Egypt. J. Chem. **Vol. 69**, No. 1 pp. 1 - 13 (2026)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Combining Gene Expression and Candidate Gene Approach for Improvement of Growth Traits in Dromedary Camels

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Abstract

The present research aimed to ascertain the relationship between gene expression and single nucleotide polymorphisms (SNPs) of the genes encoding glutamate decarboxylase (GAD1), fatty acid binding protein 4 (FABP4), transmembrane protein 95 (TMEM95), nucleobindin 2 (NUCB2), ATP binding cassette sub-family G member 2 (ABCG2), signal transducer and activator of transcription 5A (STAT5A), and uncoupling protein 1 (UCP1) may be related to growth traits in dromedary camels. Farm records documented the growth characteristics of 340 male dromedary camels. Following genotyping analysis, a total of 72 male dromedary camels were selected based on the availability of their phenotype records and similar conditions. Blood samples were obtained from the 72 selected individuals to perform the RNA extraction procedure. PCR-DNA sequencing of *UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A*, and *GAD1* revealed SNP variations in the amplified DNA regions. These SNPs were used to classify the camels into distinct genotype groups for further analysis. A significant correlation ($P < 0.01$) was observed between growth traits and the detected SNPs, accompanied by notable differences in the expression profiles (UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1) among dromedary camels, which indicates their potential as surrogates for growth rates. Furthermore, dromedary camels with high growth rates may benefit from marker-assisted selection (MAS) using the identified SNPs and the studied gene expression profiles. The results may support future MAS in camels, pending further validation.

Keywords: Candidate gene; Dromedary camels; Gene expression; Growth traits.

Introduction

The dromedary camel (Camelus dromedarius) is a crucial domestic animal in arid and semi-arid regions, as it offers high-quality sustenance under harsh conditions at comparatively moderate costs [1]. Over the past decade, there has been an increase in genetic research aimed at improving camel meat quality, which is crucial for sustaining meat consumption. The birth weights of dromedary camels vary significantly among breeds, regions, and even within the same breed, averaging around 35 kg. The birth weights of camels are known to range from 27 to 39 kg, comparable to those of tropical cattle breeds [2]. Camels were reported to weigh approximately 27, 48, 65, and 79 kg at birth, and at 30, 60, and 90 days of age, respectively. Such signifies that from birth to 90 days of age, camels exhibit a growth rate of 580 grams daily [3]. Programs for livestock selection employ MAS, which makes use of candidate gene data [4]. The initial step in the molecular marker selection procedure is identifying the candidate genes associated with the economically important feature. The SNPs within genes responsible for phenotypic variation can be found through candidate genes associated with physiological and biochemical processes. When phenotypic selection is ineffective, MAS can improve the rate of genetic gain in those species [5]. MAS can be used to increase selection differentials, shorten generation intervals, and gain genetic gain. MAS can achieve these goals by selecting young bulls before conducting progeny testing [6]. Developed spline-based growth models and analyzed variation patterns in the birth weight and weaning weight of Pakistani camel breeds. Recent technical breakthroughs have created new opportunities for exploring complex elements of camel meat quality [7]. Recent technology breakthroughs

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Receive Date: 18 May 2025, **Revise Date:** 13 July 2025, **Accept Date:** 17 July 2025
DOI: https://doi.org/10.21608/ejchem.2025.386502.11790

have created new opportunities for investigating detailed camel meat characteristics. Using the candidate gene technique, it is possible to choose genes or genomic areas that directly influence economic aspects, so increasing livestock genetic improvement via genomic selection [8]. Numerous genes have been selected as potential candidates for qualities that are economically significant in livestock due to their physiological significance in the traits of interest or because they are found in genomic areas that include previously identified quantitative trait loci (QTLs) for these traits. Using the candidate gene strategy, researchers can target specific genes or genomic regions that directly influence economically significant traits in livestock, enhancing genetic improvement through genomic selection. A multitude of genes have been found due to their physiological significance to traits of interest or their proximity to established QTLs. Current research is shifting focus from single gene associations to uncovering gene expression profiles, clusters, and associations characteristic of specific phenotypes, such as meat quality. In this respect, Table 1 details the candidate genes being studied and their physiological effects. It is hypothesized that the genes UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 significantly influence growth traits in dromedary camels based on their functions. The study aims to analyze these genes using PCR-DNA sequencing and gene expression techniques to elucidate their potential as candidates for boosting growth characteristics in this species. Is there a correlation between genetic composition and gene expression that might be leveraged to decrease the expenses associated with monitoring gene expression over time?

Table 1. Studied genes and their functions.

