Single nucleotide polymorphisms and metabolic biochemical profile of productive markers characterize three European breeds of dairy cattle

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Abstract

The objective of this study was to investigate polymorphisms of *DGAT1, FABP, OLR1* and *ATP1A1* genes using PCR-DNA sequencing, and to associate these genetic structures to changes in metabolic biochemical markers and milk composition indicators in a total of 90 dairy cows of the Holstein, Simmental, and Brown Swiss breeds (30 cows each). PCR was carried out for amplification of 411-bp of *DGAT1*, 525-bp of *FABP*, 582-bp of *OLR1*, and 300-bp of ATP1A1 genes. Three breeds' nucleotide sequence variations in the form of single nucleotide polymorphisms (SNPs) were detailed by DNA sequencing analysis. Chi-square analysis showed that the distribution of all discovered SNPs varied significantly ($P < 0.001$). Biochemical indices in cow's serum revealed no significant difference in serum total protein, albumin, and total cholesterol among the three breeds. However, triglyceride showed a significant increase in Simmental compared to either Holsteins or Brown Swiss, while the highest mean value of triiodothyronine (T3) and tetraiodothyronine (T4) was detected in Holstein dairy cows The milk composition indicators analysis revealed that milk protein, sugar, and density were significantly higher in Holsteins than both Simmental and Brown Swiss. Meanwhile, milk fat and total solids revealed a significantly higher increase in Simmental than both brown Swiss and Holstein. As a result, the metabolic biochemical markers profile along with the identified SNPs could be used as a candidate and a reference guide for effective characterization of the Holstein, Simmental, and Brown Swiss breeds, leading to the creation of a marker-assisted selection system for production traits in dairy cattle breeds.

Biochemical markers, DNA sequencing, dairy breeds, milk constituents, productive genes

The inter- and intra-differences between livestock breeds may be coupled to the effect of their evolution, origin, and history (Troy et al. 2001). Despite the fact that world-wide differences between animal breeds cannot justify classifying a species as vulnerable, reduction in the number of indigenous breeds of livestock can be considered a non-compensable and irreversible wash-away of genetic material (Taberlet et al. 2008). The use of single nucleotide polymorphisms (SNPs) or whole genome scanning may therefore reshape the findings of previous studies assessing genetic variations and genetic determinants of livestock breeds, and thus provide more in-depth understanding of molecular basis of genetic diversity (Groeneveld et al. 2010). In order to improve effective and practical selection and breeding of livestock, particularly for genetic traits including growth rate, body weight, carcass characters, feed intake, and milk production and composition, there is an increase in the application of molecular genetic technologies that represent specific DNA markers linked to various quantitative trait loci (QTL) (Spelman and Bovenhuis 1998).

Generally, most of the productivity traits such as milk production and composition have been shown to be affected by numerous polymorphisms in different loci of genes (Buitkamp and Götz 2004). For example, associations have been documented between

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contents of milk fat in cattle and gene encoding acylCoA-diacylglycerol acyltransferase1 (DGAT1) (Winter et al. 2002). According to reports, the DGAT1 enzyme is essential for the synthesis of triglycerides, the development of connective tissue that is rich in fat, gut fat absorption, and the production of lipoprotein (Cases et al. 1998). Similar to this, DGAT1 has been identified as a key gene influencing milk production and milk fat percentages (Thaller et al. 2003).

Fatty acid binding proteins (FABPs) have been given a variety of classifications of actions, including intracellular protein transport, modification of different enzymes involved in lipid metabolism, regulation and expression of genes responsive to fatty acids, and fatty acid content of cell membranes (Zimmerman and Veerkamp 2002). According to studies, BTA14 harbours FABP4 in a region (46,833,665-46,838,053) that is rich in QTL for milk production features (Ogorevc et al. 2009), indicating that BTA14 could be used as a marker candidate gene to assess milk production traits.

Bovine Na^+, K^+-ATPase protein complex is made up of several isoforms and subunits (Herrera et al. 1987). The subunit's main isoform, the kind found in red blood cells, is encoded by the *ATP1A1* gene. The 3065 nucleotides that make up the coding sequence for the bovine *ATP1A1* gene are dispersed over 22 exons. Sequence variants are known to be useful for gene mapping, defining population structure, and conducting functional research (Dybus and Grzesiak 2006). One SNP was discovered in the bovine *ATP1A1* gene by Hawken et al. (2004).

The oxidised low-density lipoprotein receptor 1 (*OLR1*) gene is responsible for encoding lectins, which are the main protein responsible for binding to, internalising, and degrading oxidised low-density lipoprotein (oxLDL) (Khatib et al. 2006). The *OLR1* gene, which has 5 exons and codes for 279 amino acids of protein, is found on chromosome 5 (BTA5) in dairy cows. Additionally, it has been discovered that the oxidised lipids affect lipid metabolism in the liver and mammary glands and decrease glucose metabolism (Liao et al. 2008). OLR1, a crucial protein for oxLDL metabolism, might be responsible for these outcomes (Komisarek and Dorynek 2009).

According to research by Nowroozi-Asi et al. (2016), dairy animals with varying physiological and health situations exhibit changes in blood chemistry and hormone levels, which can significantly alter milk output (Huhtanen et al. 2002). The composition of milk varies between species, breeds, even individuals within a breed, and physiological status, such as lactation stage and season, parity, and milking interval (Gantner et al. 2015). Therefore, changes are periodically made to the chemical components of cow's milk to satisfy customer preferences (Boichard and Brochard 2012).

Little is known about the molecular characterization of productive genes in European cow breeds (Schennink et al. 2009). The genetic variants of the *DGAT1*, *FABP*, *ATPAT1*, and *OLR1* genes have been linked to productive qualities in a single breed of cattle, but investigations on these correlations in the Holstein, Simmental, and Brown Swiss breeds of cattle are lacking. In addition, few studies have examined the biochemical markers and hormone profiles that differ between dairy cattle breeds without mentioning nutrition and management.

