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Research Article

Artificial Neural Network-Based Identification of Associations between UCP2 and UCP3 Gene Polymorphisms and Meat Quantity Traits

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In identifying mutations occurring in distinct cow breeds, genetic elements must be taken into consideration. More recently, these hereditary features have gained attention throughout the world. As in many underdeveloped nations, to bridge the deficit in molecular genetics, multiple solutions are required. The inner membrane anion carrier superfamily contains the uncoupling proteins (UCPs), vital to energy regulation. Research on heredity has shown that variations in the UCP2 and UCP3 genes are connected to obesity and metabolic syndrome. This research aimed to investigate if any mutation in the UCP 2 and UCP 3 genes are related to many characteristics in Pakistan's three indigenous cattle breeds using artificial neural network (ANN). For better analysis, the output of the ANN model is loaded into the Primer Premier 3 software. Using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and sequencing, the results of this study indicated 07 variations in the exon 4 region of the UCP2 gene and 03 variants in the exon 3 area of the UCP3 gene among 215 indigenous cow breeds. The association study revealed that the g.C35G mutation in the UCP3 gene is strongly related to meat quantity characteristics such as carcass weight and drip percentage (P0.05) but not with body height or hip width ($P > 0.05$). Sequence analysis showed five distinct diplotypes: AA, BC, AC, CC, and CD. Cattle with the novel heterozygous diplotype BC perform better in carcass trait and drip percentage than animals with other genotypes. The study's findings suggest that the UCP3 gene may be utilized for marker-assisted selection (MAS) and breed mixing in Pakistan cattle breeds to aid in the country's economic growth.

1. Introduction

Global cattle genetic diversity is comprised of species, breeds, strains, and varieties. For conservation biology aims, variation between and within breeds is always seen as more significant than species diversity. Goats are important livestock with a lengthy history of domestication and economic utility. Domestication is said to have occurred approximately 10,000 years ago, and it expanded over the globe as a result of human migrations and trade routes. China is the world's biggest goat breeder, having a wide variety of commercial, indigenous, and hybrid species [1, 2]. Energy is the fundamental requirement of cells for many life processes, and the primary energy source is within the cell mitochondria. ATP is created inside mitochondria during the redox reaction, a process known as phosphorylation. Adenosine triphosphate (ATP) synthesis is perceived in mitochondria. Although the reactions were not detected as combined processes, the transport families inside the mitochondria were not present. The family of this carrier is known as the UCP (uncoupling protein) [3]. In the inner membranes of mitochondria, the UCPs ("uncoupling proteins") of the carrier protein family are widely encountered [4].

UCPs are categorized as "UCP1, UCP2, UCP3, UCP4, and UCP5, and UCP plant" into six separate groupings. Intrinsic mitochondrial proteins with a molecular weight of roughly 31 kDa–34 kDa are uncoupling proteins. The mass of BMCP1 and uncoupling protein 4 (UCP4), the larger proteins, is 3638 kDa. Unconnected proteins are regarded to be the essential proteins around which they are isoelectric. Its structure is tripartite; in each repetition, two hydrophobic sections are accurate to a-helices. Six crossings of the lipid bilayer are performed in the polypeptide chain, and the amino carboxylic ends reach beyond the mitochondria's internal membrane [5]. In each repetition, the two helices are connected to the protein matrix by a hydrophilic hoop. The functional unit is a dimer and is composed of two comparable subunits. It has been shown that two monomers are coupled in tandem with functionally capable units for other carrier proteins [6].

