Genetic Profile of κ-Casein Gene Based on RFLP Technique in Association with Milk Traits in Egyptian Buffaloes

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Abstract: To reveal the linkage between κ-CN genotypes resulted from PCR-RFLP analysis and milk yield and composition traits, stepwise analysis was used for determining the best regression equation of those phenotypic traits on detected genotypes. 80 blood samples were used for genetic characterization of studied buffaloes, collected from five different geographical locations of Egyptian provinces with total of 340 dairy production records for 59 lactating buffaloes. Milk components profile was analyzed by MilkoScan device. Results of stepwise regression analysis showed that *Acu*I genotypes were significantly linked to most studied traits such as, TMY, FAT%, TS%, Ash%, and Humidity% with highly statistical. In some cases, the covariance between the independent variables (*Acu*I, *HpyCH4*IV, or compacted genotypes) represented the best regression equation, as with DMY and lactose%. Least-square means for studied traits showed that, heterozygous animals were significantly superior to homozygous animals, especially within *Acu*I or compacted genotyped animals. In other words, they could be considered the most superior individuals producing better milk with distinct fat%, TS%, SNF%, and Ash% traits. On contrary, homozygous animals had higher lactose percentage and humidity traits. Consequently, polymorphisms of κ-CN gene should be involved within modern selection programs as potential candidates associated with dairy performance traits referred to as gene assisted selection (GAS) that permits to select animals at an early age for breeding programs.

Keywords: PCR-RFLP, *Acu*I, *HpyCH4*IV, Stepwise regression analysis

INTRODUCTION

Buffaloes are widespread animals across many developing counties especially in Asia; the world population of buffalo in 2017 was estimated to be 200,967,747 heads, of which 97.4% are in Asia, 1.7% is in Africa, almost entirely in Egypt, 0.7% is in Americas, and 0.2% is in Europe (FAO, 2019) . Genus of Water or Asian buffaloes (*Bubalus*) are broadly classified into two main different subspecies, known as the River and Swamp types, based on their morphological, behavioral and geographical criteria (Cockrill, 1981; Groeneveld *et al.,* 2010).

Water river buffalo (*Bubalus bubalis*) is one of the important livestock animals in some developing countries due to its valuable contribution in different agricultural sectors. In Egypt, river buffalo is variably distributed all over the Nile valley and Nile delta (Hassanein *et al.,* 2013). In 2017, the total number of Egyptian buffalo was 3,375,727 head, presenting about 44% of Egypt's milk production. Approximately, 31% of buffalo population is found in three governorates, *i.e.,* El-Sharqia, El-Behaira, and El-Menoufia (FAO, 2019).

Despite their economic value, buffalo efficiency of milk production is low (8-10 kg/day) due to absence of systematic breeding strategies. The difficulties in applying a wide-scale selection programs for superior animals is due to distribution of buffaloes mostly between small-holders and small commercial farms. To achieve rapid genetic improvement in buffaloes, many aspects should be taken into account as: formation of nucleus herds and applying the modern breeding strategies which incorporated with using biotechnology tools like, artificial insemination (AI), embryo transfer and using molecular biology techniques as gene assisted selection (GAS) or marker assisted selection (MAS) technology, etc.

The majority of genetic improvement for quantitative traits in livestock has been achieved through conventional animal breeding programs which depends on phenotype-derived estimated breeding values (EBV), without acquaintance of the number of genes affecting the trait or the impacts of each gene (Eggen, 2012). The lengthiness of the generation interval in large animals prevents the achievement of a rapid genetic improvement using these traditional selection methods in animal breeding. Therefore, researchers are striving to develop efficient methods for livestock breeding, which would aid in the selection of superior individuals within a shorter time period and with greater accuracy. Modern breeding programs should involve analysis of genes responsible for quantitative characters which have some advantages in comparison with conventional selection. Such selection is directly based on identification of genotype and this facilitates selection among young animals, irrespective of their sex, and in turn would increase selection efficiency (Safronova *et al.,* 2017).

Recently, animal selection on the basis of molecular markers considered to be more reliable than any other criterion (Rachagani *et al.,* 2006; Riaz *et al.,* 2008). The using of DNA polymorphic markers permits the determination of individual genotypes at many loci and provides information on population parameters such as allelic and genotypic frequencies and referred to as marker assisted selection (Abdel Dayem *et al.,* 2009; Gouda *et al.,* 2013). For instance, κ-CN gene is commonly used as genetic marker for milk production traits on which bulls can be evaluated and selected for future breeding programs (Patel *et al.,* 2007; Abdel

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Dayem *et al.,* 2009; Alim *et al.,* 2015). Thus, involving of κ-CN genotypes within modern selection programs would significantly contribute to the improvement of milk production traits in buffaloes. Consequently selection based on genes (GAS) or selection based on markers (MAS) not only minimizes problems but also makes it possible to select animals at early age for breeding programs (Medrano and Aguilar-Cordova, 1990; Patel *et al.,* 2007).

The concept of marker assisted selection (MAS) refers to utilizing the information of polymorphic loci as an aid to selection; onwards of the 1990s, breeders used gene marker technology in the form of MAS to eliminate deleterious gene alleles or to select favorable conditions based on some marker information (Eggen, 2012). Through MAS of dairy livestock, some genes are suggested as potential candidates associated with dairy performance traits. Among different candidates, genetic variants of caseins (CN_s) have been extensively studied, and reported in cattle as important factors associated with lactation performance, milk composition and cheese yield efficiency (Hussain and Twayej, 2016).

Some researchers are focusing on detecting milk protein polymorphisms as a potential tool for genetic choice and genetic characterization of bovine breeds (Lara *et al.,* 2002). Until now, several types of molecular markers have been utilized to evaluate DNA polymorphisms, *e.g.* restriction fragment length polymorphism (RFLP), which was the first DNA-based marker for constructing genetic linkage maps; and by combining this method with PCR (PCR-RFLP), genetic polymorphisms of most important genes had been detected. The progress of PCR-RFLP technique enabled fast analysis of the polymorphisms of virtually unlimited number of genes, including that coding κ-CN gene (Yang *et al.,* 2013). Nowadays, genetic marker research applied to animal breeding and production is concentrated mainly on analyzing mutations located within candidate genes and their relation with economically substantial traits (Awad *et al.,* 2016). The present study focused on determination of the linkage between the detected genotypes of κ-CN gene with milk yield and its chemical components traits.

MATERIALS AND METHODS

Location

This study was conducted at Biotechnology Laboratory of Animal Production and Fisheries Department, Faculty of Agriculture, Suez Canal University; Ismailia, Egypt.

