactoferrin gene polymorphism was obtained with PCR-RFLP method using *EcoRI* enzyme in the Holstein-Friesian breed; with the allelic frequencies of 0.68 and 0.32, respectively and the genotypes frequencies equal to be 0.38, 0.02 and 0.6 for AA, BB and AB genotypes, respectively.

Table 12.The observed and expected number of cows in *HinfI* genotypes polymorphism, genotypic frequency, gene frequency and Chi square values ($\chi 2$) in the three herds of cattle.

Herd	**N of Animals**	Observed number			Expected number			Genotypic frequency			Allele frequency		$\chi 2$ value
		AA	AB	BB	AA	AB	BB	AA	AB	BB	A	B	
Friesian, El Karada	80	18	27	35	12	38	29	0.15	0.48	0.37	0.39	0.61	6.9*
Friesian, Sakha	62	17	29	16	16	1	15	0.26	0.50	0.24	0.51	0.49	0.3NS
Local, Elserw	38	4	8	26	2	13	24	0.05	0.33	0.62	0.21	0.79	5. 1*

NS= Non-significant, *=P<0.05

4-2-1-3 Heterozygosity and inbreeding:

The observed (H_O) and expected (H_E) heterozygosity, the polymorphic information content (PIC) and the fixation index (F_{IS}) estimated by POPGENE program for the SNP of ***HinfI*** polymorphism of lactoferrin gene in the three populations are shown in **Table 13**. The value of H_O was of medium rate 0.34 in Elkarada Friesian herd and it was in Hardy-wienberg equilibrium (**Table 13**), while the value was low in Sakha Friesian herd (0.14) and Elserw local herd (0.05). Also the value of H_E was medium value (0.44) in the three herds. These results were nearly similar to **Akyuz et**

al. (2012) who found that H_O values were 0.186, 0.038 and 0.33 in East Anatolian Red cattle population**,** Holstein, and Jersey cattle, respectively. **Brym et al. (2005)** and **Dybus et al (2005)** found that H_O values were 0.28 and 0.43 in Black and White cattle and Jersey cows, respectively.

The values of polymorphism information content **(PIC)** were similar (0.36 and 0.37) in Elkarada and Sakha Friesian herds, but in Elserw local herd PIC value was 0.26 where it was lower than the Friesian herds. The inbreeding value **(F_{IS})** were moderate(0.29 in Elkarada herd and 0.37 in local cattle). **Akyuz et al (2012)** found that F_{IS} value was 0.072 in East Anatolian Red cattle population, homozygosity was at a low rate.

Table 13, The observed (H_O) and expected (H_E) heterozygosity, the polymorphism information content (PIC) and the inbreeding coefficients (F_{IS}) for ***HinfI*** polymorphisms in the three populations of Elkarada, Sakha Friesian herds and Elserw local herd.

Population	No.of records	H_O± SE	H_E± SE	PIC	F_{IS}
Friesian, Elkarada	160	0.34±0.03	0.44±0.03	0.36	0.29
Friesian, Sakha	76	0.14±0.04	0.44±0.04	0.37	0.06
Local cattle, Elserw	360	0.05±0.02	0.44±0.02	0.27	0.37

4-2-1-4 Differences among milk traits in different genotypes of lactoferrin gene:

The least square means given in Table14 have shown the associations between different genotypes of lactoferrin gene and milk yield and compositions and AFC.

In El karada Friesian herd, the least square means and their standard errors for milk yield were 3171 ± 285 kg, 3125 ±256 kg and 3057 ±276 kg in genotypes of AA, AB and BB, respectively; the differences were not significant among the three genotypes. In Sakha Friesian herd the least square means and their standard errors for milk yield trait were 2734 ± 229 kg, 3055 ±302 kg and 2792 ±318 kg for the three genotypes AA, AB and BB, respectively. The differences were significant among the three genotypes and AB genotype was the highest genotype in milk yield and selection could be practiced for this genotype in Egypt. These results were disagreeing with **Zabolewicz et al (2014)** who revealed that the differences in 305 day milk yield of 241 cows in different lactoferrin genotypes were not significant, accounting for 8928 ± 1309 kg for AA genotype, 8783 ± 2297 kg for AC genotype and 8935 ± 2133 kg for CC genotype.

The differences in fat yield trait among the three genotypes in El karada Friesian herd were significant, the least square means and their standard errors for fat yield trait were 64.8± 10.8 kg, 58.6 ±9.2 kg and 49.9 ± 9.8 kg in genotypes of AA, AB and BB, respectively (Table 14). The AA was the highest genotype in Friesian cattle so we can select for the fat trait towards AA genotype. These results were agreeing with **Dolatabady and Shafaeipour (2014)** who stated that the bovine lactoferrin gene is a major gene that influences the milk fat percentage in Holstein cattle. Also, **Zielak-Steciwko et al (2014)** showed that positive interaction between AA genotype and fat content. On the contrary, **Nanaei et al (2016)** revealed that the B allele was associated with higher milk fat percentage ($P<0.05$) and the genotype AB had shown significantly higher fat milk percentage in comparison with AA genotype for lactoferrin gene, In Sakha Frisian herd the differences in fat yield trait among the three genotypes were not significant, the least square means and their standard errors for were 60.8± 12 kg, 67.4 ±11.5 kg and 57 ± 11.7 kg in genotypes of AA, AB and BB, respectively (Table 14).