Gene	Function
UCP1	A protein involved in cell energy metabolism, it releases energy as heat and allows protons to pass through the mitochondrial membrane. This protein affects thermogenesis and reduces reactive oxygen species (ROS) levels. [9].
TMEM95	Significantly influences male fertility, impacting reproductive traits and affecting growth traits [10].
NUCB2	Nucleotide-binding protein (NEFA/NUCB2) produces the terminal peptide nesfatin-1, which is crucial in glucose metabolism and appetite regulation [11]. This secreted protein is expressed in the hypothalamic nuclei of rats, influencing appetite control [12]. Nesfatin-1 is a powerful satiety factor that modulates ingestive behavior, with its expression diminishing in the hypothalamus paraventricular nucleus through fast [13][14]. Additionally, nesfatin-1 impacts melanocortin signaling in the hypothalamus, affecting food behaviour [15].
FABP4	The primary roles of fatty acid-binding proteins (FABPs) involve the absorption, transport, and metabolism of fatty acids [11]. So far, nine unique members of this gene family have been identified, including FABP4 [12]. FABP4 participates in lipid hydrolysis and intracellular fatty acid transport, principally via its direct contact and binding with hormone-sensitive lipase, a crucial enzyme in lipid catabolism[13][14].
ABCG2	Commonly referred to as breast cancer resistance protein, this is a cell membrane transporter that facilitates the active transfer of external chemicals into the cell by ATP hydrolysis[11, 12]. It is essential for the liberation of nutrients, including vitamin B2 (riboflavin), from milk [13, 14]. ABCG2 substantially influences milk fat composition, protein concentrations, and milk production in cattle [15 - 17]. Genome-wide studies have demonstrated that SNPs in the ABCG2 gene affect milk production in cattle [18].
STAT5A	The STAT family consists of seven structurally and functionally related cytoplasmic proteins involved in cell division, proliferation, and apoptosis. These proteins are activated by various cytokines, growth factors, and hormones [11][12]. STAT5 is a crucial regulator of growth hormone effects on target genes [13]. The mammalian STAT protein family comprises seven members: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6 [14].
GAD1	Glutamic acid decarboxylase (GAD) synthesizes a crucial substance in the brain [11]. Research suggests a relationship between animal body weight and GAD polymorphisms. GAD has been shown to stimulate the production of hormones related to insulin and pepsin, which regulate feed intake, metabolic rate. Additionally, GAD can promote the growth and development of animals [12].

Uncoupling protein 1 is denoted as UCP1, transmembrane protein 95 as TMEM95, nucleobindin 2 as NUCB2, and fatty acid binding protein 4 as FABP4. GAPDH refers to glyceraldehyde-3-phosphate dehydrogenase, GAD1 denotes glutamate decarboxylase, STAT5A signifies the signal transducer and activator of transcription 5A, and ABCG2 represents ATP binding cassette subfamily G member 2.

Materials and Methods

Animals and Experimental Samples

340 dromedary camels from the Center for Studies and Development of Camel Production in the Matrouh Governorate, Egypt, were ordered to have their development characteristics examined between 2021 and 2024. To ensure data quality and representation across weight classes, several filtration steps were implemented. Initial screening excluded records with unknown birth dates, 110 missing pedigree information, and newly added animals from other herds. In later stages, data on female animals was excluded, and the analysis was restricted to male offspring from dams in the third and fourth parity. Additionally, male offspring resulting from insemination by non-herd Maghrabian sires and those showing underweight due to health defects were also removed. After these steps, genotype analysis was conducted on 72 males.

Blood samples from male camels were collected via jugular vein punctures and placed in tubes containing the anticoagulant disodium EDTA for RNA extraction.

The research ethics committee of the Faculty of Agriculture, Assuit University, allowed the use of the animals (code number: 03-2025-0022).

Growth Performance Traits

Body weight (BW) characteristics for each camel, expressed in kilos using an electronic balance, were among the study's factors. This included yearling weight at 12 months (BW12m), birth weight (BW0), weight at 3 months (BW3m), and weight at 6 months (BW6m).

All procedures involving animals were conducted according to the guidelines of the Ethics Committee of the Faculty of Agriculture, Assiut University (No: 03-2025-0022)

Reverse Transcription, Total RNA Extraction, and Quantitative Real-Time PCR

Trizol reagent (RNeasy Mini Kit, Catalogue No. 74104) was employed to extract total RNA from camel blood following the manufacturer's instructions. A NanoDrop® ND-1000 Spectrophotometer was utilized to quantify and validate the quantity of the extracted RNA.

cDNA was synthesized from each sample following the manufacturer's instructions (Thermo Fisher, Catalogue No. EP0441). The gene expression profiles of the UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 coding regions were evaluated utilizing quantitative RT-PCR and SYBR Green PCR Master Mix (2x SensiFastTM SYBR, Bioline, CAT No: Bio-9). Relative quantification of mRNA levels was performed utilizing SYBR Green PCR Master Mix in real-time PCR (Quantitect SYBR Green PCR kit, Catalogue No. 204141). Table 2 indicates that primer sequences were derived from the nucleotide sequence of Camelus dromedarius, as reported in PubMed, serving as a model. The housekeeping gene GAPDH serves as a constitutive control for normalization purposes.

The reaction mixture included three microliters (150 ng) of total RNA, four microliters of 5x Trans Amp buffer, 0.25 microliters of reverse transcriptase, 0.5 microliters of each primer, 12.5 microliters of 2x Quantitect SYBR Green PCR master mix, and 4.25 microliters of RNase-free water, making a total of 25 microliters.

The final reaction mixture was put in a PCR, and the subsequent procedure was executed: Thirty minutes of reverse transcription at 50 °C, ten minutes of initial denaturation at 94 °C, followed by forty cycles of 94 °C for fifteen seconds, one minute at the annealing temperatures specified in Table 2, and thirty seconds at 72 °C. A melting curve analysis was conducted to confirm the specificity of the PCR product following the amplification phase. The ΔCT for the UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 genes was calculated using threshold cycle (CT) values normalized to the GAPDH gene. A reduced ΔCT indicated higher expression [11,12].

Sequencing of Real-Time PCR Products and Identification of Polymorphisms

Prior to DNA sequencing of real-time PCR results, primer dimers, nonspecific bands, and other contaminants were removed by purifying the target bands of the appropriate size using a PCR purification kit (Jena Bioscience # pp-201s/Germany), in accordance with the manufacturer's instructions.