Blood Samples and DNA Extraction

Eighty randomly blood samples were collected from five geographical locations of some Egyptian provinces (Table 1). Approximately 5 ml of blood was drawn per animal, in "K_3-EDTA" (an anticoagulant) coated sterile vacutainers and immediately preserved in a well-insulated icebox containing ice cool pack until reaching to the laboratory then stored at -20˚C to the time of DNA extraction. Genomic DNA was isolated from the whole blood using commercial purification kit, (Quick-gDNA™ MiniPrep). DNA concentration and purity was estimated using NanoDrop (Spectrophotometer ND-1000). DNA concentrations ranged from (20 to 30 ng/µl), with a purity ratio from 1.7 to 1.9 which was considered as a high DNA purity.

Collection of milk samples

A total of 340 dairy production records from 59 lactating buffaloes were used. Animals were selected from accredited farms. Milk samples were collected from pedigreed animals with the least relationship to decrease the genetic similarity and to increase chance to get more polymorphism. For each studied animal the individual milk sample was collected in Falcon tube (50 ml) immediately after milking and preserved by adding 3 ml of Potassium dichromate solution then stored at -20˚C till further analysis. Determination of milk components profile was analyzed by MilkoScan device. The studied traits were Fat %, Protein %, Lactose %, Total solids %, Solid not fat %, Ash % and Humidity %.

Table (1): List of farms used in the present study

Location/Farm	Animals No.
Farm of Agriculture College, Cairo University (C.F.)	20
Kafr El Sheikh Agricultural Research Station (K.S.)	29
Farm of Agriculture College of Menoufia University (Sh. F.)	10
Ismailia Agricultural Research Station (I.S.)	11
Sids Research Station, Beni Suef Governorate (S.S.)	10

PCR-RFLP Assay

Because of the highest degree of nucleotide sequence conservation between cattle and river buffalo, the gene primers used for amplification are basically of cattle origin (Othman *et al.,* 2011), therefore some authors used the primers designed according to cattle gene sequence for genotyping buffalo (Patel *et al., 2007*; Riaz *et al.,* 2008; El Nahas *et al.,* 2013; El Nahas and Abou Mossallam, 2015). PCR-RFLP technique included, amplification of the target sequences of κ-CN gene (453 bp) presented in Table (2) with polymorphism chain reaction (PCR) described by Barroso *et al.* (1998) and followed by digestion of these PCR products with both *Acu*I and *HpyCH4*IV (Isoschizomer for *Mae* II) restriction enzymes (New England Biolabs®inc., USA).

Restricted products were loaded on 3% agarose gel (80 ml) after staining with 0.5µl of ethidium bromide (10mg/ml) in 1x TAE as a running buffer at a voltage 100v, 40mA for two hours. For detection of κ-CN genotypes, the gel photos captured for the resulted bands of restricted products were analyzed manually by comparing with the standard DNA ladder (100 bp) co-migrated in the same gel and define the corresponding genotype according to literature review data. Detected fragments sizes corresponding to different κ-CN genotypes after digesting PCR products with different restriction enzymes are summarized in Table (3)

Table (2): Primer sequences for κ-CN genes

Gene	Sequence of used primer	References
κ-CN	F 5′-TGTGCTGAGTAGGTATCCTAGTTATGG-3′ R 5′GCGTTGTCTTCTTTGATGTCTCCT-3′	(Medrano and Aguilar-Cordova, 1990; Barroso *et al.,* 1998; Riaz *et al.,* 2008; El Nahas *et al.,* 2013; El Nahas and Abou Mossallam, 2015)

Table (3): Detected fragments size corresponding to different κ-CN genotypes using PCR-RFLP technique

Gene	Restriction Enzyme (RE)	Genotype	Fragments size (bp)
κ-CN	*Acu*I [1]	AA	453 bp (uncut product)
		AB	453, 339, and 114 bp
	*HpyCH4*IV [2]	BB	254 and 199 bp
		AB	453, 254 and 199 bp

[1] El Nahas *et al.* (2013) and El Nahas and Abou Mossallam (2015); and [2] Riaz *et al.* (2008)

The DNA patterns arising from the digestion of 453bp PCR of κ-CN gene with *Acu*I and *HpyCH4*IV enzymes were combined to create new synthesized genotype (Compacted genotype) for each animal possess two restriction patterns of the restricted enzymes as reviewed by Barroso *et al.* (1998). Cumulative restriction pattern through restriction analysis of κ-CN gene indicated different genotypes using two restriction enzymes and are presented in Table (4). Determination of κ-CN genotypes and compacted genotypes were illustrated in details through in press data of Al-Shawa *et al.* (2020).

Table (4): Cumulative restriction pattern analysis of κ-CN gene

Compacted Genotypes	Cumulative restriction pattern analysis					
	***Acu*I Enzyme**			***HpyCH4*IV Enzyme**		
	bp					
AD	453	—	—	453	—	—
AE	453	—	—	—	254	199
AF*	453	—	—	453	254	199
BD	—	339	114	453	—	—
BE	—	339	114	—	254	199
BF	—	339	114	453	254	199
CD	453	339	114	453	—	—
CE*	453	339	114	—	254	199
CF*	453	339	114	453	254	199

(*) refers to genotypes detected in the present study

Statistical analysis

Allelic frequencies per locus and population were calculated using FSTAT for windows, version 2.9.3.2 (Goudet, 2002). Genotypic frequencies per locus and population were calculated manually with the formula given below;

$$\text{Frequency of observed heterozygotes} = \frac{Heterozygote\ Number}{Total\ number\ of\ individuals}$$

Calculation of Estimated Breeding Values (EBV) for phenotypic traits:

The collected data of milk yield and composition traits were used to estimate the EBV for each animal by the use of MTDFREMEL program of Boldman *et al.* (1995) using the following model:

$$EBV_{ijo} = Y_{ijo} - (\mu + L_i + P_j + \varepsilon_{ijo})$$

Where:

EBV_{ijo}: is the estimating breeding value of the k^{th} animal on o^{th} trait

Y_{ijo}: is the observed value of the k^{th} animal

μ: is the overall mean

L_i: is the fixed regression coefficient on i^{th} Location

P_j: is the fixed regression coefficient on j^{th} parity

ε_{ijo}: is the Random error.

Linkage analysis

After identifying the κ-CN genotypes for all individuals using PCR-RFLP analysis, the linkage analysis was performed using these genotypes and EBV as a phenotypic values of milk yield and composition traits to detect the linkage between studied genes and phenotypic traits. Data of current experiment were analyzed using SAS program applying stepwise procedure (SAS, 1999).