Therefore, the goal of the current study was to analyse the genetic makeup of the Holstein, Simmental, and Brown Swiss cattle breeds using DNA sequencing to detect variation at the loci of functional genes expressing *DGAT1, OLR1, FABP*, and *ATP1A1*. The study also sought to determine the relationships between these genetic traits and serum concentrations of different metabolic biochemical indicators, such as total protein, albumin, total cholesterol, triacylglycerols, triiodot hyronine (T3) and tetraiodot hyronine (T4), as well as concentrations of different milk composition indicators, such as protein, fat, lactose, total solids, and milk density in these breeds.

Materials and Methods

Experimental animals

The Faculty of Veterinary Medicine at Mansoura University's Research Ethics Committee set standards for the care and handling of experimental animals which were followed during the sample collection and animal care phases of this investigation (code number R/23).

For this study, a total of 90 dairy cows (30 of each breed: Holstein, Simmental, and Brown Swiss) were used. The animals were from a private farm in Egypt's Ismailia Governorate, on the Ismailia Desert Road. A commercial dairy herd of about 450 animals was used to grow the animals, which were in their third lactation season. Cows were typically 3 years old and weighed 450 kg on average. A cubicle (free-stall/feedlot) barn with straw-bedded stalls and a slatted floor that was periodically scraped was used to keep the animals. They were artificially inseminated, given a total mixed ration (TMR), and milked twice daily. Each cow produced an average of 8,500 kg of milk/year after energy correction.

Sample collection

Two blood samples (5 ml each) were taken from each animal using a jugular venipuncture under strict aseptic conditions and placed in tubes with disodium EDTA as an anticoagulant for whole blood collection and without anticoagulant to separate serum. For DNA extraction, EDTA-filled tubes were utilised. The plain tubes were centrifuged for 15 min at 3500 rpm after being left at room temperature until they had clotted. The serum was then pipetted automatically into sterile, clean tubes, and stored at -20 °C for later biochemical analysis.

DNA extraction and polymerase chain reaction for productive genes

Extraction of the genomic DNA from whole blood was done using Gene JET whole blood genomic DNA extraction kit following the manufacturer's instructions (Thermo Scientific, Lithuania). Polymerase chain reaction was carried out for amplification of fragments of exon 7 of *DGAT1* (411-bp), exon 4 of *FABP* (525-bp), exon 6 of *OLR1* (582-bp), and exon 17 of *ATP1A1* (300-bp) genes.

Table 1 lists the primers used in the amplification. The polymerase chain reaction (PCR) mixture was run in a thermal cycler with a final volume of 100 µl; 6 µl of DNA, 41 µl of double-distilled water, 50 µl of PCR master mix (Jena Bioscience, Germany), and 1.5 µl of each primer were all present in each reaction volume. The first denaturation temperature of 94 °C was applied to the reaction mixture for 4 min. Thirty cycles of denaturation at 94 °C for 1 min, annealing at temperatures (as stated in Table 1) for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min were performed. At 4 °C, samples were kept.

Table 1. Forward and reverse primer sequence, length of polymerase chain reaction (PCR) product and annealing temperature for *DGAT1*, *FABP, ATP1A1* and *OLR1* genes.

Primer	Forward	Reverse	Annealing temperature	Length of PCR product	Reference
DGAT1	5′-GCACCATCCTCTTCCTCAAG -3′	5-′GGAAGCGCTTTCGGATG -3′	56 °C	411-bp	Akyüz et al. 2015
OLR1	5′TAT CCT TCA GGG ACC TGT GC -3′	5′-CAG CAA ATG TTG CAA AAA CAA -3′	60 °C	582-bp	Rychtářová et al. 2014
FABP	5′ACTTAGATGAAGGTGCTCTG-3′	CCTCAGGACTAAACAACTTATG-3′	55 °C	525-bp	Zhou et al. 2015
ATP1A1	5′ ACAAACAAAAGG GTCACAACA -3′	5′ CTTACCCTAGATCCT GGCTCAT -3′	58 °C	300-bp	Liu et al. 2010

DNA sequencing and polymorphism detection

PCR purification kit was used, and the manufacturer's instructions were followed to purify PCR products of the required size (target bands) (Jena Bioscience). PCR product quantification was done with Nanodrop (Uv-Vis spectrophotometer Q5000/USA). According to Sanger et al. (1977), PCR products with target bands were sent for DNA sequencing in forward and reverse directions using ABI 3730XL DNA sequencer based on enzymatic chain terminator technique (Applied Biosystem, USA) to detect single nucleotide polymorphisms in the productive genes between the three breeds.

Chromas 1.45 and blast 2.0 software were used to analyse the DNA sequencing data (http://www.technelysium.com.au). Single-nucleotide polymorphisms (SNPs) were identified between the PCR results of a few productive genes and GenBank reference sequences. The MEGA4 software programme was used to compare the amino acid sequence variance of the productive genes among the three breeds based on DNA sequencing data alignment.

Metabolic biochemical analysis

Serum analysis

Serum total protein and albumin were determined according to the method of Dumas and Biggs (1972) using kits (Stan bio, USA); total cholesterol and triglycerides were determined using kits (Spinract, Spain) according to Young (2001). These indicators were measured spectrophotometerically using Lambada EZ201 (Perkin Elmer,

USA). Additionally, serum T3 and T4 concentrations were determined through chemiluminescent competitive analogue assay by IMMULITE 2000 using kits (Siemens Health Diagnostics, products Ltd. Lianberis, United Kingdom) according to the technique recommended by Bayer et al. (1987).

Milk composition analysis

In the morning, milk samples were taken from cows of each breed and put into a milk scanning device (Funke Cerbber Milk Scan 2008 Labortechnik, Germany) (Zhou et al. 2015). The contents of milk protein, milk fat, lactose, total solids, and density were determined.

Statistical analysis

Data were examined for linearity, homogeneity of variance, and distribution normality. Statistical Analysis System (SAS) version 9.1 was then used to analyse them, and all results are presented as means with standard error of the mean (SEM). Data were compared using PROC General Linear Model (GLM) technique for one-way analysis of variance (ANOVA), and Duncan's Multiple Range test was performed as a *post hoc* test where it was necessary to compare the Least Square Means of various treatments (Holm 1979). Chi-square test was used to compare the frequencies of SNPs in the Holstein, Simmental, and Brown Swiss breeds. The level of significance was chosen at $P < 0.05$.