Uncoupling proteins (UCPs) are members of the superfamily of mitochondrial inner membrane anion carriers that play a crucial role in energy balance. According to genetic study, obesity and metabolic syndrome are connected to UCP2 and UCP3 gene variants. The goal of this study was to see if there was a link between UCP2 and UCP3 gene polymorphisms and economically important traits in Qinchuan cattle [7, 8]. In mammals, uncoupling proteins 2 were found in the lungs, spleen, central nervous system, intestines, kidneys, uterus, and immune cells [9]. The phenomenon by which a specific DNA sequence or a gene exists in one of the two or more variant forms with each form capable of expressing itself as an alternative phenotype. Polymorphism is the most common and dynamic form of genetic variation present throughout the human genome. It has become widespread in life processes, including mainly ROS products ("reactive species of oxygen"), feeding mechanisms, insulin regulation, immune systems, and various illnesses, e.g., atherosclerosis, cancer, diabetes mellitus, and injury to neurons [10]. UCP3 mainly was found in skeletal muscles and the heart and "BAT" very little [11]. UCPs have been postulated as candidate genes for obesity and insulin resistance in the fields of thermogenesis, obesity, diabetes, and free-radical biology. They have been proposed as important therapeutic targets for ageing, degenerative disorders, diabetes, and obesity, among other things [12, 13]. The central body mass is the muscles of the skeleton, and they contribute to thermogenesis and metabolism [14]. By separating substrate combustion in mitochondria from ATP synthesis by the mitochondrial respiration chain, dissipating the mitochondrial proton gradient, and controlling energy metabolism via inducible mitochondrial proton leak, the UCP3 protein regulates metabolic efficiency. The UCP3 gene is mostly expressed in chicken skeletal muscle and is involved in energy metabolism, reactive oxygen production, fatty acid metabolism, and body weight management [15, 16]. The uncoupling protein 3 is the one responsible for the thermal genesis in the muscles of the skeleton. The uncoupling protein 3 is strongly controlled during fasting and starvation in skeletal muscles when energy consumption for metabolism is substantially demanded [17]. The biochemical activities and biological functions of the recently identified mammalian UCP2 and UCP3 are not well known. However, recent data support a role for these UCPs in state 4 respiration, respiration uncoupling, and proton leaks in mitochondria. Moreover, genetic studies suggest that UCP2 and UCP3 play a part in energy expenditure in humans.

Livestock is a subsector of Pakistani agriculture that accounts for roughly 56% of agricultural value addition and 11% of the government's revenue (GDP). Livestock farming makes a considerable contribution to agriculture's ancillary services. In order to highlight the genuine performance of livestock production and livestock and poultry items, the study looked into the relationship between agricultural GDP and livestock product output [18, 19]. The estimated cattle population in Pakistan is 29.66 million. Almost 49 percent are present in Punjab, Pakistan. At the same time, the remaining 23% of the population are present in Sindh and 20% in NWFP. In Balochistan, just 8% are located. These percentages have varied since 1996 in various surveys. Recently, Punjab's share grew by 2.8 percent, and Sindh declined by 3.3 percent. In other Pakistani provinces, the general percentage of the livestock population is not equal [20]. The humped-type (zebu) and Bos endemic are the type of Pakistani animals (cattle). There are about 15 legitimate livestock in the country and 43% of the cattle population.

A missense variation at codon 55 of the UCP3 gene has been linked to an increased risk of obesity in humans. In Chinese Qinchuan cattle, the genetic mutation g.C4902T in the UCP3 gene has been found to be highly connected to total body measuring parameters such as nettle heights and chest depth. According to another study, the relative expression of UCP3 in beef cattle is substantially higher in the low-feed efficiency group than that in the high-feed efficiency group [21, 22]. While many researchers have been involved in regulating energy metabolism genes UCP2 and UCP3, less genetically modified information on UCP2 and UCP3 and a percentage of unique mutations in three indigenous breeds of Sindh Pakistan cattle is known. The uncoupling protein 2 (UCP2) is found in many tissues and exerts dual effects: it protects cells function from damage caused by reactive oxygen species (ROS). On the other hand, the uncoupling induced by UCP2 in mitochondria of pancreatic beta cells decreases ATP synthesis and impairs insulin secretion in response to glucose. Hyperlipidemia also prevents insulin secretion through a similar pathway, leading to hyperglycemia. The uncoupling protein 3 (UCP3) is found mostly in skeletal muscle and BAT and its absence did not alter heat production or body temperature. This protein would export fatty acids outside the mitochondrial matrix for combustion in tissues where fat is the main fuel. In humans, the uncoupling proteins may not play a leading role in energy regulation.