The association of detected κ-CN genotypes, through using *Acu*I and *Hpych4*IV enzymes, with milk yield and milk composition traits was estimated by stepwise regression analysis in which milk yield and composition traits were the dependent variables and the κ-CN genotypes were the independent variables.

Partial regression analysis was employed to detect the effects of different κ-CN genotypes linked to the total estimated breeding value (EBV) variation for milk yield and composition traits. The percentage of the total genetic variation explained by the association between each single genotype of the total genotypes used and each trait is R^2 (coefficient of determination) value. The statistical analysis model of stepwise regression is mentioned below:

$$Y_{\alpha ijk} = a_\alpha + \sum \beta_i G_j + \varepsilon_{ijk}$$

Y_{ijk}: Accession means for study trait to:

a_α: Intercept function for the set of independent variables within β within G

β_i: The partial regression coefficients that specified the empirical relationship between Y and G_j

G_j: Gene alleles (J =1, 2, 3)

ε_{ijk}: Random error.

RESULTS AND DISCUSSION

Stepwise regression analysis "Forward solution"

Stepwise regression analysis used to determine the best equation solution to estimate the regression of the studied traits (dependent variable) on the genotypes of enzymes used (independent variables).

Daily Milk Yield (DMY)

Results of stepwise analysis for DMY trait are shown in Table (5), the first forward model has the single effect of the independent variable (*HpyCH4*IV, genotypes), and the second one added the combined information of genotypes from both *HpyCH4*IV and *Acu*I, enzymes (Compact genotypes) to the equation. Applying the second equation resulted in increasing the model accuracy R^2 by (3.80%) and decreasing value of Mallow's C_p by 2.03 comparing to the first equation due to addition of compacted genotypes information, therefore the covariance between *HpyCH4*IV genotypes and compacted genotypes makes the prediction of DMY value more precisely with statistically significant ($P \leq 0.03$).

Table (5): Stepwise regression of DMY trait on different genotypes (to forward solution)

Step	Enzymes	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	***HpyCH4*IV**	10.43	-1.32	7.41	7.41	5.03	0.007
2	***HpyCH4*IV**	7.39	-1.05	7.41	11.22	3.00	0.03
	Compacted Genotypes		0.45	3.80			0.04

Total Milk Yield (TMY)

Applying stepwise regression in Table (6) showed that, the first equation was the best one due to the significant single positive effect of *Acu*I genotypes on TMY trait at ($P \leq 0.02$) comparing to the second forward equation, which combined between the effect of *Acu*I genotypes and compacted genotypes that had not any statistical effect on TMY trait. Consequently, it seems that genotyping of the κ-CN gene by using *Acu*I enzyme was successfully linked to the TMY trait with highly significant value ($P \leq 0.02$).

Total Milk Yield in 305 days (T305)

Applying stepwise regression in Table (7) clarified that there was no statistically significant effect of the independent variable (*HpyCH4*IV enzyme) on T305 trait.

Table (6): Stepwise regression of TMY trait on different genotypes (to forward solution)

Step	Enzymes	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	***Acu*I**	1090.61	239.60	1.75	1.75	2.48	0.02
2	***Acu*I**	1110.19	714.48	1.75	2.25	3.00	NS
	Compacted Genotypes		-164.82	0.49			NS

Table (7): Stepwise regression of T305 trait on different genotypes to forward solution

Step	Enzyme	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	***HpyCH4*IV**	2283.95	-147.37	1.35	1.35	1.05	NS

Milk Components traits

Milk Fat trait (FAT %)

As shown in Table (8), by applying stepwise analysis, we got two different models. The first forward model has the single effect of the independent variable (*Acu*I, genotype), and the second one has two independent variables (*Acu*I and compacted genotypes. *Acu*I genotypes alone, in first forward equation had highly significant effect at ($P \leq 0.0007$) which makes the prediction of FAT percentage value more precisely. Therefore, genotyping of κ-CN gene by using *Acu*I enzyme was successfully linked to the FAT percentage trait with highly significant ($P \leq 0.0007$).

Table (8): Stepwise regression of FAT % trait on different genotypes to forward solution

Step	Enzymes	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	***Acu*I**	8.05	1.15	3.9	3.9	2.05	0.0007
2	***Acu*I**	8.10	2.41	3.9	4.2	3.00	NS
	Compacted Genotypes		-0.44	0.3			NS

Milk Protein trait (Protein %)

As shown in Table (9), there are two forward stepwise models, the first forward model has the single effect of the independent variable (*HpyCH4*IV, genotypes) and the second one has two independent variables (*HpyCH4*IV, genotype and compacted genotypes). All applied forward solutions failed to determine the best equation to estimate the regression of protein% trait on different genotypes of used enzymes, *i.e.,* they did not make any statistically significant improvement in R^2 and this is may be due to low variations of protein% estimates between animals.

Table (9): Stepwise regression of Protein % trait on different genotypes (to forward solution)

Step	Enzymes	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	***HpyCH4*IV**	3.87	0.12	0.66	0.66	2.12	NS
2	***HpyCH4*IV**	3.73	0.13	0.66	1.01	3.00	NS
	Compacted Genotypes		0.02	0.35			NS

Milk Lactose trait (Lactose %)

Results of stepwise regression in Table (10) showed that the covariance between the two independent variables (*Acu*I, genotypes and compacted genotypes) in the second equation will improve R^2 value by 2.47% with highly statistically significant value ($P < 0.0001$). From both solutions equations, we figure out that, the partial regression coefficient estimates for *Acu*I genotypes had negative effects on lactose trait with highly statistically significant value ($P<0.0001$) while the partial regression coefficients estimate for compacted genotypes had positive effect on lactose trait with highly statistically significant value ($p \leq 0.003$), therefore addition of both independent variables to the regression model equation will increase the precision of predicting lactose trait, *i.e.,* genotyping of κ-CN gene by using *Acu*I and *HpyCH4*IV enzymes was successfully linked to lactose trait at highly statistically significant level.

Table (10): Stepwise regression of Lactose % trait on different genotypes to forward solution

Step	Enzymes	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	***Acu*I**	5.66	-0.32	6.88	6.88	9.79	<.0001
2	***Acu*I**	5.63	-1.08	6.88	9.35	3.00	<.0001
	Compacted Genotypes		0.26	2.47			0.003

Total Solids trait (TS %)

Results in Table (11) showed that, there were two equations; the first one reflected the single effect of the independent variable *Acu*I genotypes while, the second one represented the covariance between two independent variables (*Acu*I genotypes and compacted genotypes). However, increasing the accuracy model by 0.14% in the second model, but the single effect of *Acu*I genotypes is the best equation for estimating regression of TS trait on genotypes of κ-CN gene with highly

significant ($P \leq 0.002$), i.e., changes in TS trait largely depend only on the partial regression coefficient of the *Acu*I genotypes. Consequently, from results of analysis, it seems that genotyping of the κ-CN gene by using *Acu*I enzyme successfully linked to the TS trait with highly significant ($P \leq 0.002$).