The differences in protein yield trait among the three genotypes and were significant since the least square means and their standard errors were 38.4 ±7.6 , 39.6 ±6.5 and 32.8 ±6.9 kg in Elkarada Frisian herd and 48.2±8.1,54.3±7.6 and 40.5±7.9 kg in Sakha Frisian herd genotypes of AA, AB and BB, respectively (Table 14). These results indicated that AB was the highest genotype for protein yield and we can select the hetero genotype directs in selection improvement programs for protein yield. The results were agreeing with **Kaminski et al (2006)** who found that SNP at position 32 (G/C) genotype of bovine LF gene was significantly associated with milk protein content. **Nanaei et al (2016)** revealed that no significant association (P > 0.1) was evidenced between the LF polymorphism and 305-day milk yield and protein percentage.

In El karada Friesian herd, the least square means and their standard errors for age at first calving (AFC) were 30.4 ± 1.5, 33.7 ± 1.3 and 32.2 ± 1.5 month in genotypes of AA, AB and BB, respectively**.** and the differences among the three genotypes were significant. But in Sakha Friesian herd, the differences were not significant and the least square means and their standard errors were 31.8 ± 1.3, 32.3

± 1.2 and 33.4 ± 1.4 month in genotypes of AA, AB and BB, respectively**,** The best AA genotype was the best in AFC so we can select towards it in Friesian cattle in Egypt.

Tab**le14.** Least square means and their standard errors for milk, fat and protein yields and age at first calving in different patterns of ***HinfI*** restriction enzyme for Friesian and local cattle.

Trait	Genotype	El karada Friesian herd		Sakha Friesian herd		Local	
		No of records	Mean ±SE	No of records	Mean ± SE	No of records	Mean ± SE
305-day milk, kg	AA	58	3171± 258[a]	41	2734± 229[b]	2	126± 107[a]
	AB	104	3125± 256[a]	89	3054± 302[a]	11	193± 78[a]
	BB	68	3057± 276[a]	64	2791± 318[b]	53	307± 56[a]
305- day fat, kg	AA	58	64.8± 10.8[a]	41	60.8±12[a]	2	5.6± 4.3[a]
	AB	104	58.6±9.2[ab]	89	67.4± 11.5[a]	11	7.6± 3.1[a]
	BB	68	49.9± 9.8[b]	64	57±11.7[a]	53	12.1±2.3[a]
305-day protein, kg	AA	58	38.4±7.6[a]	41	48.2± 8.1[a]	2	4.5± 3.2[a]
	AB	104	39.6± 6.5[a]	89	54.3± 7.6[a]	11	6.9± 2.4[a]
	BB	68	32.8± 6.9[b]	64	40.5±7.9[b]	53	9.2±1.7[a]
AFC, Month	AA	53	30.4±1.5[b]	39	31.8± 1.3[a]	0	0
	AB	98	33.7±1.3[a]	88	32.3± 1.2[a]	9	35.2± 3.0[b]
	BB	63	32.2± 1.5[b]	53	33.4±1.4[a]	49	38.6± 2.1[a]

In the Elserw local herd, the least square means for milk yield in the genotypes AA, AB and BB were 126±107, 193±79 and 307±56 kg, respectively and the differences among the three genotypes in milk yield were not significant (Table 14). BB was the highest genotype in milk yield trait. Least square means for fat yield were 5.6 ±4.3, 7.6 ±3.1 and 12.1 ±2.3 kg for the three genotypes AA, AB

and BB, respectively where the differences were not significant. These results disagree with the results obtained for Friesian cattle in which AB genotype was the highest in milk and fat traits. For protein yield trait, the least square means were 4.5 ±3.2, 6.9 ±2.4 and 9.2 ±1.7 kg for the genotypes AA, AB and BB, respectively where the differences among the three genotypes were not significant and these results were disagreeing with the results obtained for Friesian cattle in which there were significant differences among the three genotypes in protein yield (Table 14). For age at first calving, the least square means and their standard errors were 35.2 ±3.4 and 38.6 ±2.5 month for the two genotypes AB and BB, respectively and the difference between the two genotypes was significant, i.e. AB considered as the best genotype in AFC, so that the cows of AB genotype will start milking earlier than the BB cows. Accordingly we must select towards AB genotype to improve AFC trait in local cattle.

4-2-2 Molecular analysis for genotypes polymorphism of Prolactin gene using *Nael* restriction enzyme

4-2-2-1 Molecular weights:

The PCR amplified DNA fragment of 230 bp was digested with ***Nael*** restriction enzyme and the various observed genotypes CC, CD and DD are shown in Fig 9. Also, ***Nael*** restriction analysis of the PCR product yielded banding pattern corresponding to three different genotypes, viz. CC genotype with one band (230 bp), CD genotype with three bands (230, 150 and 80 bps) and BB genotype with two bands (150 and 80 bps).

