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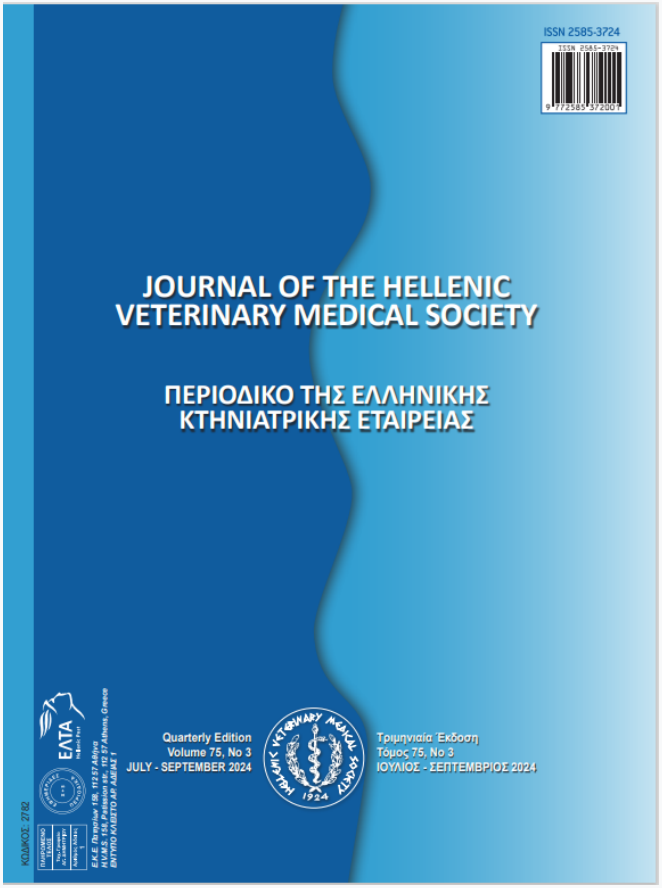

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Research article
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Combining single nucleotide polymorphisms and gene expression of *LEP*, *FABP4*, *STAT5A* and *DGAT1* genes for improvement of body weight in Holstein calves

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ABSTRACT: The objective of this study was to investigate the possible association of single nucleotide polymorphisms and gene expression of leptin (*LEP*), fatty acid binding protein (*FABP4*), signal transducer and activator of transcription 5A (*STAT5A*) and diacylglycerol acyltransferase (*DGAT1*) genes with body weight in Holstein calves. For the RNA extraction process, blood samples from 100 Holstein bull calves were taken. According to farm records, the acquired bull calves were weaned at a body weight of roughly 90 kg, with birth weights ranging from 31 to 38 kg and weaning ages ranging from 60 to 100 days. To account for the non-genetic causes, the dairy calves' body weight was changed to their 205-day body weight. Nucleotide sequence changes in the form of SNPs were found in the *LEP, FABP4, STAT5A*, and *DGAT1* genes in the investigated Holstein calves. The identified SNPs and corrected 205-day body weight showed a significant correlation ($P < 0.05$). According to ΔCT values, a significant relationship between the mRNA levels of the *LEP, FABP4, STAT5A*, and *DGAT1* genes and body weight was also found ($P < 0.05$). The effectiveness of the *LEP, FABP4, STAT5A*, and *DGAT1* genes as proxies for growth rates in Holstein dairy calves is highlighted by this study. In addition, the discovered SNPs and expression profile of investigated genes could be used as a marker assisted selection (MAS) for high body weight in cattle.

Keywords: Holstein calves; Growth-related genes; Gene expression; Single nucleotide polymorphism

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INTRODUCTION

The Holstein cow, sometimes referred to as a Holstein-Friesian or Friesian, is the breed of cow that produces the most milk in the world today (Horan *et al*., 2004). As well as in all contemporary discourse, the term "Holstein" refers to an animal descended from Northern American genetics, whereas the term "Friesian" refers to native European black and white cattle. According to Rodriguez-Venegas *et al*., 2023, Holstein-Friesian cattle were first bred in the northern Netherlands (Fries country).

Since they enable the detection, mapping, and analysis of polymorphisms of genes encoding proteins that act on metabolic pathways implicated in economically valuable features, molecular biology techniques are helpful in genetic improvement (Katsanis and Katsanis, 2013). The four primary categories of molecular markers are SNPs, RFLPs, AFLPs, and VNTRs (Vignal *et al*., 2002). Single base pair polymorphisms serve as the foundation for single nucleotide polymorphisms (SNPs). A site at which two alternative bases occur with a discernible frequency is known as an SNP (Amiteye, 2021) and DNA sequencing can be used to find them (Amiteye, 2021).