Solids not fat trait (SNF %)

From stepwise regression results of Table (12), we noticed that, accede of *Acu*I genotypes to the model increased the accuracy by 1.49% and decreased Mallow's C_P by 2.98 at significant level of ($P \leq 0.02$), which resulted in more precision of SNF% trait prediction. Therefore, using the covariance between *Acu*I and *HpyCH4*IV genotypes was successfully linked to the SNF% trait and could be considered the best regression equation, due to lowering Mallow's C_p value and increasing R^2 ratio at $P \leq 0.02$ comparing to the single effect of *HpyCH4*IV genotypes in the first equation.

Ash trait (Ash %)

From results of stepwise analysis in Table (13), only the addition of *Acu*I genotypes to the model had statistically significant positive effect at ($P \leq 0.011$).

Table (11): Stepwise regression of TS% trait on different genotypes (to forward solution)

Step	Enzymes	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	***Acu*I**	18.47	0.98	2.85	2.85	1.46	0.002
2	***Acu*I**	18.50	1.82	2.85	2.99	3.00	NS
	Compacted Genotypes		-0.29	0.14			NS

Table (12): Stepwise regression of SNF % trait on different genotypes (to forward solution)

Step	Enzymes	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	*HpyCH4*IV	8.90	0.33	2.20	2.2	5.98	0.007
2	*Acu*I	8.62	0.20	1.49	3.69	3.00	0.02
	*HpyCH4*IV		0.29	2.2			0.02

Table (13): Stepwise regression of Ash % trait on different genotypes (to forward solution)

Step	Enzyme	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	***Acu*I**	0.78	0.08	2.03	2.03	1.12	0.011

Humidity trait (Humidity %)

As shown in Table (14), the single negative effect of *Acu*I genotypes was statistically significant at ($P \leq 0.002$) on humidity% trait as mentioned in first equation, therefore, the first equation which involved only *Acu*I genotypes was the best one with highly significant effect ($P \leq 0.002$) instead of non-significant effect of acceding the compacted genotypes in the second model.

Table (14): Stepwise regression of Humidity trait on different genotypes (to forward solution)

Step	Enzymes	Intercept (a_i)	Estimate (β_i)	Partial R^2 (%)	Model R^2 (%)	Mallow's C_p	Probability (Pr ≤)
1	***Acu*I**	81.45	-0.97	2.97	2.97	2.27	0.002
2	***Acu*I**	81.39	-2.32	2.97	3.37	3.00	NS
	Compacted Genotypes		0.47	0.40			NS

Least-square means for studied milk production traits within different enzymes' genotypes using Duncan's method.

According to analysis of variance results, except for average breeding value of the percentage of protein and 305d milk yield traits, all mean squares of milk production and its chemical constituents were influenced by κ-CN polymorphisms.

The least-square means of DMY and TMY traits within different enzymes' genotypes are presented in

Table (15), while the least-square means of Fat%, Lactose%, SNF%, TS%, Ash%, and Humidity% within different enzymes' genotypes are presented in Table (16).

Non-availability of literature data on the relationship of κ-CN genotypes with both milk yield and its chemical composition traits in buffaloes may be resulted from the monomorphic profile for κ-CN gene reported by the majority of these reviewed studies and hence, it will not be possible to assess the relationship between resulted homozygotes and milk constituents; or may be due to focus only on genetic characterization of this gene in most literature review. Consequently, discussion of this part will be limited only on cattle data that are not also conclusive, and are sometimes even conflicting.

Table (15): Least-square means (LSM) and standard errors (in parentheses) for milk yield traits across different genotypes of used enzymes.

Restricted enzyme	Genotypes	Least-square means	
		DMY (kg)	TMY (kg)
***Acu*I**	AA	5.88^{ns} (0.67)	1412.62^{b} (116.52)
	AB	7.23 (0.24)	1632.63^{a} (67.66)
***HpyCH4*IV**	AB	7.30^{a} (0.33)	1440.22^{a} (52.86)
	BB	5.81^{b} (0.53)	1605.04^{b} (137.59)
Compacted Genotypes	AF	6.63^{b} (0.62)	1330.21^{b} (94.86)
	CE	6.48^{b} (0.41)	1715.05^{a} (127.03)
	CF	7.98^{a} (0.25)	1550.23^{ab} (46.65)

Daily Milk Yield (DMY)

The means of DMY trait within *HpyCH4*IV and compacted genotypes were significantly differed, whereas, they had non-significant differences within *Acu*I genotypes, i.e., heterozygous animals ("AB" and "CF" genotypes) of both *HpyCH4*IV and compacted genotypes had higher means of DMY trait, while homozygous "AA" animals were not significantly differed than heterozygous "AB" animals within *Acu*I genotypes (Table 15). Consequently, heterozygous animals showed better daily milk yield than homozygotes.

Some authors found significant effect of κ-CN genotypes on the DMY trait in cattle, as Mohammadi *et al.* (2013) who reported that, comparison of the least square means showed significant difference between "AA" and "BB" genotypes of the κ-CN gene for milk production in Iranian Holstein cattle. They found that individuals with "AA" genotype had higher significantly differences for DMY (29.08±7.36 kg) than individuals with "BB" (28.03±6.17 kg) and "AB" genotypes (28.11±5.89 kg). In addition, Wolanciuk (2015) noticed that cows with allele "A" of the κ-CN gene produced significantly more daily milk yield ($P \leq 0.05$), and the "BB" homozygotes produced 2.9-4.32 kg less milk compared with the "AA" κ-CN homozygotes. On contrary, some other investigators noticed non-significant statistical effect of different κ-CN genotypes on DMY trait, such as Kübarsepp *et al.* (2005). Additionally, Petrovska *et al.* (2017) who reported that, there was no statistical difference between different genotypes, but the highest DMY trait was observed from "AB" κ-CN genotype in Latvian Brown breed (19.7 ± 1.52 kg); while a lower daily milk yield was "BB" genotype in the same breed (14.6 ± 4.65 kg).