The aim of the study was to find UCP2 and UCP3 gene polymorphisms in three native Sindh Pakistan cattle and examine their novel mutations. The findings of this study could point to a broader hypothesis for future research on the impact of the UCP2 and UCP3 genes as well as MAS-based livestock.

2. Material and Methods

2.1. Blood Collection. In this investigation, blood was obtained from different animals of the Sahiwal cattle breed, ranging from one to five years. Five mL of blood samples were collected from each in EDTA tubes. The animals were cared for following Canada's established protocol [23]. The priority order of best samples for next stage is done using ANN logic. Best sample means sample having better information and data. Each blood sample was represented by only eight features: peak, mean value, standard deviation, kurtosis, age group, and skewness. This was because classification would be done on full blood samples rather than individual cells. Using this information, the ANN gives the sample priority list which can be used for future result analysis. A total of 45 neurons were taken as consideration for hidden layer modeling.

The need of sample priority list was necessary as for future analysis, we can support our results based on the correct sample data. Using the above based technique as shown in Figure 1, a total of 215 animal samples were selected out of many samples.

2.2. DNA Extraction and Quantification. Mini DNA extraction kit (K0781) of Thermo Scientific was used to obtain the DNA from the whole blood. It is particularly made for the speedy and efficient separation of purified genomic DNA from whole blood. A silica-based membrane in a spin column is included in the package to avoid costly and dangerous phenol-chloroform isolation and prolong the alcohol precipitation procedure. After lysing the cells, the technique takes around 20 minutes to complete and executes the highly pure DNA [24].

2.3. Primer Design and Synthesis. Primer Premier 3 software version 0.4.0 was used to design PCR primers for both UCP2 and UCP3 genes. Primers were finally synthesized from the Macrogen company of Korea. Table 1 shows two primer pairs built using the Ensemble database: UCP2 having Acc. ENSBTAG00000003692 and UCP3 with Acc. ENSBTAG00000005259 [25].

2.4. PCR Amplification and Single-Stranded Conformation Polymorphism (SSCP). The PCR reaction was performed in a 20 μL PCR tube with 50 ng/uL of DNA sample, 10 pmol of each primer, 0.2 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (Biomolecules, China). When carrying out a PCR reaction, the following conditions should be followed: The first phase of denaturation was performed. 5 minutes at 95°C, then 35 cycles of denaturation at 94°C for 30 seconds, hybridization at 58°C for 45 seconds, extension at 72°C for 60 seconds, and final extension at 72°C for 5 minutes. Combining 5 uL of PCR product with 5 uL of denaturing solution, heating at 98°C for 10 minutes, and immediately cooling on ice was used to genotype SSCP [26].

2.5. Gel Electrophoresis. Gel electrophoresis is a technique for sorting DNA fragments (or other macromolecules like RNA and proteins) based size and charge. The method of passing a current through a gel containing the molecules of interest is known as electrophoresis. The molecules will flow through the gel in different directions at varying speeds depending on their size and charge, allowing them to be separated from one another [27, 28]. For electrophoresis, hydrated gel networks have a number of advantages. They provide a wide range of mechanically robust experimental formats, including horizontal/vertical electrophoresis in slab gels as well as electrophoresis in tubes or capillaries. The mechanical stability also allows for post-electrophoretic manipulation, such as blotting, electroelution, or MS identification/finger printing of intact proteins or proteins degraded in gel slices, allowing for more experimentation. Because biochemistry gels are chemically inert, they interact with biomolecules seldom during electrophoresis, permitting separation based on physical rather than chemical differences between sample components. Agarose gel (1.5%) and polyacrylamide gel (10%) was used for electrophoresis of amplified PCR product. The gel was then put into an electrophoresis chamber, which contains 1X TBE buffer. A constant electric current of 70 V was applied for 45 min. Finally, the PCR product was observed under the UV Gel Doc system (Bio-Rad, USA) [29].