A polypeptide hormone called leptin is largely produced and secreted by fat cells (Forhead and Fowden, 2009). According to Kuenzel and Fraley (1995), leptin binds to a receptor that is mostly found on Neuropeptide — Y — neurons in the hypothalamus, which also appear to be crucial in the integration of feeding behavior with internal indications of body energy status. Leptin is regarded to be a crucial signaling molecule that links dietary status to reproductive function since leptin concentrations are highly impacted by adipose tissue mass. The *LEP* gene is found on chromosome 4 in cattle. It has two introns and four exons, however only two exons are translated to protein. Exons 2 and 3 contain the LEP gene's 501 nucleotide long coding sequence (Liefers *et al*., 2002).

The actions of fatty acid binding proteins (FABPs) have been categorized in a number of ways, including intracellular protein transport, modification of various enzymes involved in lipid metabolism, regulation and expression of fatty acid-responsive genes, and fatty acid content of cell membranes (Zimmerman and Veerkamp, 2002). According to Ogorevc *et al.,* 2009, *FABP4* is located in the region (46,833,665-46,838,053) where BTA14 is harbored, which is rich in QTL for milk production features. This suggests that *FABP4* could be employed as a marker candidate gene to evaluate production traits.

The signal transducer and activator of transcription 5 (STAT5) is the main regulator of growth hormone activity on target genes (Buitenhuis *et al.,* 2004). It is crucial for prolactin signaling intracellular modulation and can trigger milk protein gene transcription in response to prolactin (Brym *et al*., 2004). Studies on *STAT5A* polymorphisms, growth features, and milk production traits mostly focus on cattle (Brym *et al*., 2004). As a substitute growth marker, the *STAT5A* gene has been investigated (Oikonomou *et al.,* 2011).

The diacylglycerol acyltransferase 1 (DGAT1) enzyme, according to reports, is crucial for the creation of lipoprotein, the development of connective tissue that is rich in fat, the synthesis of triglycerides, and intestinal fat absorption (Sanjayaranj *et al*., 2023). Similar to this, it has been determined that *DGAT1* is a crucial gene that affects milk supply and milk fat percentages (Smaragdov, 2011).

Previous studies used qualitative genetic methods to investigatethe possible association between *LEP, FABP4*, *STAT5A,* and *DGAT1* genes polymorphism and productive traits in livestock (Liefers *et al*., 2002; Ogorevc *et al.,* 2009; Smaragdov, 2011; Oikonomou *et al*., 2011). Our study aimed at studying *LEP, FABP4*, *STAT5A,* and *DGAT1* genes variation and their association with Holstein calves via PCR-DNA sequencing and gene expression approaches.

MATERIALS AND METHODS

Animals and experimental samples

One hundred Holstein bull calves were specifically obtained for the current investigation from a private farm in Gamasa, Dakhlia governorate. According to farm records, the acquired bull calves were weaned at a body weight of roughly 90 kg, with birth weights ranging from 31 to 38 kg and weaning ages ranging from 60 to 100 days. Jugular vein punctures were used to obtain 100 fresh blood samples from Friesian bull calves raised in Egyptian circumstances and placed in tubes containing anticoagulant disodium EDTA for RNA extraction. The Ethical Committee approved the sample collection and animal care techniques utilized in this study, and they complied with Mansoura University's regulations. The Mansoura University Animal Care and Use Committee (MU-ACUC) gave its approval to the study's protocol (code VM.R.23.11.131).

Adjustment or correction of non-genetic factors

By using linear interpolation to extrapolate from the calf's birth weight, weaning weight, and age, the weaning weight was modified to 205 days of age. Gould (2015) calculations were used to calculate the 205-day.

$$A = \frac{(B-C)}{D} \times 205 + C + \text{age of dam adjusted}$$

Where A is the weight at 205 days (kg), B is the weight at weaning (kg), C is the weight at birth (kg), and D is the weaning age (days).

Total RNA extraction, reverse transcription and quantitative Real- Time PCR

Following the manufacturer's instructions, total RNA was extracted from the blood of calves using Trizol reagent (RNeasy Mini Ki, Catalogue no. 74104). Using a NanoDrop® ND-1000 Spectrophotometer, the isolated RNA's quantity was determined and validated. Each sample's cDNA was synthesized in accordance with the manufacturer's instructions (Thermo Fisher, Catalogue no. EP0441). *LEP, FABP4, STAT5A*, and *DGAT1* coding segments' gene expression patterns were evaluated using quantitative RT-PCR and SYBR Green PCR Master Mix (2x SensiFastTM SYBR, Bioline, CAT No: Bio-98002). SYBR Green PCR Master Mix was used in real-time PCR to conduct relative quantification of mRNA levels (Quantitect SYBR green PCR kit, Catalogue no. 204141). Primer sequences were designed based on the *Bos taurus* sequence that was published in PubMed, as indicated in Table 1. For normalization, the housekeeping gene *ß. actin* was utilized as a constitutive control.