Total Milk Yield (TMY)

Within *Acu*I genotypes, heterozygous individuals "AB" had been considered better milk producers than homozygous individuals "AA". On contrary, results for *HpyCH4*IV genotypes clarified that, animals with homozygous genotypes "BB" showed more significant difference for means of TMY trait than those with heterozygous genotypes "AB". Also, results of compacted genotypes signified that the compacted genotypes ("CE" genotypes) which involved heterozygotes of *Acu*I genotypes with homozygotes of *HpyCH4*IV genotypes had higher means of TMY trait with statistically significant difference, while heterozygote compacted genotypes ("CF" genotypes) had not any statistically significant difference than other compacted genotypes ("CE" and "AF" genotypes) (Table 15).

In some reviewed studies, TMY trait was significantly influenced by different κ-CN genotypes, as reported by Mir *et al.* (2014) who found that, κ-CN gene polymorphism had statistically significant ($p < 0.05$) association with milk yield; animals with κ-CN "AB" genotype had higher TMY during 1st lactation (+421.8 kg) and 2nd lactation (+588.8 kg) than those with "AA" genotype. Therefore, incorporation of "AB" genotypes for κ-CN may help to improve the milk yield in Sahiwal cattle population of Pakistan. Also, Djedović *et al.* (2015) showed that, κ-CN genotypes statistically significantly ($P \leq 0.05$) influenced the TMY; Simmental cows of "AB" genotypes had higher milk yield (+191 kg) than "AA" and (+787 kg) than "BB" genotypes. Similarly, "AB" genotyped crossbred animals have higher milk yield (+560 kg) than "AA" genotyped individuals. Similar results were obtained for Busha individuals. Also, "AB" individuals had higher milk

yield (+140 kg) than "AA" and (+289 kg) than "BB" genotypes. From the other hand, (Trakovická *et al.,* 2012; Miluchová *et al.,* 2018) reported that κ-CN polymorphism had no significant effect on evaluated TMY.

Milk Components traits

Milk Fat trait (FAT %)

Results of Table (16) showed that, animals with heterozygous genotypes "AB" within *Acu*I enzyme or individuals with compacted genotypes ("CE" genotypes) which involved heterozygotes of *Acu*I genotypes with homozygotes of *HpyCH4*IV genotypes showed significantly higher fat percentage. As well, heterozygous compacted genotypes ("CF" genotypes) showed more fat percentage than those involved heterozygotes of *HpyCH4*IV genotypes with homozygotes of *Acu*I genotypes ("AF" genotypes). Whereas, all *HpyCH4*IV, genotypes had no statistical differences between each other for fat percentage trait. From previous results, we concluded that heterozygous genotypes of *Acu*I enzyme should be involved in the breeding programs that aiming to improve the percentage of fat trait of produced milk.

From one hand, some investigations reported that, κ-CN polymorphism had significant influence on fat content or fat yield in different cattle breeds, as Kučerová *et al.* (2006) who noticed that, genotype "AA" was associated with the highest breeding value for fat % (+0.020), followed by "BB" genotype with lower fat % (+0.007). On contrary, Wolanciuk (2015) noticed that "BB" homozygotes generally correlated with higher content of fat (by 0.12: 0.52) compared with the "AA" κ-CN homozygotes. While, Botaro *et al.* (2009) demonstrated that, κ-CN genotypes had significant effect on milk fat %, i.e., "AB" cows had the highest milk fat content followed by "AA" and "BB" (3.38, 3.25, and 3.14% respectively). Also, Hamza *et al.* (2011) indicated that, κ-CN genotypes identified using SSCP genotyping method had significant ($P<0.05$) effect on fat content. They observed that cows with genotype TT had significantly higher fat (3.89%) than those of genotypes CC and TC (3.62 and 3.66%, respectively) in Chinese Holstein cattle. Moreover, Djedović *et al.* (2015) showed that, κ-CN genotypes statistically highly significantly ($P \leq 0.01$) influenced the milk fat yield. "AB" genotyped cows throughout all examined breeds and crossbreds in this study had higher milk fat content in relation to "AA" and "BB" genotypes, Simmental cows of "AB" genotypes had higher milk fat yield (+32 kg) than "AA" and (+39.8 kg) than "BB" genotypes producing through the same period, similarly, "AB" genotyped crossbred animals had higher milk fat yield (+8.8 kg) than "AA" and (+12.4 kg) than "BB" genotypes.

From the other hand, some authors such as Kübarsepp *et al.* (2005); Trakovická *et al.* (2012); Mohammadi *et al.* (2013); Djedović *et al.* (2015); Miluchová *et al.* (2018) reported that, polymorphisms of κ-CN were not associated with milk fat percentage or yield of dairy cattle. Mohammadi *et al.* (2013) found that comparison of the least square means showed non-significant difference between "AA" and "BB" genotypes of the κ-CN gene for fat content in Iranian Holstein cattle. However, animals with "AA" genotypes had the higher percentage of fat (3.44±0.53). As well, Djedović *et al.* (2015) showed that, κ-CN genotypes did not influence significantly ($P > 0.05$) the milk fat content. Also, Miluchová *et al.* (2018) suggested that, κ-CN gene polymorphism had no statistical effect on the average breeding value for fat yield as well as the percentage of milk fat. Additionally, Trakovická *et al.* (2012) found that, κ-CN polymorphism had no significant effect on evaluated fat yield (kg) in standard period of lactation; however, there was a positive indication of the "AA" genotype effect on milk fat yield.

Milk Lactose trait (Lactose %)

As presented in Table (16), animals with different genotypes of either individual enzymes or compacted genotypes showed statistical differences for lactose% trait. By using *Acu*I genotyping, animals with homozygous "AA" genotypes had higher lactose % than those with heterozygous "AB" genotypes. On contrary, by using *HpyCH4*IV, the heterozygous individuals "AB" had the higher means of lactose% comparing to homozygous individuals "BB". As well the compacted genotypes that involved heterozygotes of *HpyCH4*IV genotypes with homozygotes of *Acu*I genotypes ("AF" genotypes) showed the highest significant means of lactose% comparing to other compacted genotypes, followed by individuals involved the heterozygous genotypes from both enzymes ("CF" genotypes).

Wolanciuk (2015) mentioned that, "AA" and "BB" genotypes of the κ-CN gene showed significant difference for lactose %. In Polish Red breed, "AA" genotype had higher content of lactose% by 0.13, while, in White-backed breed "BB" genotype had the more percentage of lactose by 0.29. Hamza *et al.* (2011) indicated that, κ-CN genotypes identified that using SSCP genotyping method had significant ($P<0.05$) effect on lactose content of cows genotyped as "TC" had significantly highest lactose content (4.87%) followed by those of genotypes "CC" and "TC" (4.85 and 4.76%, respectively) in Chinese Holstein cattle. From the other hand, Botaro *et al.* (2009) found that, κ-CN genotypes had insignificant effect on lactose %. Also, Wolanciuk (2015) concluded that, κ-CN genotypes showed non-significant effect on lactose % in both Polish Holstein-Friesian and Jersey breeds

Total Solids trait (TS %)

As mentioned in Table (16), the highest mean of TS% trait was observed for animals with compacted genotypes containing heterozygous genotype of *Acu*I enzyme and homozygous genotype of *HpyCH4*IV enzyme ("CE" genotype), followed by the heterozygotes individuals ("CF" genotype). In parallel, within *Acu*I genotypes, heterozygotes animals "AB" had the highest significant mean of TS% trait. On contrary, all genotypes of *HpyCH4*IV enzyme did not statistically differ from each other.