The PCR product was quantified using Nanodrop (UV-Vis spectrophotometer Q5000/USA) to ensure adequate concentration and purity, hence ensuring high-quality products [13]. PCR products containing the target bands were subjected to bidirectional DNA sequencing using an ABI 3730XL DNA sequencer, which works by using a method called the enzymatic chain terminator approach developed by [14]. The DNA sequencing results were analyzed using Chromas 1.45 and Blast 2.0 software [15]. The SNPs were detected in the PCR products of the analyzed genes, and the GenBank reference sequence was utilized to evaluate variations in the amino acid sequences of the researched genes among the enrolled dromedary camels, based on data alignment from DNA sequencing [16].

Table 2. Oligonucleotide primers for growth-related genes.

Researched Marker	**Primer** sequences	Product size (bp)	Annealing Temperature (°C)	GenBank isolate
UCP1	F5′- ATGGTGGGGCTGACAGCCTCAG-3 R5′- TGTGGTGAAGAACTCCTGGAC-3′	315	58	XM_010979308.3
TMEM95	F5′- AGCGCTGCTTCCCAGGAAGTCA -3 R5′- AATCAGTCATAGGAGGGCCAGA-3′	504	55	XM_010996542.3
NUCB2	F5′- GCGCGGTGGGTAGAAGCACAG -3′ R5′- TGTCTTGTCTATGTCTATAGGC - 3′	360	55	XM_010977217.3
FABP4	F5′- ATGATTACATGAAAGAAGTGG -3′ R5′- AACCAACACAACCATATTGAAT- 3′	498	60	XM_010979386.3
ABCG2	F:5-AGGATAGTCATCGTCGTGACT-3 R:5- TCCGACAACATAGAAAGCCACT-3	512	58	XM_031433730.2
STAT5A	F:5-TGCGCCAGATGCAGGTGTTGT-3 R:5- CAACTCATTCTCTGTGTCCTGCGT-3	443	58	XM_031468751.2
GAD1	F:5-TCTCTTCCTGGCGCTCGCGTG-3 R:5- GCAGGTTCTTGGAGGACTGCCT-3	460	55	XM_031451630.2
GAPDH	F:5-TCGATCCCCCAACACACTTG-3 R:5- TGATGGTGCATGACAAGGCA-3	106	60	XM_010990867.2

UCP1= Uncoupling protein1, TMEM95= Transmembrane protein 95, NUCB2= Nucleobindin 2, FABP4= Fatty acid binding protein 4*, ABCG2=* ATP *binding cassette subfamily G member 2, STAT5A= Signal transducer and activator of transcription 5A, GAD1= Glutamate decarboxylase* and *GAPDH= Glyceraldehyde-3-phosphate dehydrogenase.*

Statistical Analysis

Alternative hypothesis: A correlation exists between gene expression and studied growth traits, which may function as an alternative tool for traditional genetic evaluation. The null hypothesis: There is no correlation between gene expressions and growth traits, and we can't use gene expression as an alternative tool for genetic evaluation.

The statistical significance of the variations in the growth-related gene expression profiles among the male camels under examination was evaluated for every gene. Using SAS, 2010 [17], a T-test (genes have <3 SNP groups) or one-way analysis of variance (ANOVA) (genes have ≥3 SNP groups) was used in a single model as follows: $Yijk = \mu + Gi + \varepsilon ijk$, where Yijk = overall mean for each trait, Gi = the fixed effect of animal genotype for each marker, and εijk = experimental error.

Differences were significant at $p < 0.05$. The results showed utilizing the least squares mean and standard error (LSM ± SE). Using Duncan's multiple range test, post hoc analyses were carried out among genotype classes for each marker with three or more classes in each trait to determine significance [18]. The correlation coefficient (r) was computed to assess the strength of the relationship between the growth data and ΔCT. The correlation between ΔC for every gene and growth characteristic and the amount of variation for PC1 and PC2 (Principal Component Analysis) was visualized using SRplot.

Results

Association between SNPs and growth traits

The results of PCR-DNA sequencing for the UCP1 (315-bp), TMEM95 (504-bp), NUCB2 (360-bp), FABP4 (498-bp), ABCG2 (512-bp), STAT5A (443-bp), and GAD1 (460-bp) indicated SNPs variations in amplified DNA nucleotides. By comparing the DNA sequences of reference genes obtained from GenBank with the indicators analyzed in the dromedary camel study, each SNP was verified (Figures 1 and 2).

Table 3 illustrates the distribution of a single nucleotide variation and a specific type of genetic modification associated with growth markers in the camels studied. Fisher's exact test analysis revealed significantly different occurrences of the examined markers for the SNPs ($p < 0.01$). All growth metrics examined were influenced by alterations in exonic regions, which modified the coding of DNA sequences in dromedary camels. DNA sequencing of the genes under investigation identified 18 SNPs, of which 2 are synonymous and 16 are non-synonymous.

Table 3. Distribution of single nucleotide variations and a kind of genetic modification affecting development parameters in dromedary camels

Gene	SNPs	Amino acid order and sort
UCP1	G70A	A 24T
	T170G	V 57 G
	G265C	A 89 P
TMEM95	C290T	A 97 V
NUCB2	T92C	V 31 A
	G185A	R 62 H
FABP4	G424A	V 142 I
ABCG2	T77G	L 26 R
	A147G	49 G
	G219T	L 73 F
	G366T	R 122 S
STAT5A	C56T	T 19 I
	C194T	T 65 M
	A311G	D 104 G
	G363A	M 121 I
GAD1	A93G	31 P
	G209A	R 70 K
	G276A	92 A

UCP1= Uncoupling protein1, TMEM95= Transmembrane protein 95, NUCB2= Nucleobindin 2, FABP4= Fatty acid binding protein 4, ABCG2= ATP binding cassette subfamily G member 2, STAT5A= Signal transducer and activator of transcription 5A, and GAD1= Glutamate decarboxylase. -A= Alanine; D= Aspartic acid; G= Glycine; H= Histidine; I= Isoleucine; K= Lysine; L= Leucine; M= Methionine; P= Proline; R= Arginine; S= Serine; T= Threonine; and V= Valine.