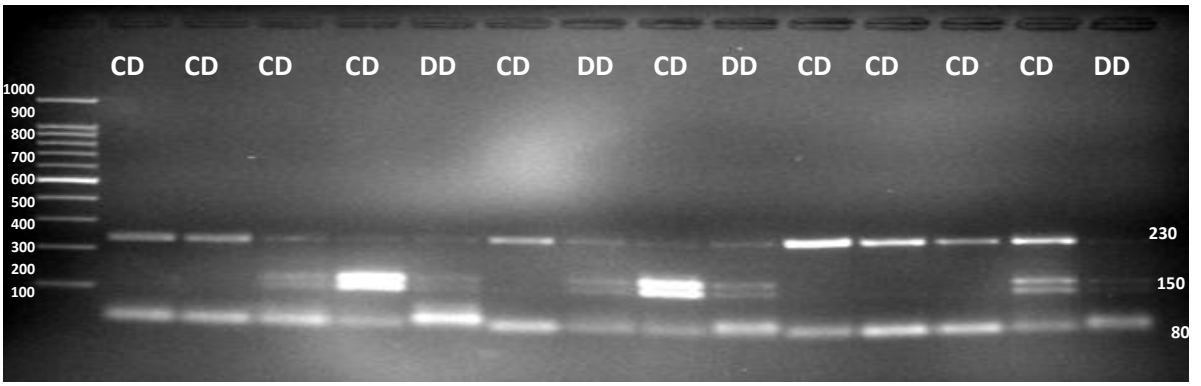


Figure 9: Representive restriction pattern at prolactin/ ***Nael*** locus on 3.0% agarose gel.

4-2-2-2 Genotypic and allelic frequencies:

Chi square values ($\chi 2$) for populations in Elkarada, Sakha and Elserw herds were non-significant, being 0.4, 1.4 and 1.1, respectively (Table 15). Genotypes from ***Nael*** restriction enzyme were of two patterns, CD and DD in Elkarada Friesian herd where the frequencies of the two genotypes were 0.13 and 0.87, respectively; indicating that DD genotype was the highest genotypic frequency. In Sakha Friesian herd, there were three genotypes and their frequencies were 0.02, 0.22 and 0.76 for CC, CD and DD genotypes, respectively, while the frequencies in Elserw local herd were 0.05, 0.35 and 0.6, respectively. These results refer that DD genotype was the highest genotype in the frequency value in both populations. **Miceikiene et al (2006)** revealed that the frequencies of prolactin allele in Lithuanian cattle breeds are shown three genotypes (AA, AB and BB) where the prolactin gene AA genotype was the most frequent in all investigated populations (0.62–0.94), followed by AB (0.06–0.37) and BB genotype was the least frequent (0.00–0.08). The same author revealed that BB genotype was not found in Lithuanian White Black and Lithuanian Light Grey cattle breeds and 75% of all the

investigated populations had AA genotype, 18% had AB genotype and 7% had BB genotype, some of the genes are proposed as potential candidates associated with milk performance traits in Marker-Assisted Selection of dairy cattle.

Table 15. The observed and expected number of cows in ***Nael*** polymorphism genotypes, genotypic frequency, gene frequency and Chi-square values ($\chi 2$) in the three herds in cattle**.**

Herd	N of animals	Observed numbers			Expected numbers			Genotypic frequency			Allele frequency		$\chi 2$ Value
		CC	CD	DD	CC	CD	DD	CC	CD	DD	C	D	
Friesian, El Karada	80	0	11	69	0.38	10.2	69.4	0.0	0.13	0.87	0.07	0.93	0.4^{NS}
Friesian, Sakha	62	0	16	46	1.03	13.9	47.0	0.02	0.22	0.76	0.13	0.87	1.4^{NS}
Local, Elserw	38	3	11	24	1.9	13.2	22.9	0.05	0.35	0.60	0.22	0.78	1.1^{NS}

NS = Non-significant

4-2-2-3 Heterozygosity and inbreeding:

The observed (H_O) and expected (H_E) heterozygosities, the polymorphic information content (PIC) and the inbreeding coefficient (F_{IS}) estimated by for SNP of the ***Nael*** polymorphism of prolactin gene in the three populations were shown in **Table 16**. The value of heterozygosity H_O for prolactin-***Nael*** polymorphism was

found to be medium in Elkarada (0.47) and Sakha (0.26) herd, but in Elserw local herd it was low (0.18). The value of H_E was 0.23 in the three populations. These results were disagreeing with the values of H_O obtained by **Akyuz et al.,(2012)** of East Anatolian Red cattle population (0.186). While **Brym et al. (2005)** found that H_O values were 0.038 in Holstein cattle and 0.33 in Jersey cattle. **Dybus et al. (2005)** found that H_O values were 0.28 in Black and White and 0.43 in Jersey cattle.

The values of **PIC** were 0.12 and 0.20 in Friesian herds in Elkarada and Sakha but it was the highest in Elserw local herd (0.28). The inbreeding value (F_{IS}) was -0.07 in Elkarada herd and -0.15 in Sakha herd but in local cattle it was the lowest value which was -0.17. These results were disagreeing with **Akyuz et al. (2012)** who found that F_{IS} value was 0.072 in East Anatolian Red cattle population.

Table 16. The observed (H_O) and expected (H_E) heterozygosity, the polymorphism information contents (PIC) and the inbreeding values (F_{IS}) for ***Nael*** polymorphisms in the three populations of Friesian and local cattle in Elkarada, Sakha and Elserw herds.