Botaro *et al.* (2009) showed that, κ-CN genotypes had insignificant effect on TS %. While, *Hamza et al.* (2011) indicated that, κ-CN genotypes identified using SSCP genotyping method had significant ($P<0.05$) effect on TS content in Chinese Holstein cattle.

Solids not fat trait (SNF %)

The highest mean of SNF% trait was observed for animals with compacted genotypes containing heterozygous genotype of *Acu*I enzyme and homozygous genotype of *HpyCH4*IV enzyme ("CE" genotype). So, within *Acu*I genotypes, heterozygous animals "AB" had the highest significant mean of SNF% trait. On contrary, homozygotes "BB" animals had the highest mean of SNF% trait for *HpyCH4*IV enzyme (Table 16). Hamza *et al.* (2011) indicated that, genotypes of κ-CN identified using SSCP genotyping method did not affect solids not fat trait in Chinese Holstein cattle.

Ash trait (Ash %)

From results of Table (16), we observed that, within *Acu*I genotypes, heterozygous animals "AB" had the highest significant mean of Ash% trait. In addition, animals with compacted genotypes containing heterozygous genotype of *Acu*I enzyme, with either homozygous or heterozygous genotype of *HpyCH4*IV enzyme, had the highest significant mean of Ash trait. While all genotypes of *HpyCH4*IV enzyme did not statistically differ from each other.

Humidity trait (Humidity %)

The highest statistical mean of humidity% trait was observed for homozygotes "AA" animals within *Acu*I genotypes. Moreover, we noticed also that animals genotyped with "AF" genotype that combined between homozygotes from *Acu*I enzyme and heterozygote from *HpyCH4*IV enzyme had the highest significant mean of humidity% trait from all compacted genotypes. On contrary, *HpyCH4*IV genotypes had not any statistical difference from each other (Table 16).

Table (16): Least-square means (LSM) and standard errors (in parentheses) for milk components traits across different genotypes of used enzymes.

Restricted enzyme	Genotypes	Least-square means					
		FAT%	Lactose%	TS%	SNF%	Ash%	Humidity%
*Acu*I	AA	9.42^{b} (0.36)	5.21^{a} (0.07)	19.60^{b} (0.36)	9.54^{b} (0.10)	0.86^{b} (0.03)	80.25^{a} (0.35)
	AB	10.52^{a} (0.21)	4.91^{b} (0.04)	20.55^{a} (0.21)	9.74^{a} (0.06)	0.94^{a} (0.02)	79.34^{b} (0.21)
*HpyCH4*IV	AB	9.75^{ns} (0.16)	5.19^{a} (0.03)	19.93^{ns} (0.16)	9.50^{b} (0.05)	0.89^{ns} (0.02)	80.03^{ns} (0.16)
	BB	10.19 (0.43)	4.93^{b} (0.09)	20.22 (0.44)	9.78^{a} (0.12)	0.91 (0.04)	79.56 (0.42)
Compacted Genotypes	AF	9.21^{b} (0.29)	5.34^{a} (0.06)	19.45^{b} (0.29)	9.40^{b} (0.09)	0.85^{b} (0.03)	80.49^{a} (0.28)
	CE	10.74^{a} (0.40)	4.78^{c} (0.08)	20.69^{a} (0.40)	9.89^{a} (0.11)	0.94^{a} (0.04)	79.11^{b} (0.39)
	CF	10.31^{a} (0.14)	5.04^{b} (0.03)	20.40^{a} (0.14)	9.60^{b} (0.04)	0.93^{ab} (0.01)	79.57^{b} (0.14)

The non-significant association between genetic variants of κ-CN gene and both of protein percentage in milk and total milk yield in 305 day traits found in this study in Egyptian buffaloes may be resulted from insufficient numbers of studied animals, minor variation in estimated values of protein% between studied animals or existence of a large-scale variety of κ-CN genotypes between cattle breeds comparing to buffaloes. Therefore, discussion will be limited here on the effect of κ-CN polymorphisms on protein% trait and 305d milk yield in cattle breeds.

Some reviewed studies conducted on cattle breeds to determine the linkage between κ-CN polymorphisms and protein % indicated that, κ-CN genotypes had significant effect on milk protein % as mentioned by Kučerová *et al.* (2006) who found that, genotype "BB" was associated with the highest breeding value for protein % (+0.038), while, "AA" genotype was associated with lower average breeding value for protein % (+0.022). As well, Mohammadi *et al.* (2013) reported that, comparison of the least square means showed significant difference between "AA" and "BB" genotypes of the κ-CN gene for milk protein percentage in Iranian Holstein cattle. Cows with "BB" genotype had higher significantly differences for milk protein percentage (3.20±0.36) than those with "AA" (3.09±0.27) and "AB" genotypes (3.19±0.13). Also, Wolanciuk (2015) showed that "BB" homozygotes was generally correlated with higher protein content (by 0.13-0.51), compared with the "AA" κ-CN homozygotes. Allele "B" of the κ-CN was also linked to higher casein content in the milk. Additionally, Miluchová *et al.* (2018) suggested that, κ-CN gene polymorphism had a measurable effect on the average breeding value for the percentage of protein in milk of Holstein cows. Statistical analysis confirmed that the "AA" genotype significantly reduces the average value of the protein content of milk (by 0.09% on average), compared with genotype "BB". While, Kübarsepp *et al.* (2005) and Botaro *et al.* (2009) reported that κ-CN genotypes had no significant effect on protein content. Similarly, Trakovická *et al.* (2012) found that, κ-CN polymorphism had no significant effect on evaluated protein yield (kg) in standard period of lactation; however, there was a positive indication of the "AA" genotype effect on protein yield. Also, *Hamza et al.*

(2011) indicated that, κ-CN genotypes identified using SSCP genotyping method had insignificant (P>0.05) effect on protein, however, cows with genotype "TT" had insignificantly higher proteins (3.50%) than those of other two genotypes (3.35 and 3.36%, respectively) in Chinese Holstein cattle. Hamza *et al.* (2010) mentioned that, different κ-CN genotypes had no significant (P > 0.05) effect on 305day milk yield of Chinese Holstein cattle.