The distribution of single base differences in growth-related genes of dromedary camels exhibited a highly significant variance ($p < 0.01$) as determined by Fisher's exact analysis. Male camels were categorized into several groups based on the discovered SNPs in each gene (Table 4). For the UCP1 gene, G70A SNP camels were labelled G1 UCP, while those with T170G and G265C SNPs were G2 UCP1; camels without identified SNPs were G3 UCP1. In the TMEM95 gene, C290T SNP camels were G1 TMEM95, and those without were G2 TMEM95. The NUCB2 gene split camels into G1 NUCB2 for T92C and G185A SNPs, with the remaining G2 NUCB2. The FABP4 gene categorized camels with G424A SNP as G1 FABP4 and those without as G2 FABP4. The ABCG2 gene created five groups: G1 for T77G, G2 for A147G, G3 for G366T, G4 for G219T, and G5 for those lacking SNPs. STAT5A gene groups included G1 for C56T, G2 for C194T and G363A, G3 for A311G, and G4 for those without SNPs. Lastly, the GAD1 gene divided camels into G1 for A93G and G276A, G2 for G209A, and G3 for those without detected SNPs.

The least square means across SNP-based genotype groups showed a significant association($P < 0.01$) between the detected SNPs and growth traits; specifically, G3 UCP1, G2 TMEM95, G1 NUCB2, G2 FABP4, G2 ABCG2, G1 STAT5A, and G3 GAD1 dromedary camels exhibited elevated BW0, BW3m, BW6m, and BW12m.

Gene expression of growth-related genes and their association with body weight

ΔCT is inversely proportional to the gene's expression profile. A significant difference ($P < 0.01$) was detected in the gene expression profile of growth-related genes among investigated camels. Levels of UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 genes expression were significantly higher in G3 UCP1, G2 TMEM95, G1 NUCB2, G2 FABP4, G2 ABCG2, G1 STAT5A, and G3 GAD1 discriminated camels, as indicated by the lower values of ΔCT (Figure 1).

There was a significant association between ΔCT values and growth traits in the studied male dromedary camels (Figure 2). A highly significant negative correlation ($P < 0.01$) was detected between ΔCT values and growth traits in male dromedary camels (Table 5). Principal Component Analysis (PCA) was used to assess the differences among various groups for each gene and to evaluate the correlation between ΔCT values and growth traits. The figure illustrates the individual loadings of the first and second principal components (PCs). PC1 effectively distinguishes between the different gene groups (distinct clusters) and captures a significant amount of variation ranging from 79.3% for UCP1 to 91.4% for ABCG2, indicating significant differences in body weight based on gene expression among the groups (Figure 2).

Table 4. Single nucleotide polymorphism in growth-related markers and their correlation with growth parameters in dromedary camels.

Gene	SNPs	No of camel harboring SNP	Camel Group harboring SNP	BW0 (kg)	BW3m (kg)	BW6m (kg)	BW12m (kg)	Pr
UCP1	G70A	26	G1 *UCP1*	36.00±0.15^{c}	79.20±0.49^{c}	137.89±0.67^{c}	195.50±1.39^{c}	0.004**
	T170G, G265C	14	G2 *UCP2*	37.06±0.10^{b}	82.16±0.34^{b}	142.43±0.44^{b}	204.70±0.85^{b}	
	-	32	G3 *UCP3*	39.08±0.14^{a}	89.13±0.41^{a}	151.73±0.65^{a}	224.13±1.35^{a}	
TMEM95	C290T	41	G1 *TMEM95*	36.40±0.13	80.35±0.41	139.62±0.58	199.01±1.18	0.002**
	-	31	G2 *TMEM95*	39.13±0.13	89.28±0.40	151.93±0.64	224.56±1.32	
NUCB2	T92C, G185A	28	G1 *NUCB2*	39.29±0.11	89.74±0.34	152.61±0.57	226.03±1.16	0.003**
	-	44	G2 *NUCB2*	36.48±0.13	80.66±0.42	140.03±0.59	199.83±1.19	
FABP4	G424A	39	G1 *FABP4*	36.34±0.13	80.14±0.40	139.34±0.57	198.44±1.17	0.002**
	-	33	G2 *FABP4*	39.04±0.14	88.98±0.43	151.52±0.66	223.70±1.38	
ABCG2	T77G	10	G1 *ABCG2*	37.31±0.03^{c}	83.20±0.21^{c}	143.62±0.24^{c}	207.20±0.51^{c}	0.004**
	A147G	15	G2 *ABCG2*	39.62±0.03^{a}	90.92±0.26^{a}	154.61±0.42^{a}	229.93±0.71^{a}	
	G219T	22	G3 *ABCG2*	35.68±0.07^{d}	78.14±0.20^{e}	136.44±0.25^{e}	192.55±0.56^{e}	
	G366T	19	G4 *ABCG2*	38.49±0.19^{b}	87.21±0.50^{b}	148.73±0.72^{b}	218.07±1.63^{b}	
	-	6	G5 *ABCG2*	36.95±0.15^{c}	81.68±0.47^{d}	141.90±0.68^{d}	203.43±1.24^{d}	
STAT5A	C56T	12	G1 *STAT5A*	39.62±0.03^{a}	90.92±0.26^{a}	154.61±0.42^{a}	229.93±0.71^{a}	0.002**
	C194T, G363A	32	G2 *STAT5A*	36.88±0.12^{c}	81.87±0.41^{c}	141.68±0.57^{c}	203.21±1.16^{c}	
	A311G	13	G3 *STAT5A*	38.91±0.18^{b}	88.38±0.43^{b}	150.31±0.70^{b}	221.52±1.63^{b}	
	-	15	G4 *STAT5A*	35.44±0.07^{d}	77.45±0.18^{d}	135.61±0.14^{d}	190.81±0.49^{d}	
GAD1	A93G, G276A	29	G1 *GAD1*	37.93±0.15^{b}	85.32±0.48^{b}	146.29±0.62^{b}	212.91±1.39^{b}	0.003**
	G209A	26	G2 *GAD1*	35.86±0.11^{c}	78.63±0.30c	137.22±0.44^{c}	194.12±0.91^{c}	
	-	17	G3 *GAD1*	39.59±0.03^{a}	90.76±0.26^{a}	154.37±0.41^{a}	229.39±0.72^{a}	