Population	No of records	H_O± SE	H_E± SE	PIC	F_{IS}
Friesian, Elkarada	160	0.47±0.03	0.23±0.03	0.12	-0.07
Friesian, Sakha	76	0.26±0.04	0.23±0.04	0.20	-0.15
Local cattle, Elserw	360	0.18±0.02	0.23±0.02	0.28	0.17

4-2-2-4 Differences among milk traits in different genotypes of prolactin gene:-

The least square mean given in **(Table 17)** have shown the associations between different genotypes of lactoferrin gene and milk yield and compositions and AFC traits**.** The least square means and their standard errors for 305 days milk yield trait for CD and DD genotypes were 3214±260 and 3023±263kg in **El karada** Friesian herd and 3085±372 and 2635±271kg **in Sakha** Friesian herd, respectively **(Table 17)**. The differences between the two genotypes detected were significant for milk yield trait in Sakha herd, not significant in Elkarada herd. These results revealed that the CD genotype was the highest genotype in Friesian cattle for milk yield trait; agreed with the results of

Othman et al (2011) who revealed that homo genotype cows were the least in milk yield relative to hetero genotypes cows, the BB genotype was the lowest in daily milk yield. The means for fat yield in Elkarada Friesian herd for the CD and DD genotypes were 58.5 ±9.5 and 57.5 ±9.4 kg, respectively but in Sakha Friesian herd were 63.6 ±14.9 and 59.9 ±8.7, respectively. (**Table 17**). The differences between the CD and DD genotypes were non-significant. **Othman et al (2011)** revealed that BB genotype was the highest in fat yield. The means for protein yield for CD and DD genotypes in El karada herd were 35.5±6.7 and 38.3±6.7 kg, respectively; i.e. the differences between the two genotypes in protein yield were not significant. In Sakha herd, the means were 50.2 ±9.8 and 45.1±6kg, respectively and the differences between the two genotypes were significant in protein yield **(Table 17)**. So we can select towards CD genotype in Sakha Friesian herd. **Othman et al (2011)** found that cows with the BB genotype recorded the highest fat compared to the other cows genotypes. Also, **Feng et al (2006)** found that cows with genotype BB had significantly the highest protein yield than that of AA genotype, while cows with AA genotype had

significant higher protein percentage than the cows of BB and AB genotypes.

For AFC, the means for CD and DD genotypes in Elkarada Friesian herd were 31.8 ±1.3 and 32.4 ±1.3month, but in Sakha Friesian herd were 31.1±1.2 and 32.7± 1.4 month, respectively **(Table 17)**. The differences between the two genotypes, DD and CD were not significant in AFC in Friesian cattle.

Table17. Least square means and their standard errors for milk, fat and protein yields and age at first calving in different genotypes of ***Nael*** restriction enzyme for Sakha and El karada herds Friesian and local cattle

Trait	Genoty	El karada Friesian herd		Sakha Friesian herd		Local	
		No of records	Mean ±SE	No of records	Mean ± SE	No of records	Mean ± SE
305-day milk, kg	CC	0	0	0	0	0	231±118 [a]
	CD	77	3213 ± 259 [a]	25	3085±371 [a]	102	226± 71 [a]
	DD	153	3022± 262 [b]	169	2635±270 [a]	322	298±53 [a]
305- day fat, kg	CC	0	0	0	0	0	9.8± 4.7 [a]
	CD	77	58.5 ± 9.5 [a]	25	63.6± 14.9 [a]	102	8.9± 2.9 [a]
	DD	153	57± 9.4 [a]	169	59.9±8.7 [a]	322	11.8±2.1 [a]
305-day protein, kg	CC	0	0	0	0	0	6.9± 3.6 [a]
	CD	77	35.5± 6.7 [a]	25	50.2±9.8 [a]	102	6.9±2.1 [a]
	DD	153	38.3± 6.7 [a]	169	45.1± 6 [b]	322	8.9± 1.6 [a]
AFC, Month	CC	0	0	0	0	0	0
	CD	76	31.8± 1.3 [a]	24	31.1± 1.2 [a]	101	33.0± 2.2 [b]
	DD	138	32.4± 1.3 [a]	156	32.7±1.4 [a]	295	39.4± 0.8 [a]

In Elserw local herd, the means of CC, CD and DD genotypes were 231±119 , 227±72 and 298±53 kg for milk

yield, 9.8±4.7, .89±2.9 and 11.8±2.1 kg for fat yield, 6.9±3.6, 6.9±2.1 and 8.9±1.6 kg for protein yield, respectively (Table 16). Means of age at first calving were 33 and 39.4 month for the two genotypes CD and DD, respectively. Therefore the differences among the three genotypes for milk, fat and protein yield were non-significant, significant between the two genotypes in AFC and CD genotype was the best one (Table17).

4-2-3-1 Molecular analysis for genotypes polymorphism of Prolactin gene using *Sm/l* restriction enzyme:

The PCR amplified DNA fragment of 180 bp was digested with ***Sm/l*** restriction enzyme and the three observed GG, GT and TT genotypes are shown in Fig10. In the Friesian cattle, ***Sm/l*** restriction analysis yielded banding pattern corresponding to three different genotypes viz. GG genotype with one band (180 bp), GT genotype with three bands (180, 123 and 57 bps) and TT genotype with two bands (123 and 57 bp).These results were agreeing with **Deepika et al (2014)** who observed three genotypes of GG, GT and TT.