The reaction mixture was carried out in a total volume of 25 µl consisted of total RNA 3 µl (150 ng), 4 µl 5x Trans Amp buffer, 0.25 µl reverse transcriptase, 0.5 µl of each primer, 12.5 µl 2x Quantitect SYBR green PCR master mix and 8.25 µl RNase free water. The final reaction mixture was placed in a thermal cycler and the following program was carried out: reverse transcription at 50 °C for 30 mins, primary denaturation at 94 °C for 10 mins followed by 40 cycles of 94 °C for 15 s, annealing temperatures as shown in Table 1 for 1 min, and 72 °C for 30 s. At the end of the amplification phase, a melting curve analysis was performed to confirm the specificity of the PCR product. ΔCT of each sample was calculated for *LEP, FABP4*, *STAT5A,* and *DGAT1*genes using threshold cycle (CT) values that were normalized to those of the *ß. actin* gene. Greater expression was indicated by a lower ΔCT (Livak and Schmittgen, 2001; Pfaffl, 2001).

Real -time PCR products sequencing and polymorphism detection

Primer dimmers, nonspecific bands, and other contaminants were eliminated prior to DNA sequencing of Real - Time PCR products. Using a PCR purification kit (Jena Bioscience # pp-201s/Germany), target bands of the expected size (as stated by Boom *et al*., 1990) were purified using the manufacturer's instructions. In order to obtain high-quality products and to guarantee sufficient concentrations and purity of the PCR products, the quantification of the PCR product was performed using Nanodrop (Uv-Vis spectrophotometer Q5000/USA) (Boesenberg-Smith *et al*., 2012). Using the enzymatic chain terminator technique pioneered by Sanger *et al.* (1977), PCR products with the target band were sent for DNA sequencing in both the forward and reverse orientations

Table 1. Oligonucleotide primers for growth-related genes.

GenBank isolate	Annealing Temperature (°C)	Product size (bp)	Primer	Researched marker
AB003143.1	58	441	F5′- GTGCCCATCCGCAAGGTCCAG-3 R5′- TCAGCACCCGGGACTGAGGTC-3′	*LEP*
NM_174314.2	60	442	F5′- AGCATCTTGCTGAAAGCTGCA -3 R5′- CTCTGGTGGCAGTGACACCAT-3′	*FABP4*
NM_001012673.1	58	360	F5′- GAACGGACATGGCGGGCTGGA -3′ R5′- TCTGTTCATTGTACAGAATGTG - 3′	*STAT5A*
NM_174693.2	58	300	F5′- GTCGCGGCCTTCGATCCAGGGC -3′ R5′- TGGATGGGGTCCACCAGGATG- 3′	*DGAT1*
AF191490.1	60	117	F:5-GCTCAGAGCAAGAGAGGCAT-3 R:5- CACACGGAGCTCGTTGTAGA-3	*ß. actin*

using an ABI 3730XL DNA sequencer (Applied Biosystem, USA).

Chromas 1.45 and Blast 2.0 software were used to analyze the DNA sequencing data (Altschul *et al.,* 1990). Single-nucleotide polymorphisms (SNPs) were identified between PCR products of the studied genes and GenBank reference sequences. The MEGA6 software program was used to compare differences in the amino acid sequence of the studied genes across the enrolled dairy calves based on data alignment from DNA sequencing (Tamura *et al*., 2007).

Statistical Analysis

H_0: Combining single nucleotide polymorphisms and gene expression of *LEP*, *FABP4*, *STAT5A,* and *DGAT1* genes could not improve body weight in Holstein's calves.

H_A: Combining single nucleotide polymorphisms and gene expression of *LEP*, *FABP4*, *STAT5A,* and *DGAT1* genes could improve body weight in Holstein's calves.

The significant distribution of SNPs for the identified genes was discovered between the study buffaloes using Fisher's exact test analysis ($p < 0.01$). Association between identified SNPs and body weight was carried out using least square method of the general linear model (GLM) procedures using SPSS software (SPSS, 2009). The following model was used:

$$Y_{ijk} = \mu + G_i + e_{ijk}$$

Where, Y_{ijk} is the phenotypic value of the trait, μ is overall population mean, G_i is the effect of *gene SNP*, and e_{ijk} is the random error effect. The statistical significance of the changes in the expression profiles of growth-related genes between the examined calves was assessed using one way analysis of variance (ANOVA). To present the findings, mean and standard error (mean ± SE) were employed. Differences were deemed significant at $p < 0.05$.