CONCLUSION

The major interest of this study was to discover the linkage between κ-CN genotypes resulted from PCR-RFLP analysis with milk yield and composition traits. Results of stepwise regression analysis signified that *Acu*I genotypes had successfully linked to most studied traits such as, TMY, FAT%, TS%, Ash%, and Humidity% with highly statistical significance. In some cases, the covariance between the independent variables (*Acu*I, *HpyCH4*IV, or compacted genotypes) represented the best regression equation, such as, with DMY and lactose%. All applied forward solutions failed to determine the best equation to estimate the regression of protein% and T305 traits on different genotypes of used enzymes. Comparisons of least-square means for studied traits showed that, heterozygous animals were significantly superior animals comparing to homozygous animals, especially within *Acu*I or compacted genotyped animals. In other words, they could be considered the most superior individuals producing better milk with distinct fat %, TS %, SNF %, and Ash % traits. On contrary, homozygous animals had higher lactose % and humidity % traits. Consequently, polymorphisms of κ-CN gene should be involved within modern selection programs as potential candidates associated with dairy performance traits referred to as gene assisted selection (GAS) that permits to select animals at early age for breeding programs. The present study recommended involving heterozygous animals in the breeding programs scheme that aiming to improve milk quantity and quality properties of the Egyptian buffalo populations. Further researches with large numbers of animals are required to accurate investigate the associations of κ-CN genotypes with milk yield and components traits for the Egyptian buffaloes.

REFERENCES

Abdel Dayem, A. M. H., K. G. M. Mahmoud, M. F. Nawito, M. M. Ayoub and S. F. Darwish (2009). Genotyping of kappa-casein gene in Egyptian buffalo bulls. Livestock Science, 122: 286-289.

Al-Shawa, Z. M., M. F. El-Zarei, A. A. Ghazy, M. A. Ayoub, S. M. Merdan and S. A. Mokhtar (2020). Polymorphism, Allelic and Genotypic frequencies of κ-Casein and β-LG genes in Egyptian Buffaloes, In Press.

Alim, M. A., D. Sun, Y. Zhang, Y., Zhang, Q. Zhang and L. Liu (2015). DNA Polymorphisms in the β-lactoglobulin and κ–casein Genes Associated with Milk Production Traits in Dairy Cattle . Biores Comm, 1: 82-86.

Awad, A., I. E. El Araby, K. M. El-Bayomi and A. W. Zaglool (2016). Association of polymorphisms in kappa casein gene with milk traits in Holstein Friesian cattle. Japanese Journal of Veterinary Research, 64(2): S39-S43.

Barroso, A., S. Dunner and J. Canon (1998). Detection of Bovine Kappa-Casein Variants A, B, C, and E by Means of Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP). Journal of Animal Science, 76(6): 1535-1538.

Boldman, K. G., L. A. Kriese, L. D. Van Vleck, C. P. Van Tassell and S. D. Kachman (1995). A manual for use of MTDFREML: a set of programs to obtain estimates of variances and covariances. Lincoln: Agricultural Research Service.

Botaro, B. G., Y. V. R. D. Lima, C. S. Cortinhas, L. F. P. E. Silva, F. P. Rennó and M. V. P. Santos (2009). Effect of the kappa-casein gene polymorphism, breed and seasonality on physicochemical characteristics, composition and stability of bovine milk. Revista Brasileira de Zootecnia, 38(12): 2447-2454.

Cockrill, W. R. (1981). The Water Buffalo: A Review. British Veterinary Journal, 137(1): 8-16.

Djedović, R., V. Bogdanović, P. Perišić, D. Stanojević, J. Popović and Brka (2015). Relationship between genetic polymorphism of κ-casein and quantitative milk yield traits in cattle breeds and crossbreds in Serbia. Genetika, 47(1): 23-32.

Eggen, A. (2012). The development and application of genomic selection as a new breeding paradigm. Animal frontiers, 2(1): 10-15.

El Nahas, S. M. and A. A. Abou Mossallam (2015). AcuI identifies water buffalo CSN3 genotypes by RFLP analysis. Journal of Genetics, 94(1): 94-96.

El Nahas, S. M., M. A. Bibars and D. A. Taha (2013). Genetic characterization of Egyptian buffalo CSN3 gene. Journal of Genetic Engineering and Biotechnology, 11(2): 123-127.

FAO (2019). Live Animals data, Food and Agriculture Organization of the United Nations, Rome, Italy, 2019

Gouda, E. M., M. K. Galal and S. A. Abdelaziz (2013). Genetic variants and allele frequencies of kappa casein in Egyptian cattle and buffalo using PCR-RFLP. Journal of Agricultural Science, 5(2):197-203. .

Goudet, J. (2002). FSTAT version 2.9.3.2, a program to estimate and test gene diversities and fixation indices. http://www2.unil.ch/popgen/softwares/fstat.htm

Groeneveld, L. F., J. A. Lenstra, H. Eding, M. A. Toro, B. Scherf, D. Pilling, R. Negrini, E. K. Finlay, H. Jianlin, E. Groeneveld, S. Weigend and C. Globaldiv (2010). Genetic diversity in farm

animals-a review. Animal Genetics, 41(1):1-26.

Hamza, A. E., Z. P. Yang, X. L. Wang, R. J. Chen, H. T. Wu and A.I. Ibrahim (2010). Kappa Casein Gene Polymorphism and its Impact on Milk Yield and Reproductive Performance Traits of Chinese Holstein Cattle . Agricultural Journal, 5(5): 283-285.

Hamza, A. E., Z. P. Yang, X. L. Wang, R. J. Chen, H. T. Wu and A. I. Ibrahim (2011). The impact of kappa casein gene polymorphism on milk components and other productive performance traits of Chinese Holstein cattle. Pakistan Veterinary Journal, 31(2): 153-156.

Hassanein, M. K., S. M. Abolmaaty, A. A. Khalil, M. O. Taqi, Y. H. Essa and H. H. Shawki (2013). Geographical Distribution and Developmental Pattern of Buffalo in Egypt. World Rural Observations, 5(4): 14-19.

Hussain, D. A. and M. H. Twayej (2016). Genetic structure analysis of kappa-casein gene/HindIII and its relationship with some productive traits in Iraqi cows population (comparative study). World Journal of Pharmaceutical Research, 5(4): 30-38.

Kübarsepp, I., M. Henno, H. Viinalass and D. Sabre (2005). Effect of κ-casein and β-lactoglobulin genotypes on the milk rennet coagulation properties. Agronomy Research, 3(1): 55-64.