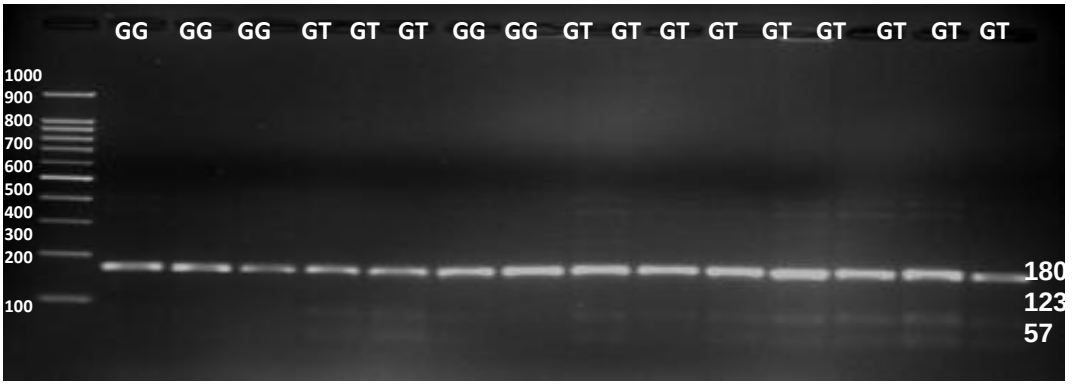


Figure 10: Representive restriction pattern at prolactin- **Sm/l** locus on 3.0% agarose gel.

4-2-3-2 Genotypic and allelic frequencies:

Chi square values (χ^2) for ***Sm/l*** gene in Elkarada, Sakha and Elserw herds were 53.3, 23.5 and 33.6, respectively (Table 18). Frequencies of genotypes from restriction enzyme of ***Sm/l*** gene were 0.03, 0.27 and 0.70 for GG, GT and TT in Elkarada Friesian herd, 0.13, 0.46 and 0.40 in Sakha Friesian herd, and 0.11, 0.44 and 0.50 in Elserw local herd, respectively; the differences milk yield trait among the three genotypes in Friesian herds were significant. **Othman et al (2011)** showed that allele frequencies of A and G were 0.113 and 0.887 in Black-and-White cows and 0.706 and 0.294 in Jersey cows, respectively

Table 18.The observed and expected number of cows, genotypes in **Sm/l** polymorphism, genotype frequency, gene frequency and their Chi-square values ($\chi 2$) in the three herds of cattle.

Herd	No of animals	Observed numbers			Expected numbers			Genotypic frequency			Allele frequency		$\chi 2$ value
		GG	GT	TT	GG	GT	TT	GG	GT	TT	G	T	
Friesian, El Karada	80	11	4	65	2.1	21.8	56.11	0.03	0.27	0.70	0.16	0.84	53.3**
Friesian, Sakha	62	17	11	34	8.2	28.7	25.17	0.13	0.46	0.41	0.36	0.64	23.6**
Local, Elserw	38	12	1	25	4.1	16.7	17.11	0.11	0.44	0.50	0.33	0.67	33.6**

**=P<0.01

4-2-3-3 Heterozygosity and inbreeding:

The observed (H_O) and expected (H_E) heterozygosities, the polymorphic information content (PIC) and the inbreeding values (F_{IS}) estimated for SNP of the ***Sm/l*** of prolactin gene in the three herds are shown in **Table19**.

In prolactin Sm/l polymorphism, the values of HO were medium in Elkarada Friesian herd (0.36) and Sakha herd (0.21), but in Elserw local herd it was low (0.09). The value of HE was 0.39 in the three herds. These results were disagreeing with Akyuz et al. (2012) who reported that the values of observed heterozygosity (HO) were 0.186, 0.038

and 0.33 in East Anatolian Red cattle, Holstein and Jersey cattle, respectively. Brym et al. (2005) and Dybus et al. (2005) found that H_O value was 0.28 in Black and White cattle and 0.43 in Jersey cattle.

The values of polymorphism information content (PIC) were moderate in values of 0.23 and 0.36 in both herds of Friesian cattle, but it was 0.34; in Elserw local herd the highest value was in Sakha herd. The value of F_{IS} was (0.82) in Friesian of Elkarada herd and it was 0.62 in Sakha herd, but it was the highest value (0.94) in the local cattle. These results were disagreeing with **Akyuz et al (2012)** who found that F_{IS} value was 0.072 in EAR cattle population. According to this value, despite the small number of the East Anatolian Red cattle population East Anatolian Red, the homozygosity was at a low rate.

Table 19: The observed (H_O) and expected (H_E) heterozygosity, the polymorphism information content (PIC) and the fixation index (F_{IS}) for the ***Sm/l*** polymorphisms populations in Friesian and local cattle in Elkarada, Sakha and Elserw herds.

Population	No.of records	H_O± SE	H_E± SE	PIC	F_{IS}
Friesian, Elkarada	160	0.36±0.03	0.39±0.03	0.23	0.82
Friesian, Sakha	76	0.21±0.04	0.39±0.04	0.36	0.62
Loclal cattle, Elserw	360	0.09±0.02	0.39±0.02	0.34	0.94

2-4-3-4 Differences among milk traits in different genotypes of prolactin gene:

The least square means given have shown in **Table 20** the associations among different genotypes of prolactin gene and milk yield and composition and AFC traits. In El karada Friesian herd, the means of milk yield in GG, GT and TT genotypes were 3152 ± 295, 3122 ± 308 and 3080 ±238 kg but in Sakha Friesian herd the means were 2918 ±306, 2996 ±472 and 2667 ±265kg respectively. There were not significant differences among the three genotypes in the milk yield. **Othman et al (2011)** stated that the genotype AG showed the highest milk yield, while the genotype GG showed the highest in fat content in Black and White cows. For ***PRL-Rsa*1** polymorphism, **Andrzej et al (2005)** showed

that *AA* genotype produced less 305-day milk and fat than *AB* and *BB* genotypes in Jersey cows. **Chung et al (1996)** reported that Holstein-Friesian cows with *AA* genotype produced milk with higher fat content than *BB* genotypes.