RESULTS

Single nucleotide polymorphisms (SNPs) and their association with body weight

SNP variations in amplified DNA nucleotides were found in the results of PCR-DNA sequencing of the *LEP* (441-bp)*, FABP4* (442-bp), *STAT5A* (360-bp) and *DGAT1* (300-bp) genes (submitted to GenBank with accession numbers gb|OR611970|, gb|OR611971|, gb|OR343252|, gb|OR343253|, gb|OR343254|, gb|OR343255|, gb|OR343256|, and gb| OR343257|). Each SNP was validated by comparing the DNA sequences of reference genes obtained from GenBank with the indicators analyzed in the study dairy calves (Figures 1-4).

```
AB003143.1      GTGCCCATCCGCAAGGTCCAGGATGACACCAAAACCCTCATCAAGACAATTGTCACCAGG 60
1               GTGCCCATCCGCAAGGTCCAGGATGACACCAAAACCCTCATCAAGACAATTGTCACCAGG 60
2               GTGCCCATCCGCAAGGTCCAGGATGACACCAAAACCCTCATTAAGACAATTGTCACCAGG 60
                ************************************ ******************

AB003143.1      ATCAATGACATCTCACACACGCAGTCCGTCTCCTCCAAACAGAGGGTCACTGGTTTGGAC 120
1               ATCAATGACATCTCACACACGCAGTCCGTCTCCTCCAAACAGAGGGTCACTGGTTTGGAC 120
2               ATCAATGACATCTCACACACGCAGTCCGTCTCCTCCAAACAGAGGGTCACTGGTTTGGAC 120
                ************************************************************

AB003143.1      TTCATCCCTGGGCTCCACCCTCTCCTGAGTTTGTCCAAGATGGACCAGACATTGGCGATC 180
1               TTCATCCCTGGGCTCCACCCTCTCCTGAGTTTGTCCAAGATGGACCAGACATTGGCGATC 180
2               TTCATCCCTGGGCTCCACCCTCTCCTGAGTTTGTCCAAGATGGACCAGACATTGGCGATC 180
                ************************************************************

AB003143.1      TACCAACAGATCCTCACCAGTCTGCCTTCCAGAAATGTGGTCCAAATATCCAATGACCTG 240
1               TACCAACAGATCCTCACCAGTCTGCCTTCCAGAAATGTGGTCCAAATATCCAATGACCTG 240
2               TACCAACAGATCCTCACCAGTCTGCCTTCCAGAAATGTGGTCCAAATATCCAATGACCTG 240
                ************************************************************

AB003143.1      GAGAACCTCCGGGACCTTCTCCACCTGCTGGCCGCCTCCAAGAGCTGCCCCTTGCCGCAG 300
1               GAGAACCTCCGGGACCTTCTCCACCTGCTGGCCGCCTCCAAGAGCTGCCCCTTGCCGCAG 300
2               GAGAACCTCCGGGACCTTCTCCACCTGCTGGCCGCCTCCAAGAGCTGCCCCTTGCCGCAG 300
                ************************************************************

AB003143.1      GTCAGGGCCCTGGAGAGCTTGGAGAGCTTGGGCGTTGTCCTGGAAGCTTCCCTCTACTCC 360
1               GTCAGGGCCCTGGAGAGCTTGGAGAGCTTGGGCGTTGTCCTGGAAGCCTCCCTCTACTCC 360
2               GTCAGGGCCCTGGAGAGCTTGGAGAGCTTGGGCGTTGTCCTGGAAGCTTCCCTCTACTCC 360
                ******************************************** ************

AB003143.1      ACCGAGGTGGTGGCCCTGAGCCGGCTGCAGGGGTCACTACAGGACATGTTGCGGCAGCTG 420
1               ACCGAGGTGGTGGCCCTGAGCCGGCTGCAGGGGTCACTACAGGACATGTTGCGGCAGCTG 420
2               ACCGAGGTGGTGGCCCTGAGCCGGCTGCAGGGGTCACTACAGGACATGTTGCGGCAGCTG 420
                ************************************************************

AB003143.1      GACCTCAGTCCCGGGTGCTGA      441
1               GACCTCAGTCCCGGGTGCTGA      441
2               GACCTCAGTCCCGGGTGCTGA      441
                ********************
```

Figure 1. Representative analysis of nitrogenous bases matching for DNA in investigated dairy calves using GenBank gb|AB003143.1| and *LEP* marker (441-bp) sequences.