Results

Molecular characterization of *DGAT1, FABP, ATP1A1* and *OLR1* genes

PCR-DNA sequencing revealed a variation in nucleotide sequence in form of SNPs between Holstein, Simmental, and Brown Swiss breeds and Chi-square analysis showed a significant difference in the frequencies of investigated genes between the three breeds (Table 2). DNA sequencing of the *DGAT1* gene (411-bp) revealed two SNPs specific for a number of Holstein cows. DNA sequencing of the *FABP* gene (525-bp) revealed 45 SNPs; one SNP specific for a number of Holstein cows, 38 SNPs specific for a number of Simmental cows, and five SNPs specific for a number of Brown Swiss cows. Additionally, one SNP seemed to characterize a number of cows in both Holstein and Simmental breeds, and common SNP in a number of Simmental and Brown Swiss cows. Regarding the *ATPAT1* gene, DNA sequencing for a fragment of 330-bp elicited three SNPs; one SNP specific for a number of Brown Swiss cows and one SNP seemed to characterize a number of Holstein cows. A common SNP was also reported to characterize a number of cows of both Holstein and Brown Swiss breeds. Concerning the *OLR1* gene, DNA sequencing of 582-bp fragment revealed three SNPs; one SNP specific for a number of Holstein cows, one SNP was common for a number of cows of both Holstein and Simmental breeds, and one SNP was detected in a number of cows of the three breeds.

Variations in the nucleotide sequence of *DGAT1*, *FABP*, *OLR1* and *ATPAT1* genes among the three breeds, as well as between these breeds and the reference sequences available in GenBank were classified as single-nucleotide polymorphisms (SNPs) (Figs 1–4). Amino acids sequence variation of the coding regions, exon 7 of *DGAT1*, exon 4 of *FABP*, exon 6 of *OLR1* and exon 17 of *ATPAT1* genes between the three breeds is shown in Table 3. The identified two SNPs in *DGAT1* gene were non-synonymous. Concerning the *FABP* gene, the identified 45 SNPs revealed six synonymous mutations; while the remaining SNPs revealed non-synonymous ones. The identified three SNPs in the *ATPAT1* gene revealed two mutations; where one was synonymous and the other was non-synonymous. Regarding the *OLR1* gene, out of the three identified SNPs, two were non-synonymous.

Metabolic biochemical indicators and milk composition

Results for the serum biochemical indicators of the three breeds are presented in Table 4. There was no difference in serum total protein, serum albumin, and total cholesterol among the three breeds of cattle, however, contents of triacylglycerols differed significantly between the Simmental and Brown Swiss breeds ($P \leq 0.01$), with Simmental showing higher levels compared to Brown Swiss. Mean values of T3 and T4 in serum differed between Holstein

Table 2. Distribution of single-nucleotide polymorphisms (SNPs) of *DGAT1*, *FABP, ATP1A1* and *OLR1* in Holstein, Simmental, and Brown Swiss cattle breeds.

Gene	SNP type	Holstein (n = 30)	Simmental (n = 30)	Brown Swiss (n = 30)	Total animals (n = 90)	Pearson Chi square	*P* value
DGAT1	G200A	28	----	----	28/90	81.290	0.0001**
	A403G	25	----	----	25/90	69.230	0.0001**
FABP	A66G		----	27	27/90	77.143	0.0001**
	T70G	----	----	24	24/90	65.455	0.0001**
	C71T	----	----	26	26/90	73.125	0.0001**
	A72C	----	----	29	29/90	85.574	0.0001**
	A72G	----	16	----	16/90	38.919	0.0001**
	G73T	----	16	----	16/90	88.919	0.0001**
	T74G	----	11	----	11/90	25.063	0.0001**
	G76A	----	18	----	18/90	45.000	0.0001**
	A77T	----	12	----	12/90	27.692	0.0001**
	T78G	----	14	----	14/90	33.158	0.0001**
	G79A	----	21	----	21/90	54.783	0.0001**
	A80T	----	16	----	16/90	38.919	0.0001**
	T81A	----	16	----	16/90	38.919	0.0001**
	A83G	----	12	----	12/90	27.692	0.0001**
	G84A	----	12	----	12/90	27.690	0.0001**
	A85T	----	19	----	19/90	48.169	0.0001**
	T86G	----	22	----	22/90	58.230	0.0001**
	G88T	----	18	----	18/90	45.000	0.0001**
	T89G	----	18	----	18/90	45.000	0.0001**
	G90C	----	16	----	16/90	38.919	0.0001**
	C91T	----	16	----	16/90	38.919	0.0001**
	T92C	----	20	----	20/90	22.500	0.0001**
	C93G	26	18	----	44/90	47.312	0.0001**
	T149C	----	16	24	40/90	40.320	0.0001**
	C396T	26	----	----	26/90	73.125	0.0001**
	T402C	----	14	----	14/90	33.158	0.0001**
	T422G	----	16	----	16/90	38.919	0.0001**
	A437C	----	12	----	12/90	27.692	0.0001**
	A438C	----	16	----	16/90	38.919	0.0001**
	G439C	-----	12	----	12/90	27.692	0.0001**
	T456A	-----	18	----	18/90	45.000	0.0001**
	C468T	----	15	----	15/90	36.000	0.0001**
	T473G	----	19	----	19/90	48.169	0.0001**
	C474G	----	21	----	21/90	54.783	0.0001**
	A475G	----	16	----	16/90	38.919	0.0001**
	G481T	----	19	----	19/90	48.169	0.0001**
	A482T	----	11	----	11/90	25.063	0.0001**
	A483T	----	16	----	16/90	38.919	0.0001**
	A494T	----	12	----	12/90	27.692	0.0001**
	A502T	----	12	----	12/90	27.692	0.0001**
	A503T	----	12	----	12/90	27.692	0.0001**
	T507C	----	20	----	20/90	51.429	0.0001**
	T516A	----	14	----	14/90	33.158	0.0001**
	A517T	----	12	----	12/90	27.692	0.0001**
	A517G	----	18	----	18/90	45.000	0.0001**
	T518G	----	----	22	22/90	58.235	0.0001**
ATP1A1	G172A	----	-----	19	19/90	48.169	0.0001**
	A305C	20	----	8	28/90	31.521	0.0001**
	T306A	17	----	----	17/90	41.918	0.0001**
OLR1	T332C	24	11	----	35/90	40.488	0.0001**
	C364T	11	----	----	11/90	25.063	0.0001**
	A447G	18	4	9	31/90	14.861	0.001*