UCP1= Uncoupling protein1, TMEM95= Transmembrane protein 95, NUCB2= Nucleobindin 2, FABP4=Fatty acid binding protein 4, ABCG2= ATP binding cassette subfamily G member 2, STAT5A= Signal transducer and activator of transcription 5A, and GAD1= Glutamate decarboxylase. BW0= Birth weight; BW3m=Weight at 3 months; BW6m=Weight at 6 months and BW12m=Weight at 12 months. Pr =probability and **=$p<0.01$); a ,b,….Different letters within the same column are significant differences.

Table 5: Pearson correlation coefficient between amount of ΔCT for each gene and growth traits

ΔCT		Growth traits			
		BW0	BW3m	BW6m	BW12m
UCP1	r	-0.29^{**}	-0.46^{**}	-0.44^{**}	-0.42^{**}
	pr	0.007	0.000	0.000	0.000
TMEM95	r	-0.47^{**}	-0.67^{**}	-0.67^{**}	-0.64^{**}
	pr	0.000	0.000	0.000	0.000
NUCB2	r	-0.57^{**}	-0.69^{**}	-0.69^{**}	-0.67^{**}
	pr	0.000	0.000	0.000	0.000
FABP4	r	-0.43^{**}	-0.58^{**}	-0.57^{**}	-0.54^{**}
	pr	0.000	0.000	0.000	0.000
ABCG2	r	-0.74^{**}	-0.91^{**}	-0.89^{**}	-0.88^{**}
	pr	0.000	0.000	0.000	0.000
STAT5A	r	-0.62^{**}	-0.74^{**}	-0.74^{**}	-0.70^{**}
	pr	0.000	0.000	0.000	0.000
GAD1	r	-0.61^{**}	-0.76^{**}	-0.75^{**}	0.73^{**}
	pr	0.000	0.000	0.000	0.000

BW0= Birth weight; BW3m=Weight at 3 months; BW6m=Weight at 6 months and BW12m=Weight at 12 months. r= Pearson correlation coefficient and Pr =probability and **=p<0.01.

Discussion

Although conventional livestock genetic enhancement has concentrated on selective breeding of superior phenotypes, employing a candidate gene strategy alongside traditional animal breeding tools is essential for refining the selection process and consequently enhancing the animal breeding program [20]. The most common kind of polymorphism in eukaryotic genomes, the SNP, is a great marker type for locating traits that are important from an economic perspective, claims [19] and [20].

Despite the conservation of several SNPs across species, our findings represent a novel contribution to camel genetics, particularly due to the lack of detailed molecular data linking gene expression and polymorphism to growth traits in dromedary camels. This study is the first to demonstrate expression patterns of UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 concerning growth performance in dromedary camels with distinct growth performance traits (BW0, BW3m, BW6m, and BW12m) in camels.

Furthermore, the study demonstrates camel-specific expression patterns and SNP combinations that correlate significantly with superior growth traits. Unlike in cattle and sheep, where similar genes have been linked mainly to fat metabolism or milk traits, our study reveals their possible contribution to early growth dynamics in camels. For instance, the expression of FABP4 and ABCG2 was significantly higher in faster-growing camels, suggesting a species-specific regulatory mechanism adapted to arid environments.

These findings contribute to advancing marker-assisted selection (MAS) approaches in camel breeding, where limited genomic resources currently restrict precision breeding programs. By identifying high-growth genotypes based on both SNPs and gene expression profiles, this research lays the groundwork for camel-specific selection indices, moving beyond extrapolated models from bovine studies.

Significant variability in dromedary camel growth characteristics may be explained by variations in growth-related genes, according to our hypothesis. This study discovered the UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 genes in the male dromedary camels under investigation by sequencing amplified real-time PCR results. The findings indicate that SNP variations in amplified DNA nucleotides were evident in the PCR-DNA sequencing data. Fisher's exact test indicated a substantial dispersion of nucleotide polymorphisms in the male dromedary camels examined (p<0.01). It is essential to recognize that, in comparison to the pertinent datasets acquired from GenBank, the polymorphisms identified and presented herein provide novel insights for the evaluated indicators.