Figure 2. Representative analysis of nitrogenous bases matching for DNA in investigated dairy calves using GenBank gb|NM_174314.2| and *FABP4* marker (442-bp) sequences.

Figure 3. Representative analysis of nitrogenous bases matching for DNA in investigated dairy calves using GenBank gb|NM_001012673.1| and *STAT5A* marker (360-bp) sequences.

Figure 4. Representative analysis of nitrogenous bases matching for DNA in investigated dairy calves using GenBank gb|NM_174693.2| and *DGAT1* marker (300-bp) sequences.

In investigated calves, table 2 displays the spread of a single base variation as well as a specific type of hereditary modification for growth indicators. Fisher's exact test examination of the SNPs indicated significantly differing occurrences of the examined markers ($p< 0.01$). Exonic region alterations affected every growth marker being studied, leading to altered coding of DNA sequences in Holstein dairy calves. DNA sequencing of the studied genes revealed 8 SNPs; 5 are non-synonymous and 3 are synonymous.

Based on identified SNPs in each gene, Holstein dairy calves were allocated into different groups (Table 3).For *LEP* gene, dairy calves harboring C42T SNP were represented as G1 *LEP*; dairy calves harboring T348C SNP were represented as G2*LEP*; while dairy calves that did not exhibit the identified SNP were represented as G3*LEP*. Regarding the identified SNPs in *FABP4* gene, dairy calves were distinguished into four groups; dairy calves harboring T117G, A280G SNPs were represented as G1 *FABP4*, dairy calves harboring T117G A222CSNPs were represented as G2 *FABP4*, dairy calves exhibiting A222C SNP were represented as G3 *FABP4*, and dairy calves did not harbor either of identified SNPs were represented as G4*FABP4*. In the same line, identified SNPs in *STAT5A* gene discriminated dairy calves into three groups; C92TSNP was exhibited by a group of dairy calves and were represented as G1 *STAT5A*; dairy calves exhibiting C236G SNP were represented as G2 *STAT5A*. Meanwhile the other groups did not exhibit denoted SNP were represented as G3 *STAT5A*. *DGAT* gene polymorphisms divided calves into main groups; dairy calves elaboratingT70C SNP were represented as G1*DGAT1*, and calves did not harbor identified SNP were represented as G2*DGAT1*. Least square means of SNPs discriminated groups elicited that there was a significant association ($P < 0.05$) between identified SNPs and corrected 205 body weight (Kg); where G2*LEP*, G2*FABP4*, G1 *STAT5A*, and G1 *DGAT1* dairy calves had higher corrected 205 body weight (Table 4).

Table 2. Single base difference dispersal as well as sort of inherited change for growth markers in Holstein dairy calves.

Amino acid order and sort	kind of inherited change	SNPs	Gene
I 14	Synonymous	C42T	*LEP*
A 116	Synonymous	T348C	
D to E 39	Non-synonymous	T117G	*FABP4*
S 74	Synonymous	A222C	
I to V 94	Non-synonymous	A280G	
S to L 31	Non-synonymous	C92T	*STAT5A*
S to W 79	Non-synonymous	C236G	
W to R 24	Non-synonymous	T70C	*DGAT1*

Single base difference dispersal for growth related genes in dairy calves showed a highly significant variation ($p< 0.01$) according to Fisher's exact analysis.

Table 3. Single nucleotide polymorphisms in growth related markers and their association with corrected 205 body weight in Holstein dairy calves.

Corrected 205 body weight (Kg)	Calf group harboring SNP	No of calves harboring SNP	SNPs	Gene
154.85±3.61^{c}	G1 LEP	47	C42T	LEP
212.51±2.45^{a}	G2 LEP	22	T348C	
183.71±1.72^{b}	G3 LEP	31	-	
187±2.68^{b}	G1 FABP4	37	T117G, A280G	FABP4
192±4.94^{a}	G2 FABP4	18	T117G A222C	
189±1.28^{b}	G3 FABP4	29	A222C	
186.84±2.84^{b}	G4 FABP4	16	-	
209.76±2.43^{a}	G1 STAT5A	21	C92T	STAT5A
168.51±2.42^{c}	G2 STAT5A	44	C236G	
181.62±3.58^{b}	G3 STAT5A	35	-	
179.92±1.39^{a}	G1 DGAT1	62	T70C	DGAT1
156.78±2.61^{b}	G2 DGAT1	38	-	

Gene expression and its association with body weight

ΔCT is inversely proportional to the genes expression profile. Levels of *LEP*, *FABP4*, *STAT5A* and *DGAT1* genes expression were significantly higher in G2*LEP*, G2*FABP4*, G1 *STAT5A*, and G1 *DGAT-1*discriminated dairy calves, as indicated by the lower values of ΔCT (Figure 1). A significant ($P < 0.05$) up-regulation of *LEP*, *FABP4*, *STAT5A* and *DGAT1* was detected in G2*LEP*, G2*FABP4*, G1 *STAT5A*, and G1 *DGAT1* SNPs identified calves as compared with other groups, as indicated by significantly lower ΔCT (Table 4).

