University of Sistan and Baluchestan

Journal of Epigenetics

Online ISSN: 2676 - 4350

Homepage: https://jep.usb.ac.ir

Iranian Genetics Society

Alterations in the expression of pituitary solute carrier (SLCs) families, ubiquitin-protease system, and ATP pump genes of high and low weight broilers using a microarray database analysis

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ARTICLE INFO

Article type:
Research Article

Article history:
Received:
Revised:
Accepted:

Keywords:
Microarray,
Broiler Line,
Pituitary,
Gene Expression .

A B S T R A C T

Selecting for juvenile body weight over the long term from a shared original population resulted in the creation of two distinct chicken lines: the low-weight selected line (LWS) and the high-weight selected line (HWS). Transport proteins encoded by solute carrier families operate within the inner mitochondrial membrane, facilitating the transport of metabolites. Ubiquitination, a dynamic and reversible process, plays a crucial role, alongside ATP-related genes, in maintaining cellular energy balance. The aim of this research was to clarify the distinction in pathway and system gene expression between high and low-weight broiler lines using microarray data previously documented on the GEO website (GEO Accession GSE122519). During weeks 1, 3, 5, and 7 of age, investigations revealed a notable reduction in SLC7A2 gene expression among the genes associated with the SLC families in HWS broilers during the first week of age. In the third week of age, there is an upregulation of SLCO2A1, SLC25A6, and SLC39A13, while SLC41A1 and SLC35B4 are downregulated. By the fifth week, SLC16A6 is upregulated along with SLC25A6, and in the seventh week, SLC35B4 is downregulated while SLC39A11 is upregulated compared to the LWS line ($P<0.01$). In the first week of age, there was an upregulation observed in relation to UBAP2L in the LWS line. By the third week, USP16 and RCJMB04_2 showed upregulation, followed by USP18 and USP16 in the fifth week. Additionally, HERPUD1 exhibited upregulation in the seventh week. In comparison to the HWS line, NUB1 and RCJMB04_3 displayed downregulation ($P<0.01$). Genes associated with ATP pumps exhibited 18 significant changes in gene expression. Specifically, 8 ATP pump genes were found to be highly expressed during the 7th week of age, while 4 genes showed increased expression during the third week of age in the HWS line compared to the LWS line ($P<0.01$). The comparisons revealed increased activity of the ubiquitination system and ATP pump-related genes in the anterior pituitary of LWS and HWS, respectively. Additionally, the SLCs gene families displayed both upregulation and downregulation of five different genes each during the production period. The results will inform future research aimed at optimizing broiler breeding strategies and improving poultry production efficiency based on a deeper understanding of molecular mechanisms underlying weight regulation.

Introduction

The breeding of meat-type chickens has led to a notable difference of more than tenfold in both body weight and feed intake. This breeding process has given rise to two distinct groups: high weight lines (HWS) marked by overeating and obesity, and low weight lines (LWS) recognized for their

DOI: https://doi.org/ 10.22111/JEP.2024.49968.1074

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How to Cite: Badakhshan, Y., Roudbari, Z., & Yousefi, M. (2024). Alterations in the expression of pituitary solute carrier (SLCs) families, ubiquitin-protease system, and ATP pump genes of high and low weight broilers using a microarray database analysis. *Journal of Epigenetics*, 5(2), 29-40. https://doi.org/ 10.22111/JEP.2024.49968.1074

reduced appetite. Additionally, certain LWS chickens exhibit severe anorexia.

The pituitary gland is crucial for maintaining energy balance by releasing a variety of hormones. The hypothalamus produces hormones that either stimulate or inhibit the release of these pituitary hormones, which are essential for regulating energy levels (Devesa, 2021). Pituitary dysfunction can impact energy regulation in diverse ways. This gland secretes hormones crucial for controlling metabolism, growth, and energy equilibrium.

These two statements offer distinct reactions to the injection of peripheral or central hormones, neurotransmitters, and amino acids. For instance, Hagen et al. (2013) discovered that food consumption following the central introduction of galanin showed similar patterns in both low-body weight (LWS) and high-body weight (HWS) broiler lines (Hagen et al., 2013).

In specific instances, like with calcitonin, alpha-melanocyte-stimulating hormone, and neuropeptide S, the LWS line showed a stronger decrease in food consumption after being administered compared to the HWS line (Cline, Prall, et al., 2008; Cline, Nandar, et al., 2008; Cline et al., 2010).