For fat yield, the means in Elkarada Friesian herd were 68 ±11, 51 ±11 and 55 ±8 kg for the three genotypes GG, GT and TT, respectively and the differences among the three genotypes in the fat yield were significant and GG genotype was the best one, while the corresponding means in Sakha herd for the three genotypes were 79 ±12, 53 ±19 and 53 ±9kg. The differences among the three genotypes in this herd were not significant. In Elkarada Friesian herd, the means for protein yield were 45 ±8, 28 ±8 and 38 ±6 kg for the three genotypes GG, GT and TT, respectively and The differences among the three genotypes were significant and the GG genotype was the highest one for the protein yield **(Table 20)**. So we can select towards GG genotype in this trait. In Sakha Friesian herd, the differences among the three genotypes in protein yield were not significant.

The means of age at first calving in Elkarda Friesian herd were 32 ±2, 33 ±2 and 32 ± 1 month for GG, GT and TT genotypes, respectively, the differences among the three

genotypes were not significant but in Sakha Friesian herd the differences among the three genotypes were significant and the means were 31±2, 35±2 and 33±1 month and the GG genotype was the best one in this trait.

Table 20. The least square means and their standard errors for milk, fat and protein yields and age at first calving in different patterns of ***Sm/I*** restriction enzyme of prolactin gene for Sakha and Elkarada Friesian herds and local cattle.

Trait	Genotype	El karada Friesian herd		Sakha Friesian herd		Local	
		No of records	Mean ±SE	No of records	Mean ± SE	No of records	Mean ± SE
305-day milk, kg	GG	52	3152 ± 294 [a]	40	2917±305 [a]	17	265±77 [a]
	GT	39	3122± 308 [a]	10	2996±471 [a]	2	122±88 [a]
	TT	139	3080 ± 237 [a]	144	2667±265 [a]	47	288±53 [a]
305- day fat, kg	GG	52	67.8± 11.2 [a]	40	79.2±11.9 [a]	17	10.7± 3.1 [a]
	GT	39	50.7 ±11.4 [b]	10	53.3±18.5 [a]	2	5.5±2.5 [a]
	TT	139	54.8± 8.4 [ab]	144	52.8±9 [a]	47	11.4± 2.2 [a]
305-day protein, kg	GG	52	44.5± 7.8[a]	40	51.8±7.9 [a]	17	8.0±2.3 [a]
	GT	39	28.4 ± 8[b]	10	47.8±12.3 [a]	2	3.4±2.6 [a]
	TT	139	37.8 ± 5.9[a]	144	43.5±6.2 [a]	47	8.7±1.6 [a]
AFC, Month	GG	45	31.5± 1.6 [a]	39	31.3± 1.7 [b]	17	37.0± 3.3 [a]
	GT	38	32.6± 1.7 [a]	9	34.7± 1.8 [a]	2	37.0± 7.2 [a]
	TT	131	32.1± 1.2 [a]	132	32.7± 1.1[ab]	47	38.0± 2.5 [a]

In Elsew local herd, the means for GG, GT and TT genotypes were 266 ±78, 122 ±88 and 289 ±54 kg for milk yield, respectively **(Table20)**. The means for fat yield were

10.7 ±3.1, 5.5 ±2.5 and 11.4 ±2.2 kg for GG, GT and TT genotypes and 8 ±2.3, 3.4 ±2.6 and 8.7 ±1.6 kg for protein yield, respectively i.e. the differences among the three genotypes in milk, fat and protein yields were not significant. **Khatami et al (2005)** revealed that the AB and BB genotypes produced 76.3g of milk per day more than the BB genotype. The means for AFC were 37.4±3.3, 37.1±7.2 and 38.2±2.5 month for GG, GT and TT genotypes, respectively and the differences among the three genotypes in local cattle were not significant.

5- SUMMARY

The aim of this study was to detect genetic and phenotypic trends for test day milk, fat and protein yields in Frisian cattle in Egypt applying the random regression model (RRM) and determining the genetic and phenotypic trend. Data of 5237 test days (TD) milk yield traits were recorded for 953 Friesian cows, daughters of 208 sires and 944 dams from two herds belonging to the Animal Production Research Institute, Egypt. Ten-month classes of lactation days were considered for the test-day yields.

The model included the random effects of direct additive genetic, permanent environment and error, while the fixed effects were herd, year and season of calving, parity which was modeled by orthogonal Legendre polynomials. The additive genetic variance estimates at first test day for milk, fat, protein yields and age at first calving traits were respectively 2.5kg, 10g, 2.5g and 1.5month increased until the fourth (5kg, 35g, 13g and 2months), decreased thereafter, reaching the lowest value at the tenth test day for milk but fat and protein yields were reaching the lowest value at the ninth test day. Age at first calving reached at the lowest value at the sixth test day (1month) then increased until tenth test day (8months).