The findings indicated that the UCP1 gene (315 bp) contains three SNPs: G70A, T170G, and G265C. The Basic Local Alignment Search Tool (BLAST) reveals that the database retains the sheep-mutated G70A bases (GenBank accession number CP162296.1). Furthermore, the T170G and G265C SNPs (GenBank accession number CP027085.1) are included in databases for cattle. The 504-bp nucleotide sequence of the TMEM95 gene had one recurrent SNP, C290T. Goats (GenBank accession number CP162227.1), sheep (GenBank accession number CP162297.1), and cattle (GenBank accession number CP027093.1) were found to have preserved versions of the detected SNPs. Similarly, two recurring SNPs, T92C and G185A SNPs, were discovered in the NUCB2 gene (360-bp) when our DNA sequencing data were compared to those of closely related species. The two modified nucleotides (GenBank accession codes CP162280.1 and CP162285.1, respectively) are preserved in the sheep genome. One recurrent SNP (G424A) in the FABP4 gene (498-bp) was found using DNA sequencing.

The altered nucleotide was found to be preserved when compared to the genomes of goats and cattle (GenBank accession numbers JQ031288.1 and CP027087.1, respectively).

The ABCG2 gene (512 bp) showed four repeated synonymous SNPs: T77G, A147G, G219T, and G366T, which we found by comparing our DNA sequencing results with those of similar species. The T77G, A147G, and G219T SNPs (GenBank accession codes CP162290.1, CP162281.1, and CP162291.1) are conserved in the sheep genome. The cow genome still has the G366T SNP (GenBank accession number XM_059887579.1). Four recurrent SNPs were found in the STAT5A gene (443-bp) using DNA sequencing. The C56T SNP was matched by the GenBank sequences for goats and cattle with accession numbers CP027083.1 and CP155510.1. Whereas the C194T SNP (GenBank accession number OY997252.1) shared the same altered base as the cattle sequence. The sheep database was found to align the A311G and G363A SNPs, which had GenBank numbers NM_001009402.2 and CP162286.1, respectively. DNA sequencing revealed three recurrent SNPs in the 460-bp GAD1 gene: A93G, G209A, and G276A. By contrasting the three identified

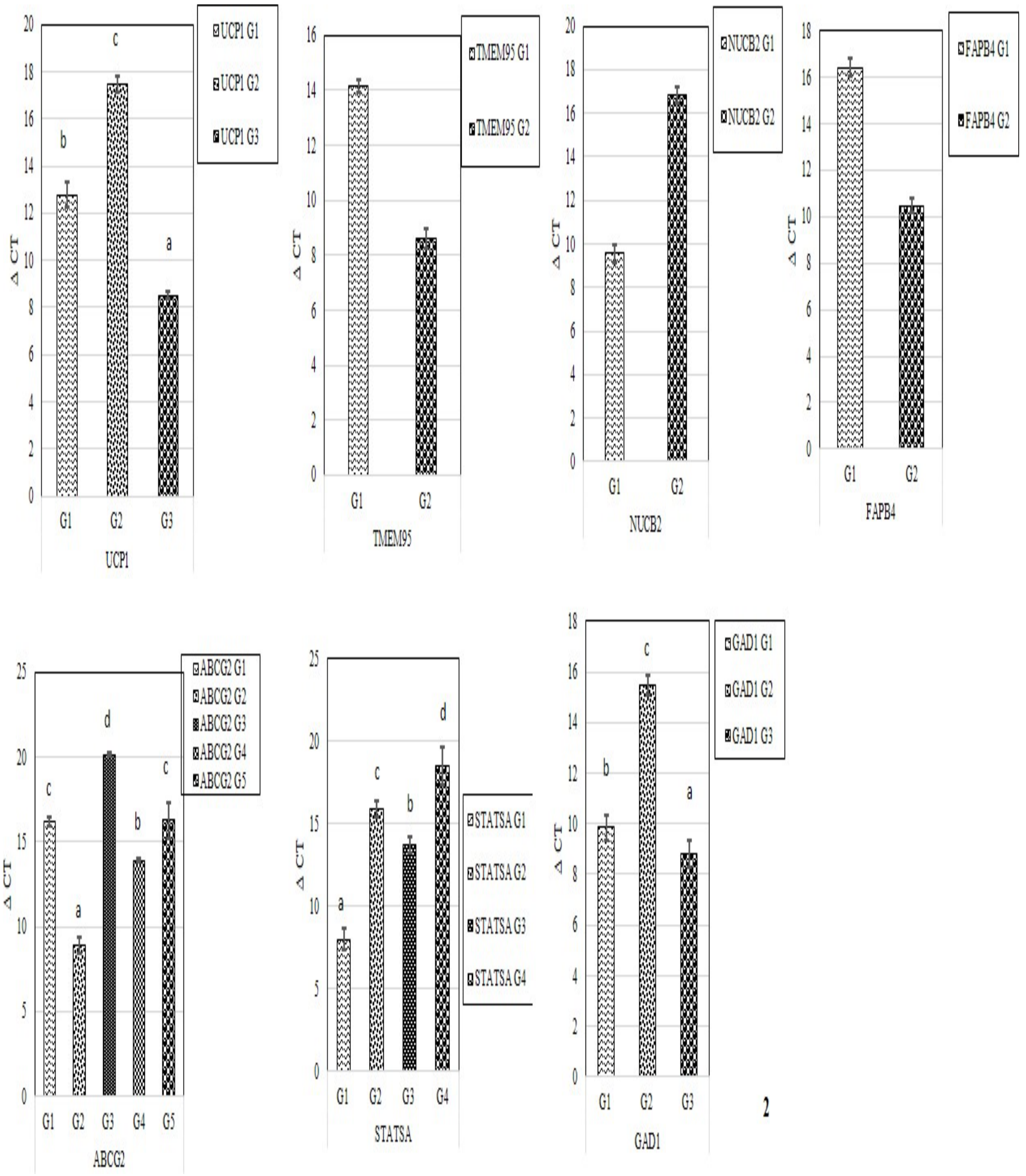

Figure 1. UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 genes expression in dromedary camels. Data is shown as ∆CT ± SE. Lower ∆CT values indicate increased expressions.