Kučerová, J., A. Matějiček, O. M. Jandurová, P. Sørensen, E. Němcová, M. Štipková, T. Kott, J. Bouška and J. Frelich (2006). Milk protein genes CSN1S1, CSN2, CSN3, LGB and their relation to genetic values of milk production parameters in Czech Fleckvieh. Czech Journal of Animal Science, 51(6): 241-247.

Lara, M. A. C., L. T. Gama, G. Bufarah, J. R. B. Sereno, E. M. L. Celegato and U. P. De Abreu (2002). Genetic polymorphisms at the k-casein locus in Pantaneiro cattle. . Archivos de zootecnia, 51(194): 99-105.

Medrano, J. F. and E. Aguilar-Cordova (1990). Genotyping of bovine kappa-casein loci following DNA sequence amplification. Nature Biotechnology, 8(2), 144-146.

Miluchová, M., M. Gábor, J. Candrák, A. Trakovická and K. Candráková (2018). Association of HindIII-polymorphism in kappa-casein gene with milk, fat and protein yield in Holstein Cattle. Acta Biochimica Polonica, 65(3): 403-407.

Mir, S. N., O. Ullah and R. Sheikh (2014). Genetic polymorphism of milk protein variants and their association studies with milk yield in Sahiwal cattle. African Journal of Biotechnology, 13(4): 555-565.

Mohammadi, Y., A. A. Aslaminejad, M. R. Nassiry and A. Esmailizadeh Koshkoieh (2013). Allelic polymorphism of K-casein, β-Lactoglobulin and leptin genes and their association with milk production traits in Iranian Holstein cattle. Journal of Cell and Molecular Research, 5(2): 75-80.

Othman, E. O., F. A. Zayed, A. A. El Gawead and M. R. A. El-Rahman (2011). Genetic polymorphism of three genes associated with milk trait in Egyptian buffalo. Journal of Genetic Engineering and Biotechnology, 9(2): 97-102.

Patel, R., J. Chauhan, K. Singh and K. Soni (2007). Genotyping and allelic frequencies of κ-CN and β-LG in Indian river buffalo bulls. Buffalo Bull, 26(3): 63-66.

Petrovska, S., D. Jonkus, J. Zagorska and I. Ciprovica (2017). The influence of kappa-casein and beta-lactoglobulin gneotypes on milk coagulation properties in Latvia dairy breed. Research for Rural Development, 2: 74-80

Rachagani, S., I. D. Gupta, N. Gupta and S. C. Gupta (2006). Genotyping of β-Lactoglobulin gene by PCR-RFLP in Sahiwal and Tharparkar cattle breeds. BMC genetics, 7(1): 31.

Riaz, M. N., N. A. Malik, F. Nasreen, S. Sadaf and J. A. Qureshi (2008). Molecular Marker Assisted Study of Kappa-Casein Gene in Nili-Ravi (Buffalo) Breed of Pakistan. Buffalo Bull, 27(3): 240-244.

Safronova, O. S., E. A. Babich, L. Y. Ovchinnikova and A. A. Ovchinnikov (2017). Polymorphism of Kappa-Casein, Somatotropin, Beta-Lactoglobulin, Prolactin, and Thyreoglobulin Genes of Black and White Cattle of North Kazakhstan. Journal of Pharmaceutical Sciences and Research, 9(5): 568.

SAS (1999). SAS' Procedure Guide. Versin 6.12 Edition. SAS Institute, INC, Cary, NC, USA.SAS.

Trakovická, A., N. Moravčíková and A. Navrátilová (2012). Kappa-casein gene polymorphism (CSN3) and its effect on milk production traits. Acta Fytotechnica et Zootechnica, 15(3): 61-64.

Wolanciuk, A. (2015). The association of genetic variants of β-lactoglobulin and κ-casein with yield and chemical composition of milk obtained from four breeds of cow. Scientific Annals of Polish Society of Animal Production, 1: 21-32.

Yang, W., X. Kang, Q. Yang, Y. Lin and M. Fang (2013). Review on the development of genotyping methods for assessing farm animal diversity. Journal of Animal Science and Biotechnology, 4(1): 2.

التنميط الوراثي لجين الكابا كازين باستخدام تقنية PCR-RFLP وارتباطه بصفات إنتاج اللبن في الجاموس المصري

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تهدف هذه الدراسة لاكتشاف الارتباط بين الأنماط الوراثية المختلفة لجين الكابا-كازين والناتجة من استخدام تقنية PCR-RFLP وأنزيمات القطع مع كلاً من محصول اللبن ومكوناته الكيميائية باستخدام تحليل الانحدار المتعدد التدريجي. حيث تم تجميع ٨٠ عينة دم من عشائر الجاموس المصري محل الدراسة للتوصيف الوراثي لجين الكابا-كازين من خمس مناطق جغرافية مختلفة بجمهورية مصر العربية بإجمالي ٣٤٠ سجل إنتاجي وسجلات نسب لعدد ٥٩ حيوان حلاب. تم تحليل عينات اللبن باستخدام جهاز Milkoscan. تشير نتائج تحليل الانحدار المتعدد التدريجي لأهمية الارتباط بين التراكيب الوراثية الناتجة عن القطع بأنزيم *AcuI* ومعظم الصفات المدروسة مثل محصول اللبن الكلي، نسبة الدهن، الجوامد الكلية، الرماد، الرطوبة، بينما نجد في بعض الحالات أن التباين المشترك بين المتغيرات المستقلة (*Acu*I، *HpyCH4*IV، Compacted Genotypes) يمثل أفضل معادلة لتقدير انحدار بعض الصفات مثل محصول اللبن اليومي ونسبة اللاكتوز على التراكيب الوراثية الناتجة. كما أن متوسط القيم التربوية لمعظم الصفات المدروسة للحيوانات ذات التراكيب الوراثية الخليطة أفضل منها في الحيوانات متماثلة التراكيب الوراثية حيث تعتبر أفضل حيوانات منتجة لحليب أعلى إنتاجاً ذو خصائص مميزة في نسبة الدهن، الجوامد الكلية، الجوامد الغير دهنية والرماد. في حين تتميز الحيوانات متماثلة التركيب الوراثي في نسبة اللاكتوز والرطوبة. ووفقاً للنتائج المستخلصة من هذه الدراسة فإنه يجب أن تتضمن برامج الانتخاب الحديثة تعدد الأشكال المظهرية لجين الكابا-كازين كجينات مساعدة لعملية الانتخاب وذلك من خلال السماح باختيار الحيوانات في عمر مبكر لضمان التحسن السريع في الأداء الإنتاجي للحيوانات.