In a previous study, these lines exhibited a significant difference in genes associated with cellular transporters and transduction, which were predicted to influence the release of pituitary hormones (Ellestad et al., 2019). A different research study found notable alterations in molecular transport, intercellular communication, kinase function, and cellular growth due to intense selection between HWS and LWS (Zhang et al., 2015).

The Solute Carrier (SLC) protein family is vital for numerous cellular processes, facilitating the movement of amino acids, nutrients, and neurotransmitters. These transporters are crucial for preserving cell structure, and any malfunctions can impact conditions like cancer, inflammation, and autoimmunity. The SLC transporters are significantly important for upholding cell integrity.

Approximately one-third of identified human proteins are membrane proteins, belonging to various large families. Among these, the solute carriers (SLCs) stand out as one of the largest families with over 384 confirmed members, and new members are regularly discovered through ongoing research. SLCs serve as passive transporters, ion-coupled transporters, and exchangers, categorized into 48 distinct families (Jacobsson et al., 2007; Jacobsson et al., 2010; Sreedharan et al., 2011). Studies have shown the significance of SLCs in various poultry tissues and organs, including the small intestine (Zeng et al., 2011) . In the hypothalamus (Piórkowska et al., 2018), Pituitary (Tang et al., 2022), oviduct (Lim et al., 2012). breast muscle(Yu et al., 2018).

The ubiquitin-proteasome (UP) pathway is the primary system for breaking down proteins, responsible for degrading around 40 to 50% of proteins. Ubiquitination, a process within this pathway, is dynamic and reversible. Deubiquitinating enzymes (DUBs) play a vital role by undoing the work of E3 ligases, removing ubiquitin chains from target proteins. This action prevents protein degradation and reverses the functional changes induced by ubiquitination. Moreover, DUBs are essential for maintaining a balance in ubiquitin levels through their participation in processes like ubiquitin maturation, editing, and recycling. Several research findings have linked mutations in ubiquitin ligase to broiler muscle dystrophy (Matsumoto et al., 2008). In the context of heat stress conditioning, Li et al. (2011) observed an increase in ubiquitin genes such as Ubiquitin B (UBB), Ubiquitin C (UBC), and Ubiquitin-specific proteinase 45 (USP45) in the breast muscle of broilers, suggesting a role in heat regulation (Li et al., 2011). Similarly, Hassanpour et al. (2016) noted an elevation of ubiquitin genes in the midbrain of broilers in response to cold stress induction (Hassanpour et al., 2016). The Na+, K+-ATPase (NKA) is a versatile transmembrane protein present in all mammalian cells, primarily situated on the basolateral side of the cell membrane. It plays a crucial role in establishing an ion gradient essential for maintaining fluid balance within the cell. This pump consists of a catalytic α subunit and a noncatalytic β subunit. The β subunit aids in the proper maturation and targeting of the α subunit to the cell membrane. Studies have demonstrated the significance of the Na+, K+-ATPase pump in regulating calcium, sodium, and potassium levels in the brains of broiler chickens (Hertz et al., 2015). Additionally, this pump plays

a crucial role in the muscle growth of chickens in a tissue culture medium (Lopatina et al., 2023).

Genes that play a role in cell membrane solute carriers, ATP pumps, and membrane potential are crucial for the survival and proper development of animals. The pituitary gland, a key structure in the brain, regulates various functions including development, growth, reproduction, appetite, and immune response. Therefore, the consistent regulation of gene expression for solute carriers and ion channels is essential. the aim of this study is to investigates alterations in gene expression profiles related to pituitary functions in high and low weight broilers using a microarray database analysis. The focus is on three key gene families: solute carrier (SLC) transporters, ubiquitin-protease system components, and ATP pumps.

Material and Methods

In this study, we examined the gene expression profile linked to pituitary tissue in chickens. The data was retrieved from the GEO database, specifically under accession number GSE122519. This dataset published by Ellested et al. in 2019, explored gene expression differences in chickens of different weights at four developmental stages: weeks one, three, five, and seven. 263 genes were expressed differently between two broiler strains.