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EU348567.1  ACCATCCTCTTCCTCAAGCTGTTCTCCTACCGGGACGTCAACCTCTGGTGCCGAGAGCGC 60
H           ACCATCCTCTTCCTCAAGCTGTTCTCCTACCGGGACGTCAACCTCTGGTGCCGAGAGCGC 60
S           ACCATCCTCTTCCTCAAGCTGTTCTCCTACCGGGACGTCAACCTCTGGTGCCGAGAGCGC 60
B           ACCATCCTCTTCCTCAAGCTGTTCTCCTACCGGGACGTCAACCTCTGGTGCCGAGAGCGC 60
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EU348567.1  AGGGCTGGGGCCAAGGCCAAGGCTGGTGAGGGCTGCCTCGGGCTGGGGCCACTGGGCTGC 120
H           AGGGCTGGGGCCAAGGCCAAGGCTGGTGAGGGCTGCCTCGGGCTGGGGCCACTGGGCTGC 120
S           AGGGCTGGGGCCAAGGCCAAGGCTGGTGAGGGCTGCCTCGGGCTGGGGCCACTGGGCTGC 120
B           AGGGCTGGGGCCAAGGCCAAGGCTGGTGAGGGCTGCCTCGGGCTGGGGCCACTGGGCTGC 120
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EU348567.1  CACTTGCCTCGGGACCGGCAGGGGCTCGGCTCACCCCCGACCCGCCCCCTGCCGCTTGCT 180
H           CACTTGCCTCGGGACCGGCAGGGGCTCGGCTCACCCCCGACCCGCCCCCTGCCGCTTGCT 180
S           CACTTGCCTCGGGACCGGCAGGGGCTCGGCTCACCCCCGACCCGCCCCCTGCCGCTTGCT 180
B           CACTTGCCTCGGGACCGGCAGGGGCTCGGCTCACCCCCGACCCGCCCCCTGCCGCTTGCT 180
            ************************************************************

EU348567.1  CGTAGCTTTGGCAGGTAAGGCGGCCAACGGGGGAGCTGCCCAGCGCACCGTGAGCTACCC 240
H           CGTAGCTTTGGCAGGTAAGACGGCCAACGGGGGAGCTGCCCAGCGCACCGTGAGCTACCC 240
S           CGTAGCTTTGGCAGGTAAGGCGGCCAACGGGGGAGCTGCCCAGCGCACCGTGAGCTACCC 240
B           CGTAGCTTTGGCAGGTAAGGCGGCCAACGGGGGAGCTGCCCAGCGCACCGTGAGCTACCC 240
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EU348567.1  CGACAACCTGACCTACCGCGGTGAGGATCCTGCCGGGGGCTGGGGGGACTGCCCGGCGGC 300
H           CGACAACCTGACCTACCGCGGTGAGGATCCTGCCGGGGGCTGGGGGGACTGCCCGGCGGC 300
S           CGACAACCTGACCTACCGCGGTGAGGATCCTGCCGGGGGCTGGGGGGACTGCCCGGCGGC 300
B           CGACAACCTGACCTACCGCGGTGAGGATCCTGCCGGGGGCTGGGGGGACTGCCCGGCGGC 300
            ************************************************************

EU348567.1  CTGGCCTGCTAGCCCCGCCCTCCCTTCCAGATCTCTACTACTTCCTCTTCGCCCCCACCC 360
H           CTGGCCTGCTAGCCCCGCCCTCCCTTCCAGATCTCTACTACTTCCTCTTCGCCCCCACCC 360
S           CTGGCCTGCTAGCCCCGCCCTCCCTTCCAGATCTCTACTACTTCCTCTTCGCCCCCACCC 360
B           CTGGCCTGCTAGCCCCGCCCTCCCTTCCAGATCTCTACTACTTCCTCTTCGCCCCCACCC 360
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EU348567.1  TGTGCTACGAGCTCAACTTCCCCCGCTCCCCCCGCATCCGAAAGCGCTTCC     411
H           TGTGCTACGAGCTCAACTTCCCCCGCTCCCCCCGCATCCGAAGGCGCTTCC     411
S           TGTGCTACGAGCTCAACTTCCCCCGCTCCCCCCGCATCCGAAAGCGCTTCC     411
B           TGTGCTACGAGCTCAACTTCCCCCGCTCCCCCCGCATCCGAAAGCGCTTCC     411
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Fig. 1. Representative DNA sequence alignment of the *DGAT1* gene (411-bp) among Holstein, Simmental, and Brown Swiss cattle and the reference sequence available in GenBank gb|EU348567.1|. Asterisks represent similarity. H - Holstein, S - Simmental, B - Brown Swiss