2.6. Purification, Sequencing, and Bioinformatics Analysis. Purification and sequencing of amplified PCR products were forwarded to the Korean company Macrogen. By blasting on the sequence alignment tool, the sequence findings were evaluated online using the ensemble.org genome database. At the same time, the percentage of the mutation was calculated by using Microsoft Excel sheet. The data for meat quantity association traits were analyzed through the SPSS software [30]. A heritable and often deleterious change in a DNA sequence or a gene, which usually has higher penetrance (expressivity) and hence higher ability to affect phenotype is known as mutation. Mutations (Lit. change) are the basic events, which are responsible for the generation of almost all genetic variations (GVs). In its contextual definition, as endorsed by many scientists, mutation is defined as the irreversible sequence variation in the DNA which essentially encompasses all types of variations occurring in the human genome spontaneously or non-spontaneously [31, 32]. It is defined as the presence of two or more alternative forms of an allele in the genome of any individual, which results in distinct phenotypes in the same population [33].

The overall process flow is shown in Figure 2. The sample is first passed through the ANN sample priority model for better results. Then, the ANN model output acts as an input for the mini-DNA extraction kit input. The information gathered is then passed to the Primer Premier 3 software where PCR analysis is performed using gel electrophoresis. After the PCR, the data are purified and the sequence is a model for bioinformatics analysis.

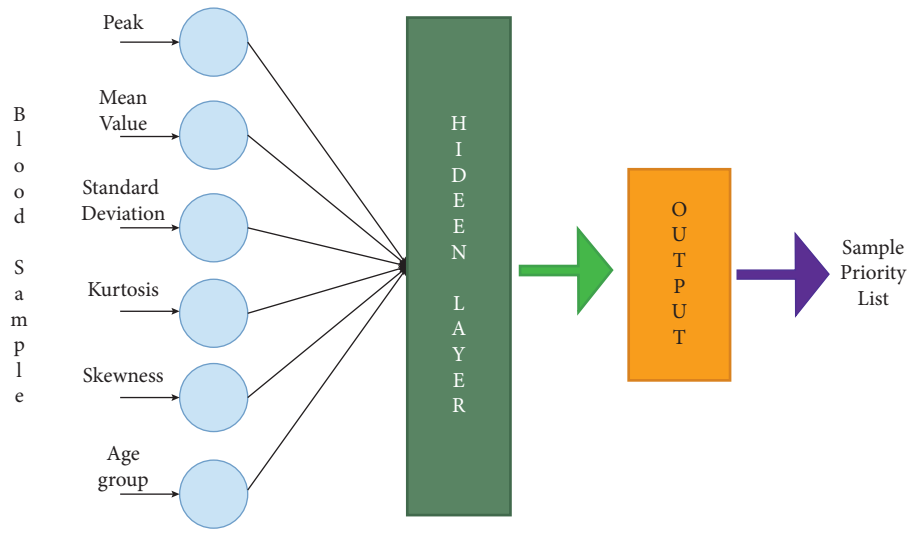

Figure 1: ANN technique for sample priority for better results.

Table 1: Shows the primer details for UCP2 and UCP3 genes.

Primers	Primer sequence	Tm (°C)	Location	Amplicon size
UCP2 primer	F: 5-TGCAGATCCAAGGAGAAAGG-3 R: 5-GCTTGACGGAGTCGTAGAGG-3	57	5′ UTR region	620 (bp)
UCP3 primer	F-GCCTCTACGACTCCGTCAAG-3 R-CTCCTCATGCTTCAGCTTCC-3	58	Exon 3, Intron 2, Exon 4	950(bp)

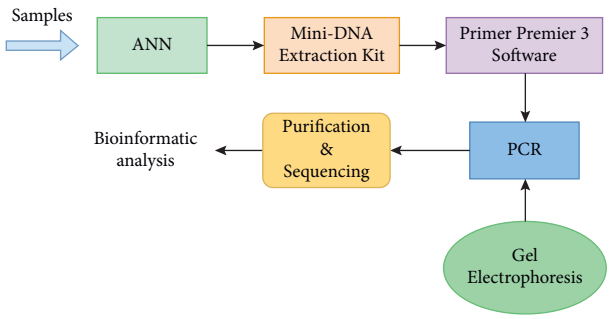

Figure 2: Flow chart of the investigation model.