Initially, Ellested et al. (2019) carefully isolated the chicken pituitary glands from the subjects. RNA was then extracted from these tissues to ensure a pure sample for further analysis. The RNA samples were hybridized on Affymetrix microarrays, a technique that enables a comprehensive assessment of gene expression levels across the entire genome. This method is essential for identifying the specific genes active in pituitary tissue at various stages of development and in chickens with different weight profiles. After creating the gene expression profile, we proceeded with data preprocessing and expression analysis. These tasks were conducted using GEO2R software, a web tool that allows users to compare two or more groups of samples in a GEO series to identify genes differentially expressed across experimental conditions. This software is particularly beneficial for our study as it automates many of the standardization steps and ensures consistent and reliable identification of significant genes. In our analysis, we strictly followed the default settings provided by GEO2R software. This approach ensures reproducibility and allows other researchers to replicate our study using the same parameters. Once the differentially expressed genes were identified, we transferred the data to Excel for further analysis and visualization. Using Excel allows for detailed examination and manipulation of the data, facilitating the identification of genes with significant differential expression. We focused on genes with a p-value of less than 0.05, indicating statistical significance. Additionally, we considered genes with a log fold change (LogFC) between -1 and 1, representing the magnitude of change in gene expression levels. These thresholds help identify genes that are not only statistically significant but also biologically relevant. To better understand the functional implications of these differentially expressed genes, we used the Gene Ontology Resource database. This database provides a comprehensive set of functional annotations for genes, helping to elucidate the biological processes, cellular components, and molecular functions associated with the genes identified in our study. By linking our findings to the Gene Ontology terms, we can gain insights into the roles these genes play in pituitary tissue and how they might contribute to differences in growth and development in chickens with varying weights.

Results

SLC Gene

A recent study comparing the pituitary glands of high and low weight broilers found that ten SLC genes showed notable differences in gene expression. At three weeks of age, high weight broilers had three SLC genes that were upregulated and two that were downregulated compared to low weight broilers. At five and seven weeks of age, two specific SLC genes were both upregulated and downregulated respectively.

In the first week of age, only the SLC7A2 gene showed a significant drop in expression in the pituitary glands of high weight broilers compared to low weight broilers ($P<0.01$). By the third week, in high weight broilers, SLC41A1 and SLC35B4 were downregulated, while SLCO2A1, SLC25A6, and SLC39A13 were upregulated ($P<0.01$).

At the fifth week of age, SLC25A6 and SLC16A6 showed differential expression, and by the seventh week of age, SLC39A11 and SLC35B4 exhibited upregulation and downregulation, respectively. This study highlights the dynamic changes in gene

expression in the pituitary glands of broilers of varying weights at different stages of development (P<0.01). Figure 1

Ubiquitin genes

The expression of genes associated with ubiquitin showed notable changes during the growth process. Specifically, there were 6 genes that were downregulated and 2 genes that were upregulated. The UBAP2L gene exhibited downregulation during the first week of age, while USP16 and RCJMB04_2 showed downregulation in the third week. In the fifth week, USP18 and USP16 displayed downregulation, and HERPUD1 in the 7th week also had significant downregulation compared to the LWS line (P<0.01). On the other hand, NUB1 and RCJMB04_3 showed a significant increase in gene expression during the third week of age (P<0.01). These findings are illustrated in Figure 2.

ATP related genes

Eighteen genes linked to ATP exhibited significant changes in expression levels between the HWS and LWS lines. Among these genes, 14 showed an increase in expression while 4 displayed a decrease. Specifically, upregulation was noted in LOC772225 during the first week of age, ATPBD1C, LOC772225, ATP6V0A1, and ATP6V1D in the third week, ATP5G1 in the fifth week, and ATPBD1C, ATP6V1D, ATP6V0E1, ATP5H, ATP1B1, ATAD1, ABCF2, and ABCC5 in the seventh week (P<0.01). Conversely, downregulation was observed in LOC418468 and ATP1A1 during the fifth week, and ATP1A1 and ABCA8 in the seventh week. Figure 3(P<0.01). gene ontology of different genes exhibited in table 1.

Discussion

Animals like commercial broilers serve as effective models for studying variations in body fat and thinness. Recent research has demonstrated significant similarities between human and poultry genomes. Investigating the genomes and transcriptomes of these thin and fat poultry models has enhanced our understanding of genomic alterations, gene expression regulation, and the activation of genetic pathways. Additionally, poultry are ideal subjects for research due to their short reproductive cycles and the ability to study embryonic development outside the mother's body. difference in growth rate between high weight selected (HWS) and low weight selected (LWS) broilers was accompanied by variations in the expression of genes in various body organs. The anterior pituitary, a crucial regulator of body functions, has been examined in different broiler strains. A concise overview of solute carrier family, ATP pump, and ubiquitination system genes revealed notable distinctions between the anterior pituitary of HWS and LWS line.