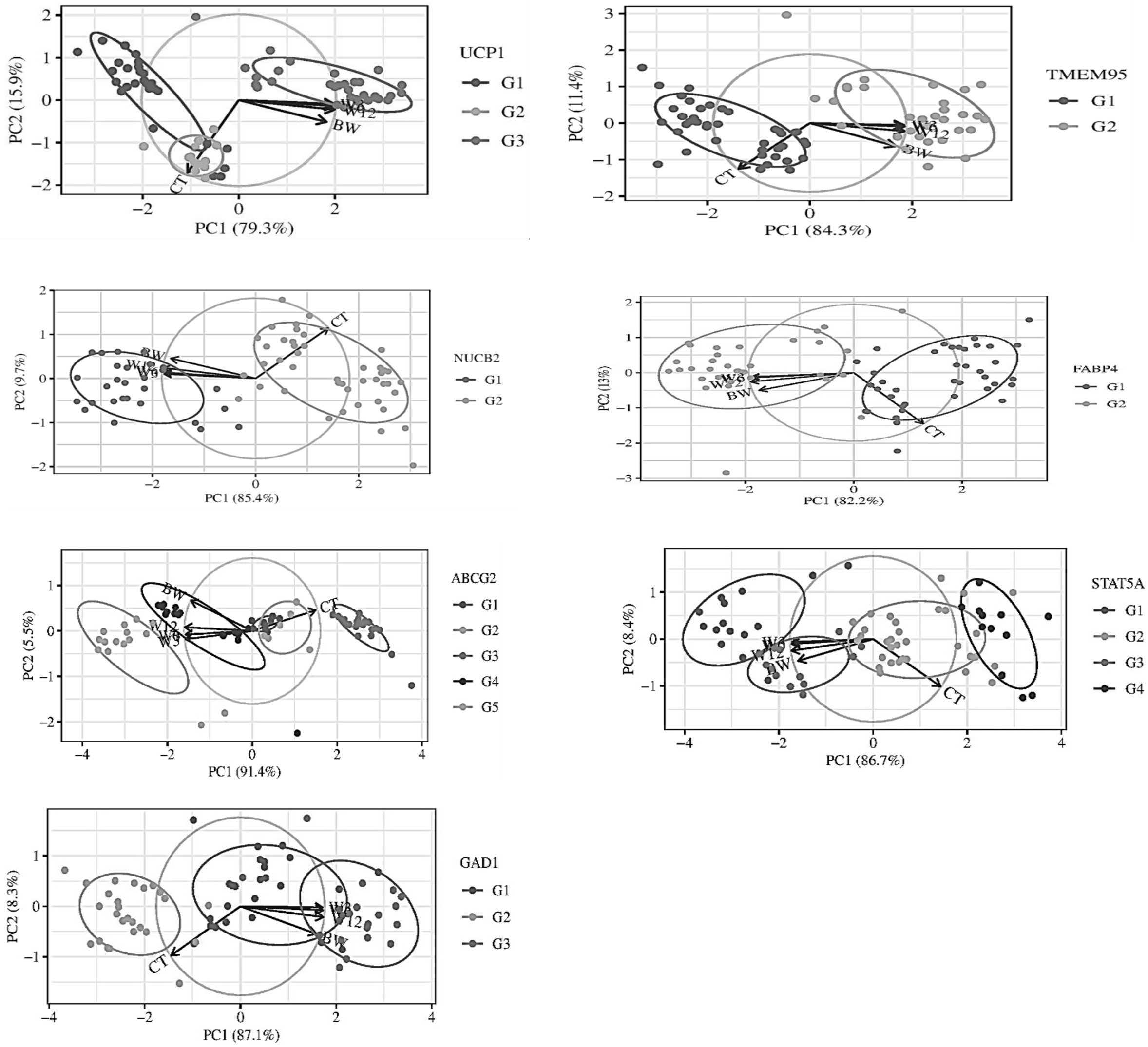

Figure 2. Principal Component Analysis (PCA Biplot) showing the correlation between ΔCT values of UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 genes with body weight and amount of variation (PC1 and PC2).

GenBank accession numbers for SNPs to sheep DNA sequences are CP162296.1, CP162286.1, and CP162283.1, respectively. The close kinship of ruminant species, of which there are more because of genetic resource conservation programs that also aid in the preservation of significant gene reservoirs, can be used to explain the conservation behaviour in the altered bases [21]. Another reason is that the growth-related genes being studied have their conserved region (CDS) sequenced [22].

Our research demonstrated that the growth genes with identified SNPs classified calves into various groups, with the G3 UCP1, G2 TMEM95, G1 NUCB2, G2 FABP4, G2 ABCG2, G1 STAT5A, and G3 GAD1 dromedary camels displaying considerably greater BW0, BW3m, BW6m and BW12m (Table 4). Therefore, these genes may serve as molecular indicators of growth performance in dromedary camels. To our knowledge, our study is the first that explores UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 gene polymorphisms and growth traits in dromedary camels. One limitation of the current study is the absence of phenotypic traits such as feed intake, fat deposition, muscle mass, or metabolic biomarkers, which are critical for understanding the physiological mechanisms underlying growth. While the study demonstrates statistical associations between gene expression/SNP variants and body weight, these should be interpreted as preliminary signals. Future studies incorporating comprehensive performance and physiological traits will be necessary to confirm the biological function of these genes in growth regulation and to determine their practical relevance for genetic improvement in camels.