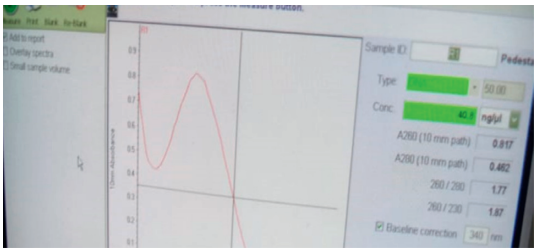

Figure 3: DNA quantification for UCP2 gene.

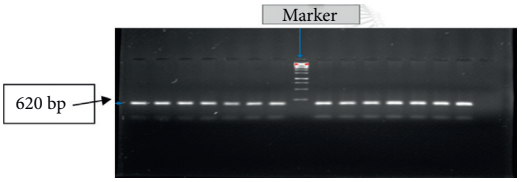

Figure 4: Amplified PCR product of the UCP2 gene.

3. Results

The sample obtained using the ANN priority list is passed through mini-DNA kit and Primer Premier 3 software for genetic analysis and other similar analysis to obtain the final results.

3.1. Genetic Analysis of UCP2 and UCP3 and Identification of Mutations. Through analyzing DNA sequence alignments on ensemble.org, mutations were discovered. The results of the quantification of DNA are depicted in Figure 3. PCR-amplified product gel photo is also shown in Figure 4. Results of the type of identified mutation, its percentage, and type of amino acid deduced are given in Tables 2 and 3. Moreover, the position of SNPs and location are described in Figure 5.

3.1.1. SNPs Description in UCP2. Seven distinct variations were discovered with respect to our query sequence when queried for the cow gene UCP2 known as ENSBTAG00000003692 and shown under Figure 5.

(1) Transversion mutation (guanine to cytosine) was noted at nt. 35 bp.

(2) Transition mutation (cytosine to thymine) was noted at nt. 57 bp.

(3) Transition mutation (cytosine to thymine) was noted at nt. 125 bp.

Table 2: Mutation percentage of three different cattle breeds.

Name of gene	Name of breed	Found SNP	Percentage method	Total percentage (%)
UCP2	Dhanni	2	2 × 100/192	1.041
	Cholistani	2	2 × 100/192	1.041
	Sahiwal	3	3 × 100/192	1.562
UCP3	Sahiwal	3	3 × 100/157	1.910
	Cholistani	0	0	0
	Dhanni	0	0	0

According to the definition of polymorphism in genetics, if rate of change of mutation is greater than 1% in any population, then, it is concluded as polymorphism and if rate of change is less than 1% in any population, then, it is concluded as just mutation.

Table 3: Genetic code-based identified mutations and deduced amino acids.

Sample ID	Position of mutation (bp)	Original codon	Modified codon	Original amino acid	Modified amino acid	Identified mutation
UCP2.2D1	27	GTG	GTA	Valine (E)	Valine (E)	Silent mutation
	41	CTG	CGG	Leucine (E)	Arginine (E)	Missense mutation
UCP2.2C1	60	CCC	CCT	Proline (N)	Proline (N)	Silent mutation
	80	GCT	GTT	Alanine (N)	Valine (E)	Missense mutation
UCP2.2S1	35	GGC	GCC	Glycine (N)	Alanine (N)	Missense mutation
	57	CGC	CGT	Arginine (E)	Arginine (E)	Silent mutation
	125	TCC	TTC	Serine (N)	Phenylalanine (E)	Missense mutation
UCP3.3S1	18	GGA	GGG	Glycine (N)	Glycine (N)	Silent mutation
	35	CTG	CGG	Leucine (E)	Arginine (E)	Missense mutation
	119	CAT	CGT	Histidine (E)	Arginine (E)	Missense mutation

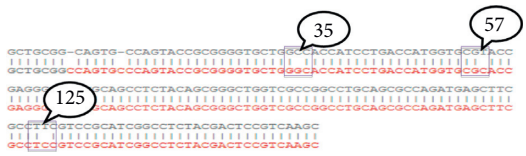

Figure 5: Position of the mutation in the UCP2 gene.