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CP027082.1  ACTTAGATGAAGGTGCTCTGGTACAAGTACAAAACTGGGATGGAAAATCAACCACCATAA 60
S           ACTTAGATGAAGGTGCTCTGGTACAAGTACAAAACTGGGATGGGAAATCAACCACCATAA 60
B           ACTTAGATGAAGGTGCTCTGGTACAAGTACAAAACTGGGATGGGAAATCAACCACCATAA 60
H           ACTTAGATGAAGGTGCTCTGGTACAAGTACAAAACTGGGATGGGAAATCAACCACCATAA 60
S           ACTTAGATGAAGGTGCTCTGGTACAAGTACAAAACTGGGATGGGAAATCAACCACCATAA 60
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CP027082.1  AGAGAAAACTC-GTGGATGATAAGATGGTGCTGGTGAGTATCTTCTCACTACTTAATTCT 120
S           AGAGAAAACTCGTGGATGATAAGATGGTGCTCGGTGAGTATCTTCTCACTACTTAATTCT 120
B           AGAGAGAACGTCGTGGATGATAAGATGGTGCTCGTGAGTATCTTCTCACTACTTAATTCT 120
H           AGAGAAAACTCAGTGGATGATAAGATGGTGCTGGTGAGTATCTTCTCACTACTTAATTCT 120
S           AGAGAAAACTCAGTGGATGATAAGATGGTGCTCGTGAGTATCTTCTCACTACTTAATTCT 120
            ***** ***     *      *    *      ****************************

CP027082.1  AGATTTTAGTGCTAGGTCATCCCATAATCGTTATCCTACCTAGAGAAATAGACAATCGCC 180
S           AGATTTCAGTGCTAGGTCATCCCATAATCGTTATCCTACCTAGAGAAATAGACAATCGCC 180
B           AGATTTCAGTGCTAGGTCATCCCATAATCGTTATCCTACCTAGAGAAATAGACAATCGCC 180
H           AGATTTCAGTGCTAGGTCATCCCATAATTGTTATCCTACCTAGAGAAATAGACAATCGCC 180
S           AGATTTCAGTGCTAGGTCATCCCATAATTGTTATCCTACCTAGAGAAATAGACAATCGCC 180
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CP027082.1  CTTGTAGAATGAAAAGTTAGTCTATTGGGATTATGGTTTCACTCTGACAATTATCCTTCT 240
S           CTTGTAGAATGAAAAGTTAGTCTATTGGGATTATGGTTTCACTCTGACAATTATCCTTCT 240
B           CTTGTAGAATGAAAAGTTAGTCTATTGGGATTATGGTTTCACTCTGACAATTATCCTTCT 240
H           CTTGTAGAATGAAAAGTTAGTCTATTGGGATTATGGTTTCACTCTGACAATTATCCTTCT 240
S           CTTGTAGAATGAAAAGTTAGTCTATTGGGATTATGGTTTCACTCTGACAATTATCCTTCT 240
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CP027082.1  AAGCTCCGTCTAGGTATACTGTGCCCCCAGCAGTATTTTCTTATCCCTCTCAATGTGAAC 300
S           AAGCTCCGTCTAGGTATACTGTGCCCCCAGCAGTATTTTCTTATCCCTCTCAATGTGAAC 300
B           AAGCTCCGTCTAGGTATACTGTGCCCCCAGCAGTATTTTCTTATCCCTCTCAATGTGAAC 300
H           AAGCTCCGTCTAGGTATACTGTGCCCCCAGCAGTATTTTCTTATCCCTCTCAATGTGAAC 300
S           AAGCTCCGTCTAGGTATACTGTGCCCCCAGCAGTATTTTCTTATCCCTCTCAATGTGAAC 300
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CP027082.1  CATATTGTATTGTGCATTTCTAATTATGTTTTTCACTCACCACATAGATGGTAAGATTCC 360
S           CGTATTGTATTGTGCATTTCTAATTATGTTTTTCACTCACCACATAGATGGTAAGATTCC 360
B           CGTATTGTATTGTGCATTTCTAATTATGTTTTTCACTCACCACATAGATGGTAAGATTCC 360
H           CGTATTGTATTGTGCATTTCTAATTATGTTTTTCACTCACCACATAGATGGTAAGATTCC 360
S           CGTATTGTATTGTGCATTTCTAATTATGTTTTTCACTCACCACATAGATGGTAAGATTCC 360
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CP027082.1  TTGAGGCCAAGTCTTGTATCTTCTTGATCTTTGTGTCTCCCTAGTTTATTACAATATCAG 420
S           TTGAGGCCAAGTCTTGTATCTTCTTGATCTTTGTGCCTCCCCAGTTTATTACAATATCAG 420
B           TTGAGGCCAAGTCTTGTATCTTCTTGATCTTTGTGCCTCCCTAGTTTATTACAATATCAG 420
H           TTGAGGCCAAGTCTTGTATCTTCTTGATCTTTGTGTCTCCCTAGTTTATTACAATATCAG 420
S           TTGAGGCCAAGTCTTGTATCTTCTTGATCTTTGTGCCTCCCTAGTTTATTACAATATCAG 420
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CP027082.1  GTATATAAGAAGAGCCAAGAGGGAATATCTTTTGATGAACATTTTTTCCTGCTCAACATT 480
S           GGATATAAGAAGAGCCCCCAGGGAATATCTTTTGAAGAACATTTTTTTCTGCGGGACATT 480
B           GTATATAAGAAGAGCCAAGAGGGAATATCTTTTGATGAACATTTTTTCCTGCTCAACATT 480
H           GTATATAAGAAGAGCCAAGAGGGAATATCTTTTGATGAACATTTTTTCCTGCTCAACATT 480
S           GTATATAAGAAGAGCCAAGAGGGAATATCTTTTGATGAACATTTTTTCCTGCTCAACATT 480
            * ***************   ***************** *********** ****   *****

CP027082.1  GAAGGAGACAATAAATAAATAAAACATAAGTTGTTTAGTCCTGAG 525
S           TTTGGAGACAATATATAAATATTACACAAGTTGTTTTTTCCTGAG 525
B           GAAGGAGACAATAAATAAATAAAACATAAGTTGTTTAGTCCTGAG 525
H           GAAGGAGACAATAAATAAATAAAACATAAGTTGTTTATTCCTGAG 525
S           GAAGGAGACAATAAATAAATAAAACATAAGTTGTTAGTTCCTGAG 525
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Fig. 2. Representative DNA sequence alignment of the *FABP* gene (525-bp) among Holstein, Simmental, and Brown Swiss cattle and the reference sequence available in GenBank gb|CP027082.1|. Asterisks represent similarity. H - Holstein, S - Simmental, B - Brown Swiss