DISCUSSION

National breeding programs frequently employ DNA marker-based technologies, which significantly influence the improvement of growth features (Bahrami *et al*., 2013). The main issues facing cattle breeding globally are the development of techniques for better utilizing the gene pools of current breeds of sheep, lowering feed costs, implementing genetic control mechanisms, finding more reserves, and enhancing the sector's economic performance (Ghanem *et al.,* 2022). It is crucial to evaluate the prospective genes in charge of cattle productive features given the significance of molecular-genetic studies (Ibrahim *et al.,* 2023). According to Song

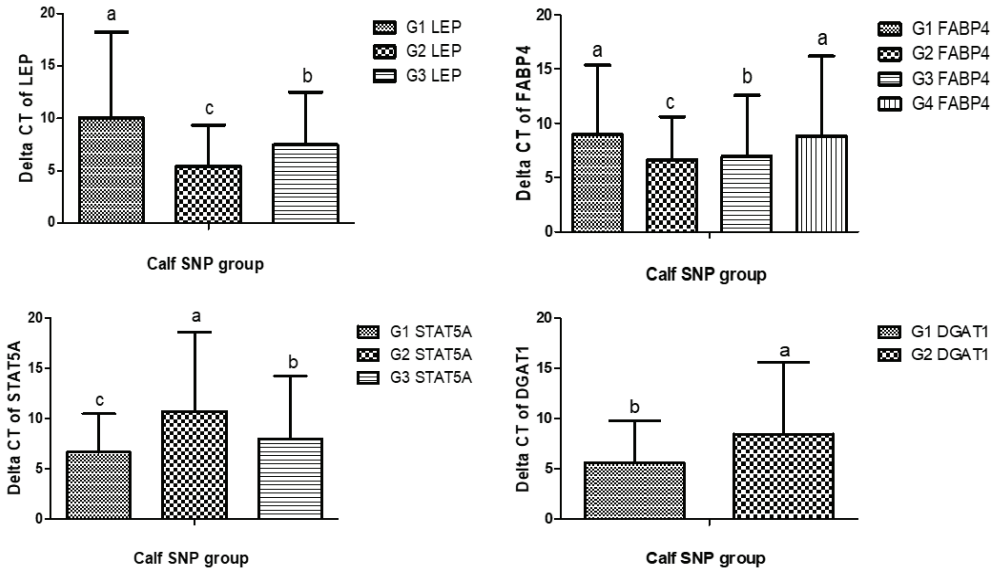

Figure 1. *LEP, FABP4, STAT5A* and *DGAT1* genes expression in dairy calves. Data shown as ΔCT ± SE. Lower ΔCT values indicate increased expressions.

Table 4. Expression profile of growth-related genes and their association with corrected 205 body weight in Holstein dairy calves indicated by values of ΔCT.

Corrected 205 body weight (Kg)	ΔCT	Calf group harboring SNP	No of calves harboring SNP	SNPs	Gene
154.85±3.61c	18.24±1.84a	G1 LEP	47	C42T	LEP
212.51±2.45a	9.38±1.46c	G2 LEP	22	T348C	
183.71±1.72b	12.51±2.43b	G3 LEP	31	-	
187±2.68b	15.36±2.514a	G1 FABP4	37	T117G, A280G	FABP4
192±4.94a	10.61±2.64c	G2 FABP4	18	T117G A222C	
189±1.28b	12.58±1.36b	G3 FABP4	29	A222C	
186.84±2.84b	16.18±1.51a	G4 FABP4	16	-	
209.76±2.43a	10.49±2.58c	G1 STAT5A	21	C92T	STAT5A
168.51±2.42c	18.64±2.81a	G2 STAT5A	44	C236G	
181.62±3.58b	14.26±1.73b	G3 STAT5A	35	-	
179.92±1.39a	9.81±1.41b	G1 DGAT1	62	T70C	DGAT1
156.78±2.61b	15.62±1.32a	G2 DGAT1	38		

et al. (2015), and Ateya *et al*., 2016, the SNP is the most frequent type of polymorphism in eukaryotic genomes that can be used as a superior marker type for identifying traits that are important economically.