3.1.2. SNPs Description in UCP3. The results of DNA quantification for UCP3 gene are shown in Figure 6, whereas Figure 7 shows the amplified PCR product of the UCP3 gene. Three distinct variations were discovered concerning our query sequence when queried for the cow gene of UCP3 cow gene ENSBTAG00000005259 as shown in the sequence alignment in Figure 8.

(1) Transition mutation (adenine to guanine) was observed at nt. 18 bp

(2) Transversion mutation (thymine to guanine) was noted at nt. 35 bp.

(3) Transition mutation (adenine to guanine) was observed at nt. 119 bp.

According to the definition of polymorphism in genetics, if the rate of change of mutation is greater than 1% in any population, it is concluded as polymorphism. If the rate of change is less than 1% in any population, it is concluded as just mutation.

The silent mutation does not change an amino acid, but in some cases can still have a phenotypic effect. This change would have no effect on the protein's structure. As a consequence of the degeneracy of the genetic code, a point mutation will commonly result in the same amino acid being incorporated into the resulting polypeptide despite the sequence change. This change would have no effect on the protein's structure and is thus called a silent mutation. A missense mutation results in a different amino acid being incorporated into the resulting polypeptide. The effect of a missense mutation depends on how chemically different the new amino acid is from the wild-type amino acid.

3.2. Association Analysis. The association analysis of CGG mutation at exon 3 of the UCP3 gene shows all 05 diplotypes with 04 meat quantity traits in the Sahiwal cattle breed. Cattles with genotype BC had higher body height than those with genotype AA. The genotype AC shows lowest body height (138.7 ± 1.2) among all the other genotypes, e.g., AA, BC, CC and CD. Carcass weight (CW) shows the lowest P value (0.04) as compared to other diplotypes, and body height (BH) shows the highest P value (0.5) in the meat quality traits. Animals having BC genotypes were significantly linked with carcass weight ($P = 0.04$). Significant association of carcass weight and drip percentage was observed for CGC polymorphism in the UCP3 gene and nonsignificant association between body height and body width. The meat quantity-controlling traits such as carcass weight were measured according to the evaluation of Agriculture Canada, 1992 [34] (see Table 4).

4. Discussions

Genes that control metabolism and energy distribution are the source of the genetic variation in farm animals, which may be advantageous when improving productivity. Various

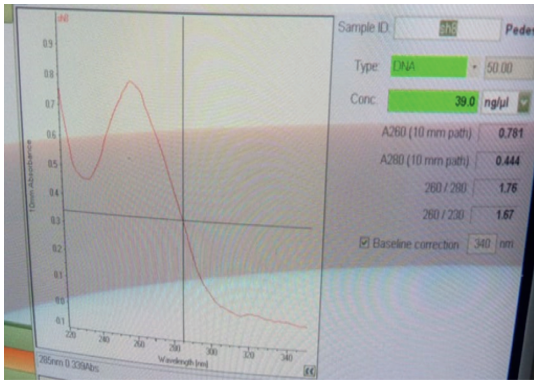

Figure 6: DNA quantification for UCP3 gene.

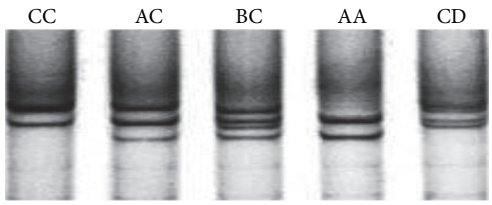

Figure 7: Amplified PCR product of the UCP3 gene on 10% PAGE.

Figure 8: Position of the mutation in the UCP3 gene.

research studies have proved that UCP2 and UCP3 are engaged, by way of physiological and pathological processes, in the control of metabolized energy [35], body mass index [36], oxidative stress [37], and obesity [38]. Gene mutation was also linked to human obesity, insulin resistance, meat quality in the past research [39–41], and pig meat yield [42–44]. According to the results of these above studies, UCP2 and UCP3 genes may be considered the crucial gene that affects cattle meat yield characteristics.