Heritability estimates at first test day were 0.12, 0.25, 0.25 and 0.05 for TDMY, TDFY, TDPY and AFC traits

respectively and increased until the third test day for TDFY, TDPY and AFC but the TDMY trait reached to fourth test day which reached to 0.25, 0.32, 0, 32 and 0.08 for TDMY, TDFY, TDPY and AFC traits respectively then TDMY, TDFY TDPY decreased until tenth test day were (0.14, 0.25, 0.25) respectively, but AFC trait reached at the lowest value at sixth test day (0.04), and increased until the tenth test day which reached to 0.2.

The range in phenotypic values of TDMY decreased from the first to fifth TDMY, were 26 to 18kg then it increased until tenth test day milk yield. The range in phenotypic values of TDFY and TDPY started by 44g and 18g respectively until the fourth test day which were 38g and 110g for TDFY and TDPY respectively then decreased until ninth test day which reached to 60g and 20g respectively. The genetic trends were slightly positive for all traits instead of protein trait indicating that the selection program performs correctly. For all traits, the phenotypic trends showing deteriorating trends indicating the presence of some environmental inadequacies especially for nutritional level.

Records of 180 milking cows (38 local cows and 142 Friesian cows) raised at three experimental farms (Serw, Elkarda and Sakha) belonging to APRI, Ministry of Agriculture, Egypt were used to investigate association of

prolactin and lactoferrin genes with the milk, fat, protein yields and age at first calving. Traits were milk, fat and protein yields and age at first calving. PCR-RFLP method was used to get single nucleotide polymorphism (SNP). Three enzymes were used to restrict DNA product: ***HinfI*** for Lactoferrin gene and ***Nael*** and ***Sm/l*** for prolactin gene. Fixed effects were herd-year-season, parity and H, M and S genotypes and the animal additive genetic effect and permanent environmental effect of cow were used as random effects.

Genotypes from restriction enzyme ***HinfI*** were AA, AB, and BB. Genotype AB was the best one for milk, fat and protein yields and was not significant for AFC trait in Frisian. In local cattle, there were no significant differences among the three genotypes for milk traits but age at first calving trait differed significantly among the three genotypes. Genotypes from restriction enzyme ***Nael*** were CD and DD in Friesian. There were no significant differences between the two genotypes in Elkarada herd for milk and fat yield, but protein yield and age at first calving were affected. In local cattle, there were no significance differences among the two genotypes for milk traits but age

at first calving was significantly affected. Genotypes from restriction enzyme ***Sm/l*** were GG, GT and TT. There were significance differences among the three genotypes in Friesian for milk traits, but age at first calving was not affected. In local cattle, milk, fat, and protein yield and age at first calving traits were not significantly affected.

6-Conclusions

1) The test-day milk yield during the first three to five months of lactation could be adopted as an early selection criterion to increase milk yield.
2) Random regression model (RRM) was considered to be efficient model in detecting the fluctuations in genetic variance during the lactation period. It would permit better modeling for repeated records throughout the lactation period and could be chosen as an accurate method for predicting breeding values.
3) Genetic trend showing the improvement of test-day milk, fat, protein yields and age at first calving. So selection program was practiced correctly in this herd.
4) For all traits, the phenotypic trends showing deteriorating trends indicating the presence of some environmental inadequacies especially for nutritional level.
5) The lactoferrin and prolactin genes could be considered as the essential major genes for milk yield and components in dairy cattle.

6) The strong associations among genotypes of prolactin and lactoferrin genes and

7) For lactoferrin gene, In Elkarada Friesian herd, AA was the best genotype for milk, fat and age at first calving traits but in protein AB genotype was the best. In Sakha Friesian herd, AB genotype was the highest in milk, fat and protein yields but AA genotype for age at first calving was the best, so we can select towards AA and AB genotypes in Egypt.

8) For prolactin gene in Friesian Sakha and Elkarada herds, the CD genotype was the highest genotype in milk and protein yields so this genotype must be the main target in selection. Also the GG genotype was the highest genotype in the three milk traits. So these genotypes can be used in early selection.

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الملخص العربي

الهدف من هذه الدراسة هو تقدير المقاييس الوراثية والمظهرية لصفات انتاج اللبن ومكوناته من دهن وبروتين وذلك بإستخد يوم الاختبار الشهرى بطريقة دورية ومنتظمة وذالك بتسجيل إنتاج اللبن والدهن والبروتين في الأبقار الفريزيان في مصر،وتطبيق نموذج الانحدار العشوائي (RRM) وتحديد الاتجاه المظهري والوراثي .(phenotypic and genetic trend) حيث تم تسجيل بيانات 5237 سجل اختبار (TD) لصفات اللبن ومكوناته لـ 953 من الأبقار الفريزيان والتي هي بنات ل208 طلوقة و 944 أم من قطعان الأبقار التابعة لمعهد بحوث الإنتاج الحيواني بمصر. قسمت مدة الحليب والتي مدتها 305 يوم إلى عشرة إختبارات شهرية .