We postulate that significant variations in the adjusted 205-day body weight of Holstein calves may be explained by variations in genes involved in growth. In this investigation, amplified PCR products that had been sequenced were used to identify the *LEP, FABP4, STAT5A*, and *DGAT1* genes in Holstein dairy calves. The findings show that PCR-DNA sequencing data (submitted to GenBank with accession numbers gb|OR611970|, gb|OR611971|, gb|OR343252|, gb|OR343253|, gb|OR343254|, gb|OR343255|, gb|OR343256|, and gb|OR343257|) contained SNP variations in amplified DNA nucleotides. The analyzed dairy calves displayed a substantial nucleotide polymorphism dispersion, according to the Fisher's exact test ($p< 0.01$). It must be emphasized that the polymorphisms discovered and made available in this context provide new data for the evaluated indicators when compared to the appropriate datasets gathered from GenBank. Our research demonstrated that the growth genes with identified SNPs classified calves into various groups, with the G2 *LEP*, G2 *FABP4*, G1 *STAT5A*, and G1 *DGAT1* dairy calves displaying considerably greater adjusted 205 body weight groupings. As a result, the genes *LEP, FABP4, STAT5A*, and *DGAT1* may be used as substitute indicators for the growth performance of dairy calves.

Our findings demonstrated the presence of two SNPs, C42T and T348C, in the *LEP* gene (441-bp). According to the results of the basal local alignment search tool (BLAST), the sheep database's altered mutated C42T base are conserved (GenBank accession number CP011904.1). Additionally, databases for buffalo, sheep and goats both have the T348C SNP (GenBank accession numbers MF490277.1, MN305996.1, and MH716185.1, respectively).Three recurring SNPs, T117G, A222C, and A280G SNPs, were identified in the 442-bp nucleotide sequence of the *FABP4* gene. The identified SNPs were found to be conserved in other closely related species by nucleotide sequence alignment. For example, the sheep has a conserved T117G SNP (GenBank accession number CP011894.1), the buffalo have a conserved A222C SNP (GenBank accession number XM_010863410.1), and the buffalo, sheep, and goat have a conserved A280G mutated base (GenBank accession numbersM_001290961.1, OR136941.1, and JQ031288.1, respectively).

Similarly, when comparing our DNA sequencing results with those of closely related species, two recurrent SNPs, C92T SNP, and C236G, were found in the *STAT5A* gene (360-bp). In the genome of buffalo, sheep, and goat, the two altered nucleotides are conserved (GenBank accession numbers MN712202.1, NM_001009402.2, and JN688205.1, respectively). DNA sequencing led to the discovery of one recurrent SNP (T70C) in the *DGAT1* gene (300-bp). When compared to the genome of buffalo, sheep, and goat the changed nucleotide was shown to be conserved (GenBank accession numbers DQ886485.1, EU301803.1, and LT221856.1, respectively). The conservation behavior in the changed bases can be explained by the close relatedness of ruminant species, of which there more thanks to genetic resource conservation programs that also help to preserve important gene reservoirs (Kasprzak-Filipek et al., 2018). Sequencing on the conserved region (CDS) of the growth related genes under investigation is another reason (Singh et al., 2014).The polymorphism of the *LEP, FABP4, STAT5A*, and *DGAT1* genes has been linked to productive qualities in livestock in previous research. For instance, Liu*et al.* (2010) showed a correlation between carcass features in Chinese Qinchuan cattle and variation in the bovine *LEP* gene exon 2. Kulig, 2005 extended on the relationships between the *LEP* gene polymorphism and characteristics of cattle's milk production. Additionally, Kulig and Kmieć, 2009 investigated the relationship between growth traits in Limousin cattle and *LEP* gene polymorphisms. *LEP* gene polymorphism and its relationship to body weight and other parameters in Madura cattle were demonstrated by Kuswati *et al*., 2002.Azhar*et al*., 2020 observed impacts of *FABP4* gene polymorphism on the chemical composition of meat in cull female Aceh cattle, which is relevant to *FABP4* gene polymorphisms and their association with productive qualities. Single nucleotide polymorphisms in *FABP4* were discovered to be correlated with meat quality and lipid metabolism gene expression in Yanbian yellow cattle by Yin*et al*., 2020. In addition, single-nucleotide polymorphisms in the *FABP4* gene related with growth features in Egyptian sheep were observed by Shafey *et al*., 2020.