In the current study, using ANN sampling priority and through PCR-SSCP, DNA sequencing and gel electrophoresis were performed to identify seven mutations in the UCP2 gene and three in the UCP3 gene. Three silent mutations and two missense mutations are found on the fourth exon of the UCP2 gene and two missense mutations are found on exon 3 of the UCP3 gene, whereas one silent mutation is found and no changes have been made to the protein sequence. Among Dhanni cattle, 27 bp of UCP2 GTG has changed to GTA; both these variants code for valine, and the change was quiet mutation because of that. The coding of another amino acid, leucine, has changed from CTG to CGG, and this alteration was not manifested. The missense mutation has occurred.

In Cholistani, in the 60 bp domain of UCP2, there is an undetectable CCC mutation (proline) and an observable

Table 4: Effect of UCP1 gene polymorphism ($P < 0.05$) on meat quantity traits.

Diplotypes (mean ± SE)	Meat quantity traits			
	BH (cm)	HW (cm)	CW (kg)	DP (%)
AA ($n = 40$)	139.3 ± 1.5	46.8 ± 1.04	257.03 ± 9.9^{a}	52.3 ± 1.1^{a}
AC ($n = 47$)	138.7 ± 1.2	46.07 ± 0.8	266.6 ± 7.8^{ab}	53.5 ± 0.9^{ab}
BC ($n = 56$)	141.1 ± 1.0	46.8 ± 0.6	281.2 ± 6.5^{b}	55.5 ± 0.5^{b}
CC ($n = 46$)	139.8 ± 1.2	46.8 ± 0.8	255.1 ± 8.2^{a}	53.1 ± 0.9^{ab}
CD ($n = 26$)	139.0 ± 1.5	48.6 ± 1.04	252.04 ± 9.9^{a}	54.5 ± 1.1^{ab}
P value	0.5	0.45	0.04	0.11

Superscripts a and b used in the above Table 4 show significant difference ($P < 0.05$). BH: body height; HW: hip width; CW: carcass weight; DP: dressing percentage; SE: standard error of means.

GCT mutation (alanine) resulting in an effectively silent amino acid change. In the 80 bp domain, there is an observable GCT mutation (valine) and an undetectable GCT mutation (tryptophan) that causes an observable amino acid change. However, results of only silent mutations and missense mutations are shown in both graphs of mutations, whereas previous studies also support the role of silent mutations for gene function and expression as reported in [45].

Furthermore, our results revealed a significant association of mutation in UCP3 gene for drip percentage trait and carcass weight trait, whereas no significant relationship was found for body height and body width traits in bovine. The genotype BC appears to be favorable compared with other genotypes for growth and carcass trait performance. While comparing with the already published results of this study are inconsistent with the work described in [46]. In summary, correlations between genetic polymorphisms and carcass weight, drip %, body height, and body width characteristics in cattle are critical for understanding the genetics of complex economically significant variables. While the findings of this research indicate that these genes affect economically significant characteristics, because most of these genetic modifications do not result in amino acid changes, the mechanisms by which they arise are unknown. Additional information on how noncoding sequences influence gene activity becomes known, and it may become clear how these SNPs contribute to variance in these characteristics. Additionally, they may be associated with other causal mutations that have not been identified yet.

5. Conclusions

Technological innovations and advances in biological sciences have catapulted forensic genetics into a new era of DNA intelligence in recent years. For analysis in biomedical field, we need a good sample to increase the result prediction more toward true values with less error. This is only possible using artificial intelligence-based system. In this research, ANN is used to collect the best sample and ordering the sample according to the best suitability for further experiment process. With the help of ANN, the research identified ten mutations in total, seven from UCP2 and three mutations from UCP3. A significant association of UCP3 gene mutation for carcass weight and drip percentage traits and a

nonsignificant implication with body height and body width traits in bovine was noted. For growth and carcass trait performance, the genotype BC appears to be superior to those with other genotypes. The Veterinary and Animal Sciences Department can use the information to improve cow breeds for the benefit of the bovine industry. Major goal for future research will be the identification of the structure of these novel UCPs as well as the analysis of mice null for the UCP2 and UCP3 gene. In general, the genetic polymorphisms and SNPs, in particular, possess a great potential in unraveling the mechanism of how genotype affects phenotype by gene-gene and gene-environment interactions.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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