In a recent investigation that compared the pituitary glands of high and low weight broilers, researchers observed distinct variations in gene expression among ten SLCs. At three weeks of age, three SLCs showed increased activity while two exhibited decreased activity in high weight broilers when compared to their low weight counterparts. By the time the broilers reached five and seven weeks of age, two specific SLC genes displayed both upregulation and downregulation. A prior study also highlighted a decrease in SLC18A2 expression in the hypothalamus of high weight broilers in contrast to low weight ones, which was linked to prolonged selection for differing body weights. When subjected to insulin treatment, two lines showcased downregulation of SLCO1C1 and SLCO2A1 genes, alongside upregulation of SLC6A4 and SLC18A2 genes in the hypothalamus (Zhang et al., 2015). Furthermore, in the hypothalamus of 45-day-old Ross 308 strain, there was a notable rise in SLC5A7 and SLC1A expression (Piórkowska et al., 2018). When comparing the pituitary glands of Shitou geese (fast growth) to Wuzong geese (slow growth), researchers observed downregulation of SLC30A2 and SLC26A11, while SLC5A8 and SLC16A10 were upregulated (Tang et al., 2022).

The ubiquitin–proteasome system (UPS) is the primary mechanism for protein breakdown in eukaryotic cells, overseeing various cellular functions like DNA repair, stress responses, and cell growth. This system involves specific enzymes that attach ubiquitin molecules to protein targets, marking them for degradation by 26S proteasomes (Mata-Cantero *et al.*, 2015). The process of ubiquitin conjugation to substrates involves a series of steps facilitated by E1, E2, and E3 enzymes (Wondrak, 2015).

Recent research has highlighted that UBAP2L is highly expressed in various cancers, attracting considerable attention from researchers in recent years. Its abnormal expression is a common

characteristic across different tumor types, suggesting a role in promoting cell proliferation through the regulation of cell cycle signaling pathways (Guerber *et al.*, 2022). UBAP2L belongs to both the Ubiquitin-binding and RNA-binding protein families, indicating its potential involvement in numerous cellular processes. It has been found to enhance the translation of specific mRNAs, indicating its function as a ribosome-binding protein crucial for protein synthesis (Luo *et al.*, 2020). Notably, in high weight broiler chickens during the first week of age, there is a significant decrease in UBAP2L gene expression compared to low weight broilers. This downregulation is believed to be a preventive measure to curb excessive cell proliferation in the pituitary gland and reduce the risk of tumor formation, as UBAP2L is considered an oncogene that promotes cell proliferation.

In the ubiquitination process, ubiquitin or ubiquitin-like proteins are covalently attached to target proteins by ubiquitin-activating enzymes. NUB1 functions as a negative regulator of ubiquitin-like proteins, particularly targeting NEDD8, a highly similar protein to ubiquitin. By inhibiting the ubiquitylation of various substrates, NUB1 influences critical cellular functions. Overexpression of NUB1 in an experiment suppressed gastric cancer proliferation, migration, and invasion. The expression of the NUB1 gene positively correlated with the p27Kip1 gene, which hinders proliferation and blocks the transition from G1 to S phase in gastric cancer cells (Zhang *et al.*, 2020).

Elevated ubiquitylation levels in tissues or cells result in increased protein degradation, and NUB1 is recognized for its anti-proliferative effects. The primary role of NUB1 lies in negatively regulating the ubiquitylation process, thereby impeding the degradation of multiple target proteins associated with ubiquitin-like proteins (Tanaka *et al.*, 2003). Essentially, ubiquitination leads to the silencing of cell proliferation genes, potentially contributing to tumor or cancer development. Therefore, upregulation of NUB1 in high-weight broilers could serve as a strategic approach to control cell mitosis.

Ubiquitin-specific peptidase 16 (USP16) is a deubiquitinase that is involved in regulating gene expression, cell cycle advancement, and various cellular functions. Initially recognized as a key deubiquitinase for histone H2A, USP16 has been found to deubiquitinate a wide array of substrates, encompassing proteins in both the cytoplasm and nucleus. During cell division, USP16 undergoes phosphorylation upon entry into mitosis and subsequent dephosphorylation during the metaphase/anaphase transition. The identification of multiple oncogene products as substrates for USP16 suggests its potential as a therapeutic target (Zheng et al., 2023).