A limitation of the present study is the relatively small sample size of certain genotype groups (e.g., n = 6-10), particularly for rare SNP alleles. While these sizes reflect natural allele frequency distribution in the population, they may affect the reliability and statistical power of certain comparisons. Therefore, these results were interpreted cautiously and emphasised patterns that are consistent across multiple genes and traits. Future studies with larger, more balanced genotype groups and independent validation populations will be essential to confirm these associations and further assess their utility in selection programs. However, polymorphism of the association with growth traits [35] and milk production [36] was observed in cattle. Polymorphisms in the FABP4 gene have been associated with growth and carcass characteristics in cattle [37, 38] and lambs [39]. The milk production qualities in cattle were influenced by the ABCG2 gene through selection using molecular markers [40-42]. In Holstein cattle, milk production parameters were associated with mutations in the STAT5A gene [43, 44]. Polymorphism in the GAD1 gene in cattle and their association with growth traits were documented [45, 46]. The present study indicates that genetic variations in an individual's transcriptional response to these conditions may influence the growth characteristics of dromedary camels. Real-time PCR was used to quantify the UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 genes in the male dromedary camels that were the subject of the study. The gene expression profile of growth-related genes among the camels under investigation showed a significant difference ($P < 0.01$). Levels of UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 genes expression were significantly higher in G3 UCP1, G2 TMEM95, G1 NUCB2, G2 FABP4, G2 ABCG2, G1 STAT5A, and G3 GAD1 discriminated camels, as indicated by the lower values of ΔCT. There was a significant association between ΔCT values and growth traits in the studied male dromedary camels. A highly significant negative correlation ($P < 0.01$) was detected between ΔCT values and growth traits in male dromedary camels. We hypothesize the reasons for this situation that there may be differences in the expression of the investigated genes related to the different physiological periods of the tissues in high and low-growth rate dromedary camels. Real-time PCR was used for the first time to measure important growth indicators in dromedary camels. While the observed negative correlation between ΔCT values and body weight traits suggests that higher gene expression levels may be linked to improved growth, results indicate that ΔCT is a relative and indirect measure. The absence of protein-level validation or direct performance traits like feed efficiency or carcass yield limits the certainty of these associations. Therefore, the current findings should be interpreted as preliminary indications of gene expression relevance, providing a molecular basis for future targeted studies involving phenotypic and biochemical validations.

In this study, the analysis revealed that the first principal component (PC1) explained a very high proportion of the variance in ΔCT values across all genes, ranging from 79.3% in UCP1 to 91.4% in ABCG2. This indicates that a single underlying axis is responsible for the majority of observed differences among individuals. Importantly, this axis strongly separated genotypic groups that differed in both gene expression and growth performance, as visualised in the Principal Component Analysis (PCA) biplots. For instance, genotype groups with high growth rates (e.g., G3 UCP1, G2 FABP4, G2 ABCG2) consistently clustered on the negative side of PC1, where ΔCT values were lower, confirming higher gene expression.

The PCA loading plots also revealed that the growth traits (BW0, BW3m, BW6m, BW12m) had strong vector alignments opposite to ΔCT, supporting the negative correlation between gene expression and growth. This suggests that gene expression, as captured by PCA components, is a major driver of phenotypic variation in growth performance. Additionally, the tight clustering of genotypes with higher expression and body weight along PC1 indicates that gene expression signatures may have predictive potential for growth outcomes.

To our knowledge, this study is the first to utilise real-time PCR to quantify gene expression related to growth traits in dromedary camels. As a result, male dromedary camels are well-versed in the mechanisms driving the investigated gene regulation. Our study is the first to demonstrate the tendency for UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 gene expression in dromedary camels.

However, it has been observed that the expression profile of the examined markers is linked to productive traits in livestock. For example, NUCB2 expression was upregulated in the forestomach of calves, suggesting that peptides from these genes may be important for the development of the forestomach in cows [47]. [48] 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 [49]. In addition, [50] 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 [51], the STAT5A gene is expressed in dairy cattle's milk during lactation and is necessary for the development of mammary glands.

Conclusions

In the examined male dromedary camels, nucleotide sequence variations in the form of SNPs were detected in the UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 genes. There was a significant association between the identified SNPs and growth traits. The mRNA level of these indicators significantly affected growth traits indicated by lower values of ΔCT. This study highlights that UCP1, TMEM95, NUCB2, FABP4, ABCG2, STAT5A, and GAD1 are candidate genes for growth traits enabling marker-assisted selection for high growth rate dromedary camels.

This study confirms functional polymorphisms in key growth-related genes in camels and identifies gene expression profiles for high-growth individuals. By analysing SNP variation and gene expression for phenotypic performance, the study establishes a rapid approach to genetic improvement in camels, enabling more precise selection in this economically important species.

While current findings provide promising targets for further research, the use of these genes in MAS requires additional studies, including heritability estimation, functional validation, and predictive genetic modelling across larger and independent camel populations.

Data Availability Statement: Upon justifiable demand, the supportive data for the study's conclusions will be given by the corresponding author. Informed Consent Statement: To contribute to the experiment, the Animal Production Research Institute, Ministry of Agriculture, Egypt gave its informed consent.

Conflicts of interest

The authors declare that there is no conflict of interest.

Author`s contributions

All Authors collaborate equally.

Acknowledgments

Appreciation is extended to the Animal Production Research Institute, Agriculture Research Center, Egypt, for their provision of facilities.

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