Selvaggi *et al*. (2009) investigated the genetic polymorphism of *STAT5A* and its associations with production attributes and milk composition in Italian Brown cattle in order to identify specific genomic loci of the *STAT5A* gene that could explain variations in productive traits in cattle. Oikonomou *et al*. (2011) investigated the impact of SNPs at the *STAT5A* gene locus on Holstein cow reproductive, milk production, and lameness. Bovine *STAT5A* gene polymorphism and its im-

pact on growth parameters in the Podolica breed were elaborated by Selvaggi *et al*., 2014. Khan *et al*. (2021) found a relationship between *DGAT1* gene polymorphisms and variables related to the production of milk and meat in cattle, buffalo, goats, and sheep. A single nucleotide polymorphism in the *DGAT1* gene and the fatty acid composition of cows were both mentioned by Sanjayaranj *et al*., 2023. Sedykh *et al*. (2017) discovered *DGAT1* gene polymorphism had an impact on the eating characteristics of bull calves. According to Smaragdov (2011), cow milk production and the *DGAT1* gene polymorphism in bulls are related.

A transcriptome is the entire collection of genes in the genome that can be consistently and effectively expressed under different physiological circumstances (Kukurbaand Montgomery, 2015). The current study suggests that genetic variations in an individual's transcriptional response to such circumstances may affect the body weight of Holstein dairy calves. Real-time PCR was used to quantify the *LEP*, *FABP4*, *STAT5A* and *DGAT1* genes in the dairy calves that were the subject of the study. The lower values of ΔCT in our results demonstrated that levels of *LEP, FABP4, STAT5A*, and *DGAT1* gene expression were considerably greater in G2 *LEP*, G2 *FABP4*, G1 *STAT5A*, and G1 *DGAT1* discriminating dairy calves. In comparison to other groups, G2 *LEP*, G2 *FABP4*, G1 *STAT5A*, and G1 *DGAT1* SNPs discovered calves showed a significant ($P< 0.05$) up-regulation of *LEP*, *FABP4*, *STAT5A* and *DGAT1*, as shown by considerably reduced ΔCT. Our analysis of gene polymorphism utilizing SNP genetic markers and gene expression has overcome the drawbacks of past investigations. Real-time PCR was used for the first time to measure important growth indicators in Holstein dairy calves to address this problem. As a result, dairy calves are well-versed in the mechanisms driving the investigated gene regulation.

Our study is the first to demonstrate the tendency for *LEP, FABP4, STAT5A*, and *DGAT1* gene expression in Holstein dairy calves. However, it has been observed that the expression profile of the examined markers is linked to productive traits in livestock. For instance, Mota *et al*. (2017) study on Nellore cattle with differing feed efficiency revealed that muscle *LEP* gene expression was higher in high feed-efficient animals in line with the plasma levels. Regarding *FATBP4*, Zhang *et al*. (2017) noted that ectopic expression of the *FABP4* gene can trigger adipogenesis in bovine muscle-derived stem cells. The up-regulation of *FABP4* in fast-growing and intermediate-growing lambs, which facilitates protein accretion, energy expenditure, and fatty acid partitioning necessary for muscle growth, was also shown by Ghanem *et al*., 2022. In addition, Elis et *al*., 2013 examined the expression of genes related to lipid metabolism and adipokines in the adipose tissue of dairy cows that differed in a quantitative trait locus related to female fertility. The results showed that during body reserve restoration, the *FABP4* gene was overexpressed. According to Yang et *al*. (2016), the *STAT5A* gene is expressed in dairy cattle's milk during lactation and is necessary for the development of mammary glands. Fink et al., 2020 hypothesized that the variety of lactation effects seen at the *DGAT1* gene mutations is caused by transcriptional effects of *DGAT1*. It's important to note that the gene expression profile of growth and lipogenic markers was used to track the growth performance of Brown Swiss, Holstein, and Simmental calves. Hendam et *al*., 2023 found that the Simmental calves had significantly higher levels of insulin-like growth factor 1, lipoprotein lipase, stearoyl-CoA desaturase, and acetyl-CoA carboxylase alpha than the Holstein Friesian and Brown Swiss calves, as evidenced by lower delta threshold cycle values.

CONCLUSION

In the examined Holstein calves, nucleotide sequence variations in the form of SNPs were detected in the *FABP4*, *STAT5A,* and *DGAT1*genes. There was a significant association between the identified SNPs and corrected 205-day body weight. The mRNA level of these indicators significantly affected corrected 205-day body weight indicated by lower values of ΔCT. This study highlights that *FABP4*, *STAT5A,* and *DGAT1* are candidate genes for growth traits in Holstein dairy calves enabling marker-assisted selection for body weight.

CONFLICT OF INTEREST

None declared

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