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AJ496457.1  GAAGAACCTGGAGGCTGTGGAGACCTTGGGATCCACGTCCACCATCTGCTCAGACAAAAC 60
H           GAAGAACCTGGAGGCTGTGGAGACCTTGGGATCCACGTCCACCATCTGCTCAGACAAAAC 60
S           GAAGAACCTGGAGGCTGTGGAGACCTTGGGATCCACGTCCACCATCTGCTCAGACAAAAC 60
B           GAAGAACCTGGAGGCTGTGGAGACCTTGGGATCCACGTCCACCATCTGCTCAGACAAAAC 60
B           GAAGAACCTGGAGGCTGTGGAGACCTTGGGATCCACGTCCACCATCTGCTCAGACAAAAC 60
            ************************************************************

AJ496457.1  TGGAACTCTGACCCAGAACCGAATGACAGTGGCCCACATGTGGTTCGACAACCAAATCCA 120
H           TGGAACTCTGACCCAGAACCGAATGACAGTGGCCCACATGTGGTTCGACAACCAAATCCA 120
S           TGGAACTCTGACCCAGAACCGAATGACAGTGGCCCACATGTGGTTCGACAACCAAATCCA 120
B           TGGAACTCTGACCCAGAACCGAATGACAGTGGCCCACATGTGGTTCGACAACCAAATCCA 120
B           TGGAACTCTGACCCAGAACCGAATGACAGTGGCCCACATGTGGTTCGACAACCAAATCCA 120
            ************************************************************

AJ496457.1  CGAGGCGGACACGACAGAGAATCAGAGCGGTGTTTCATTTGACAAGACTTCGGCCACGTG 180
H           CGAGGCGGACACGACAGAGAATCAGAGCGGTGTTTCATTTGACAAGACTTCGGCCACGTG 180
S           CGAGGCGGACACGACAGAGAATCAGAGCGGTGTTTCATTTGACAAGACTTCGGCCACGTG 180
B           CGAGGCGGACACGACAGAGAATCAGAGCGGTGTTTCATTTGACAAGACTTCAGCCACGTG 180
B           CGAGGCGGACACGACAGAGAATCAGAGCGGTGTTTCATTTGACAAGACTTCGGCCACGTG 180
            ************************************************** ********

AJ496457.1  GCTCGCTCTGTCCAGAATTGCAGGTCTTTGTAACAGGGCCGTGTTTCAGGCTAACCAGGA 240
H           GCTCGCTCTGTCCAGAATTGCAGGTCTTTGTAACAGGGCCGTGTTTCAGGCTAACCAGGA 240
S           GCTCGCTCTGTCCAGAATTGCAGGTCTTTGTAACAGGGCCGTGTTTCAGGCTAACCAGGA 240
B           GCTCGCTCTGTCCAGAATTGCAGGTCTTTGTAACAGGGCCGTGTTTCAGGCTAACCAGGA 240
B           GCTCGCTCTGTCCAGAATTGCAGGTCTTTGTAACAGGGCCGTGTTTCAGGCTAACCAGGA 240
            ************************************************************

AJ496457.1  TAACCTGCCTATCCTGAAGCGGGCTGTAGCGGGTGATGCCTCAGAGTCTGCGCTCCTGAA 300
H           TAACCTGCCTATCCTGAAGCGGGCTGTAGCGGGTGATGCCTCAGAGTCTGCGCTCCTGAA 300
S           TAACCTGCCTATCCTGAAGCGGGCTGTAGCGGGTGATGCCTCAGAGTCTGCGCTCCTGAA 300
B           TAACCTGCCTATCCTGAAGCGGGCTGTAGCGGGTGATGCCTCAGAGTCTGCGCTCCTGAA 300
B           TAACCTGCCTATCCTGAAGCGGGCTGTAGCGGGTGATGCCTCAGAGTCTGCGCTCCTGAA 300
            ************************************************************

AJ496457.1  GTGCCATGAGGTGTGCTGCGGTTCTGTGAA 330
H           GTGCCATGAGGTGTGCTGCGGTTCTGTGAA 330
S           GTGCATTGAGGTGTGCTGCGGTTCTGTGAA 330
B           GTGCATTGAGGTGTGCTGCGGTTCTGTGAA 330
B           GTGCCTTGAGGTGTGCTGCGGTTCTGTGAA 330
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Fig. 3. Representative DNA sequence alignment of the *ATP1A1* gene (330-bp) among Holstein, Simmental, and Brown Swiss cattle and the reference sequence available in GenBank gb|AJ496457.1|. Asterisks represent similarity. H - Holstein, S - Simmental, B - Brown Swiss

Fig. 4. Representative DNA sequence alignment of the *OLR1* gene (582-bp) among Holstein, Simmental and Brown Swiss cattle and the reference sequence available in GenBank gb|XM_027541303.1|. Asterisks represent similarity. H - Holstein, S - Simmental, B - Brown Swiss

Table 3. Type and position of single-nucleotide polymorphisms (SNPs) in *DGAT1*, *FABP*, *ATP1A1* and *OLR1* genes and corresponding amino acid number and type.