اشتمل نموذج التحليل على التأثيرات العشوائية للظواهر الوراثية المباشرة والبيئية الدائمة والخطأ في حين كانت التأثيرات الثابتة هي القطيع والسنة وموسم الولادة. كانت تقديرات التباين الوراثي المضيف في يوم الاختبار الأول لمحصول اللبن والدهن و البروتين والعمر عند أول ولادة على التوالي 2.5 كجم و 10 جرام و 2.5 جرام و 1.5 شهرًا وبدأت ترتفع حتى يوم الإختبار الرابع حيث وصلت إلي أعلي معدل لها (5 كجم ، 35 جرام 13 جرام و 2 أشهر) ثم انخفضت بعد ذلك وصولاً إلى أدنى قيمة في يوم الإختبار العاشر بالنسبة للبن لكن الدهن والبروتين وصلت إلى أدنى قيمة في يوم الإختبار التاسع. بلغ العمر عند أول ولادة أدنى قيمة له في يوم الاختبار السادس (1 شهر) ثم زاد حتى يوم الاختبار العاشر(8 أشهر) بلغت قيم التباين الوراثي في

يوم الاختبار الأول 0.12 و 0.25 و 0.25 و 0.05 للصفات TDMY و TDFYو TDPY و AFC على التوالي وزادت حتى يوم الاختبار الرابع لـجميع الصفات TDFY و TDPY و AFC TDMY حتى وصلت إلى 0.25 و 0.32 و 0 و 32 و 0.08 على التوالي ثم انخفضت قيم TDFY و TDPYحتى اليوم العاشر من الاختبار حيث وصلت إلى (0.14 ، 0.25 ، 0.25) على التوالي ، ولكن صفة العمر عند أول ولادة وصلت الي أدنى قيمة لها في يوم الاختبار السادس (0.04) شهر ثم زادت حتى يوم الاختبار العاشر حيث وصلت فيه إلى 0.2شهر.

انخفض المدى في التباين المظهري ل TDMYمن أول إختبار $TDMY_1$ إلى الخامس ، وكان 26 حتى وصل الي 18كجم ثم زاد العائد من اللبن حتي يوم الاختبار العاشر. بدأ التباين في القيم المظهرية لـ $TDFY_1$ و $TDPY_2$ بـ 44جم و18 جم على التوالي حتى يوم الاختبار الرابع الذي وصل الي 38 و 110 g لـ TDFY و TDPY على التوالي ثم انخفض حتى يوم الاختبار التاسع الذي وصل إلى 60جم و 20جم على التوالي. كانت الاتجاهات الوراثية إيجابية بالنسبة لجميع الصفات ويشير ذلك إلى أن برنامج الانتخاب يؤدي بشكل صحيح بالنسبة لجميع الصفات ولكن الاتجاهات المظهرية اتجاهات هابطة وذلك يشير إلى وجود بعض أوجه القصور البيئية خاصةً بالنسبة للمستوى التغذوي للحيوانات.

تم عمل تحليل جينى ل180 بقرة حلابة (38 بقرة محلية و 142 بقرة من الفريزيان) في ثلاث مزارع تجريبية سخا والقرضا فريزيان والسرو أبقار محلية تنتمي إلى معهد بحوث الإنتاج الحيوانى –مركز البحوث الزراعية وزارة الزراعة ، مصر لتقدير الارتباط بين جين البرولاكتين وجين اللاكتوفيرين مع صفات انتاج اللبن والدهن و البروتين والعمر عند أول ولادة.. تم استخدام طريقة PCR-RFLP لتحديد النقاط الفردية المضيئة على شريط المادة الوراثية ال .DNA (SNP) تم استخدام ثلاثة إنزيمات لتقطيع الحمض النووي(DNA) وهي على التوالى *HinfI* :لجين اللاكتوفيرين و *Nael* و *Sm/l* لجين البرولاكتين. وضعت التأثيرات الثابتة من موسم الحليب والقطيع وموسم وسنة الولادة والتراكيب الوراثية H ، M و S ،للحيوان نتيجة

لإستخدام إنزيمات القطع السابقة فى نموذج التحلل كما تم استخدام التأثير الوراثي المضيف للحيوان والأثر البيئي الدائم للبقرة كآثار عشوائية.

كانت التراكيب الجينية الناتجة من إنزيم القطع *HinfI* هي AA و AB و BB.وكان التركيب الوراثي AB هو أفضل تركيب وراثى فى صفة إنتاج اللبن والدهن والبروتين ولم يكن ذا أهمية بالنسبة لصفة AFC في الأبقار الفريزيان أما فى الأبقار المحلية ، لم تكن هناك فروق ذات دلالة إحصائية بين التراكيب الوراثية الثلاثة لصفات إنتاج اللبن إلا فى صفة العمر عند أول ولادة وجد اختلاف بشكل ملحوظ بين التراكيب الوراثية الثلاثة. كانت التراكيب الجينية من إنزيم القطع *Nael* هى CD و DD في أبقار الفريزيان. لم تكن هناك فروق معنوية بين التركيبين الجينيين في قطيع القرضا لصفتي إنتاج اللبن والدهن ، ولكن كانت هناك فروق معنوية بين التركيبين الجينيين لصفتي إنتاج البروتين والعمر عند أول ولادة.ولكن في الأبقار المحلية ، لم تكن هناك فروق معنوية بين التركيبين الجينيين لصفة إنتاج اللبن ولكن العمر عند أول ولادة كانت هناك فروق معنوية بين التركيبين الجينيين.ونتجت ثلاثة من الطرز الجينية من إنزيم القطع *SM/l* وكانت كالأتى GG و GT و TT وكانت هناك فروق معنوية بين التراكيب الوراثية الثلاثة في الفريزيان لصفة إنتاج اللبن ، ولكن العمر عند الولادة الأولى لم يكن هناك تأثير معنوي. في الماشية المحلية لم يكن هناك فروق معنوية بين الثلاثة تراكيب وراثية لصفات إنتاج اللبن، والدهن ، وإنتاج البروتين والعمر عند أول ولادة.