Gene	SNP type	SNP position	Breed/no. of animals harbouring SNP	Type of mutation	Amino acid number and type
DGAT1	G/A	200	Holstein/28	Non-synonymous	67 G to D
	A/G	403	Holstein/25	Non-synonymous	135 S to G
FABP	A/G	66	Brown Swiss/27	Synonymous	22 E
	T/G&C/T&A/C	70&71& 72	Brown Swiss/24&26&29	Non-synonymous	24 S to V
	A/G	72	Simmental/16	Synonymous	24S
	G/T&T/G	73&74	Simmental/16&11	Non-synonymous	25 V to W
	G/A&A/T&T/G	76&77&78	Simmental/18&12&14	Non-synonymous	26 D to M
	G/A&A/T& T/A	79&80&81	Simmental/21&16&16	Non-synonymous	27 D to I
	A/G&G/A	83&84	Simmental/12	Non-synonymous	28 K to R
	A/T&T/G	85&86	Simmental/19&22	Non-synonymous	29M to W
	G/T&T/G&G/C	88&89&90	Simmental/18&18&16	Non-synonymous	30 V to C
	C/T&T/C	91&92	Simmental/16&20&	Non-synonymous	31 L to S
	C/G	93	Holstein& Simmental /26&18	Synonymous	31 L
	T/C	149	Simmental/16&Brown Swiss/ 24	Non-synonymous	50 L to S
	C/T	396	Holstein/26	Non-synonymous	132 C
	T/C	402	Simmental/14	Synonymous	135 P
	T/G	422	Simmental/16	Non-synonymous	141 V to G
	A/C&A/C	437&438	Simmental/12&16	Non-synonymous	146 Q to P
	G/C	439	Simmental/12	Non-synonymous	147 E to Q
	T/A	456	Simmental/18	Non-synonymous	152 D to E
	C/T	468	Simmental/15	Synonymous	156 F
	T/G&C/G	473&474	Simmental/19& Simmental/21	Non-synonymous	158 L to R
	A/G	475	Simmental/16	Non-synonymous	159 N to D
	G/T&A/T&A/T	481&482& 483	Simmental/19&11&16	Non-synonymous	161 E to F
	A/T	494	Simmental/12	Non-synonymous	165 K to I
	A/T&A/T	502&503	Simmental/12	Non-synonymous	168 K to L
	T/C	507	Simmental/20	Synonymous	169 H
	T/A	516	Simmental/14	Non-synonymous	172 F to L
	A/T	517	Simmental/12	Non-synonymous	173 I to F
	A/G	517	Simmental/18	Non-synonymous	173 I to V
	T/G	518	Brown Swiss/22	Non-synonymous	173 I to S
ATP1A1	G/A	172	Brown Swiss/19	Non-synonymous	58 G to S
	A/C &T/A	305& 306	Holstein/20&Brown Swiss/8	Synonymous	P
			Holstein/17	Non-synonymous	102 H to P
OLR1	T/C	332	Holstein/24&Simmental/11	Non-synonymous	111 P to L
	C/T	364	Holstein/11	Non-synonymous	122 S to P
	A/G	447	Holstein/18&Simmental/4& Brown Swiss/9	Synonymous	R

Table 4. Mean ± standard error of the mean (SEM) serum total protein, serum albumin, serum total cholesterol, serum triglycerides, serum triiodothyronine (T3) and serum thyroxine (T4) concentrations in Holstein, Simmental, and Brown Swiss cattle.

Serum indicator	Holstein	Simmental	Brown Swiss	F value	*P* value
Total protein (g/dl)	4.4 ± 0.12	4.7 ± 0.09	4.5 ± 0.02	3.076	0.076
Albumin (g/dl)	2.6 ± 0.07	2.8 ± 0.06	2.7 ± 0.06	1.827	0.195
Total cholesterol (mg/dl)	92.3 ± 20.2^{a}	95 ± 15.1^{b}	91.7 ± 0.76^{a}	2.162	0.080
Triglycerides (mg/dl)	28 ± 2.2^{ab}	33.7 ± 3.5^{a}	22 ± 0.97^{b}	5.744	0.010
T3 (ng/dl)	122.3 ± 2.5^{a}	102 ± 12.6^{ab}	75.7 ± 1.5^{b}	9.778	0.002
T4 (ng/dl)	6.6 ± 0.26^{a}	6.0 ± 0.72^{ab}	4.6 ± 0.04^{b}	5.352	0.010

Means of different levels within the same row having different superscript are significantly different ($P \leq 0.05$).

Table 5. Mean ± standard error of the mean (SEM) of milk protein, milk fat, milk lactose, total solids and milk density in Holstein, Simmental, and Brown Swiss cattle.

Milk constituent	Holstein	Simmental	Brown Swiss	F value	*P* value
Milk protein %	2.81 ± 0.04^{a}	2.73 ± 0.04^{b}	2.47 ± 0.01^{b}	30.221	< 0.0005
Milk fat %	1.6 ± 0.27^{b}	2.8 ± 0.39^{a}	2.7 ± 0.02^{a}	5.602	0.015
Milk lactose %	4.0 ± 0.06^{a}	3.9 ± 0.05^{a}	3.5 ± 0.02^{b}	35.444	< 0.0005
Total solids %	34.5 ± 0.60^{b}	37.5 ± 0.56^{a}	33.7 ± 0.06^{b}	17.113	< 0.0005
Milk density	26.9 ± 0.48^{a}	25.5 ± 0.28^{a}	22.7 ± 0.19^{b}	40.565	< 0.0005

Means of different levels within the same row having different superscript are significantly different ($P \leq 0.05$).

and Brown Swiss ($P \leq 0.01$) with Holstein displaying higher values compared to Brown Swiss breed. Milk composition indices for the three breeds are presented in Table 5. Milk protein % was higher in Holsteins ($P \leq 0.0005$) compared to the Simmental and Brown Swiss breeds. Milk fat % was higher ($P \leq 0.01$) in Simmental and Brown Swiss compared to the Holstein breed. Additionally, milk sugar (lactose) % and density ($P \leq 0.0005$) were higher in Holstein and Simmental compared to the Brown Swiss breed. Total solids % was higher ($P \leq 0.0005$) in Simmental compared to Holstein and Brown Swiss breeds.

Discussion

In this context, PCR-DNA sequencing was carried out for molecular characterization of fragments of exon 7 of *DGAT1* (411-bp), exon 4 of *FABP* (525-bp), exon 6 of *OLR1* (582-bp), and exon 17 of *ATP1A1* (300-bp) genes in the Holstein, Simmental, and Brown Swiss breeds of cattle exposed to the environmental conditions of Egypt. There is little information on the molecular characterization of productive genes in European breeds of cattle (Schennink et al. 2009). Moreover, this is the first study that reports genetic polymorphisms of *DGAT1*, *FABP, ATPAT1* and *OLR1* genes in the Holstein, Simmental, and Brown Swiss breeds.

Studies have reported an association between *DGAT1*, *FABP, ATPAT1, OLR1* gene polymorphism and milk production and components in cattle (Khatib et al. 2006; Schennink et al. 2009; Ibrahim et al. 2019). However, unlike our study, all of these studies investigated the association of gene polymorphism in only one breed of cattle. Also, all previous studies reported gene polymorphisms using other genetic markers as restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP). DNA sequence alignment revealed novel single nucleotide polymorphisms in *DGAT1*, *FABP*, *OLR1, ATP1A1* genes when matched with the GenBank reference

sequence (Figs 1–4). Interestingly, our findings showed that the discovered polymorphisms are being reported for the first time here. The remaining portion of the amplified fragments' similarity and the shared SNPs among the three breeds may be due to the use of PCR-DNA sequencing on a conserved exon of the investigated genes, which enables precise molecular characterization of the genes and identifies physiological variations in the breeds' milk production and constituents (Singh et al. 2014). Other factors could include the historical breed gene flow and close geographic proximity (Filipek et al. 2018).

The cow breed and genotype affect the milk composition and characteristics, which have a big impact on the yield and quality of dairy products (Murphy et al. 2016; Cheruiyot et al. 2018). Additionally, because of the need for milk synthesis, milk production may physiologically alter haemato-biochemical factors in cows (Tanritanir et al. 2009). Although there was no significant variation in the contents of serum total protein and albumin among the three breeds, our study showed a considerable increase in milk protein in Holsteins compared to Simmental and Brown Swiss breeds. In order for Holsteins to sustain high levels of milk production and milk protein, we initially anticipated a decline of serum total protein and albumin levels. Due to the Holsteins' higher protein content and more effective protein synthesis than Simmental and Brown Swiss, there have been no changes in serum protein and albumin concents (Sarker et al. 2015). Nevertheless, Peterson and Waldern et al. (1981) found that lactating cows had lower serum total protein and albumin levels than non-lactating cows. The findings on milk protein are comparable to those of Manuelian et al. (2018), who noted that the Simmental breed had lower casein and protein contents than Holstein and Brown Swiss breeds. Contrarily, Bobbo et al. (2014) reported that the Brown Swiss breed had a higher milk protein content and the Holstein breed had a lower milk protein content.

However, milk fat % in the current study varied among the three breeds with Brown Swiss and Simmental displaying a higher fat content; and serum triacylglycerol content was affected by the breed, being higher in Simmental. Cholesterol levels did not differ significantly among the breeds. This variation may be attributed to the difference in genetics and physiological status of the animals (Frank 1988). Belewu (2006) concluded that variations in milk fat % is an inherited character which implies that breeds with a higher fat content produce less milk quantity than those with a low fat content. However, the work of Manuelian et al. (2018) reported a higher fat content in Holsteins and Brown Swiss compared to the Simmental breed. Bobbo et al. (2014) reported a higher mean in Simmental (3.82% in 4.28%) and a lower mean in Ren (3.39–3.73%). Cheruiyot et al. (2018) did not find significant differences in milk fat among different breed. Thyroid hormones are also well-known for their important involvement in milk production as a result of dairy cows' increased metabolic rates (Blum et al. 1983). According to our research, Holstein and Simmental breeds have higher serum concentrations of T3 and T4 than the Brown Swiss breed. This suggests that both Simmental and Holstein breeds have a higher milk production and metabolic rates.

Simply known as milk sugar, lactose is a crucial source of energy (Gambelli 2017). Because of the drop in concentration that occurs during mastitis, it may also be utilised as a measure of udder health (Macciotta et al. 2012). In our study, concentrations of milk lactose were higher in Holstein and Simmental breeds compared to Brown Swiss. Our findings are somewhat similar to those of Manuelian et al. (2018) who reported a lower lactose content in Holstein and Brown Swiss breeds compared to the Simmental breed. However, our results disagree with those of Adesina (2012) and Cheruiyot et al. (2018) who reported no differences. Total solids in milk reported in the current study showed a significant increase in Holsteins and Brown Swiss compared to the Simmental breed. Conversely, Cheruiyot et al. (2018) did not find significant differences in contents of milk components among the Admixed Dairy Cattle in Tanzania. Additionally, milk

density in the current study showed an increase in Holstein and Simmental breeds. This may be attributed to variation in the fat content which is known as the main cause of milk density variation (Walstra and Jemess 1984).

It has been suggested that one gene related to milk yield and composition traits exists in all autosomal chromosomes in cows. The most important genes affecting the amount and percentage of milk fat are found on *Bos taurus* autosomal chromosomes (BTA) 5, 6, 9, 14, 20 and 26 (Khatkar et al. 2004). In the current study, the reported nucleotide sequence variation in *DGAT1, FABP, ATPAT1* and *OLR1* genes and its subsequent amino acids between the three breeds may account for the variation in the values of metabolic biochemical indicators and milk composition components. It is worth mentioning that *DGAT1, FABP, ATPAT1* and *OLR1* genes are candidate genes for milk production traits and composition. It has also been reported that variation in these genes could affect levels of milk yield, protein and fat as well as milk energy content (Schennink et al. 2009; Ibrahim et al. 2019). Genetic polymorphisms of the investigated markers were also associated with traits other than milk production, suggesting that phenotypic variation in these traits among the three cattle breeds may be attributed to this cause. For instance, Liu et al. (2010) reported that variation in the *ATP1A1* gene was associated with heat tolerance traits in dairy cattle. Polymorphisms of the *ATP1A1* gene were also associated with mastitis in dairy cattle (Liu et al. 2012). Kowalewska-Łuczak (2018) reported relatedness of the *OLR1* gene polymorphisms with reproductive performance in dairy cattle. Relatedness of the *OLR1* gene polymorphisms with growth and carcass traits in beef cattle has also been indicated (Fonseca et al. 2015; Gui et al. 2019).

In conclusion, variability in *DGAT1*, *FABP, ATPAT1* and *OLR1* genes and the metabolic profile could be used as biomarkers for characterization of cattle breeds and may lead to a brief and rigorous selection within and between breeds of dairy cattle. Furthermore, variability at these markers makes it possible to assess the predisposition of animals to a specific type of production.

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