Cyclin-dependent kinase-1 (CDK1) has been demonstrated to phosphorylate USP16 at Ser552, leading to its retention in the nucleus and facilitating proper cell cycle progression and cell proliferation. Notably, the depletion of USP16 in HeLa cells has been shown to impede cell growth by disrupting the mitotic cell cycle (Zhang et al., 2014). The activation of USP16 coincides with the onset of mitosis, and the downregulation of USP16 in high weight broilers has been linked to the prevention of pituitary cell tumorigenesis, highlighting the potential role of USP16 in regulating cell proliferation. Ubiquitin-specific peptidase 18 (USP18) is responsible for removing ubiquitin from its target proteins. It is prominently expressed in the liver, spleen, and thymus, with detectable levels also found in lung tissue, adipose tissue, and bone marrow (Malakhov et al., 2002). When USP18 is deficient, there is an increase in the differentiation of Treg cells, potentially disrupting the balance of peripheral T cells. This deficiency also leads to a decrease in INF-γ, IL-2, and IL-17A production by CD4+ T cells, as well as a reduction in INF-γ production by CD8+ T cells (Yang et al., 2021).

USP18 is itself upregulated in response to interferons (IFNs) through the activation of the JAK-STAT signaling pathway. Upon upregulation, USP18 binds specifically to the IFNAR2 subunit, inhibiting the phosphorylation of JAK1 by blocking the interaction between JAK1 and IFNAR2, thereby affecting downstream signaling (Malakhova et al., 2006).

Interestingly, studies have shown that the loss of USP18 can decrease lung cancer metastasis, and knocking down USP18 expression can reduce the proliferation, migration, and invasion of lung cancer cells (Chen et al., 2019).

The ubiquitin-conjugating enzyme (E2) is crucial for transferring ubiquitin from the ubiquitin-activating enzyme (E1) to both the ubiquitin-ligase

enzyme (E3) and the substrate. In a study comparing three-week-old high-weight broiler pituitaries to those of low-weight broilers, it was found that the E2 enzyme was downregulated in the high-weight broilers. Ubiquitin-conjugating enzymes are integral to the ubiquitination process and are often disrupted in conditions like breast cancer. Specifically, the ubiquitin-conjugating enzyme (E2) is implicated in various aspects of carcinogenesis, including breast cancer development, as highlighted in Voutsadakis' research in 2013 (Voutsadakis, 2013).

E2 enzymes play crucial roles in multiple processes that promote tumors, such as DNA repair, apoptosis, cell cycle advancement, and oncogenic signaling pathways. Due to the significant impact of ubiquitination on determining the outcomes of substrates, the increased presence of E2 enzymes is commonly detected in various cancer samples and is linked to unfavorable outcomes for patients (Bui et al., 2021).

The high weight broilers show a likely notable reduction in UPS gene expression compared to low weight broilers, resulting in increased deubiquitinase activity, which is crucial for heightened activity in anterior pituitary cells. This suggests that inhibiting the ubiquitin-proteasome pathway can lead to an increase in ACTH secretion, as indicated by Sesta et al (Sesta et al., 2020). Conversely, elevated deubiquitinating activity is a consequence of the downregulation of the ubiquitin-proteasome system (UPS). The Na+, K+-ATPase (NKA) is a crucial transmembrane protein found in all mammalian cells, primarily situated at the basolateral surface of the cell. Its main function is to establish a directional ion gradient essential for maintaining fluid balance within the cell. This pump consists of an enzymatic α subunit and a regulatory β subunit. In high weight broiler pituitary glands, there is a notable decrease in ATPase, Na+/K+ transporting, alpha 1 polypeptide (ATP1A1) and an increase in ATPase, Na+/K+ transporting, beta 1 polypeptide (ATP1B1).

ATP1A1, a transmembrane protein, plays a key role in actively transporting Na+ and K+ ions, thereby regulating the intracellular ion balance in epithelial cells. Overexpression of ATP1A1 has been observed in tumor samples from patients with medulloblastoma (Suñol et al., 2011) and glioblastoma (Lefranc and Kiss, 2008). Conversely, overexpression of the β1 subunit (ATP1B1) enhances tight junction formation in cell membranes, improving the barrier function of lung alveolar cells (Bai et al., 2021). Additionally, ATP1B1 has been linked to peptide-mediated transdermal drug delivery in epidermal cells (Wang et al., 2015).

Studies have shown that ATP1A1 overexpression is associated with esophageal tumor progression and has been identified as a potential target for anticancer therapy (Wu et al., 2016). The observed upregulation of ATP1B1 and downregulation of ATP1A1 in the pituitary of high weight broilers compared to low weight broilers is a rational process. This modulation may help prevent carcinogenesis and ensure cell membrane integrity during the rapid growth phase in high weight broilers.

ATP binding cassette (ABC) transporters are a diverse group of transmembrane proteins present across various life forms. Initially thought to primarily function as exporters in eukaryotes, some members of the ABCC sub-family have evolved to regulate ion channels. The family comprises 48 known proteins categorized into seven groups (Wang et al., 2021). Notably, in high-weight broiler pituitaries, subfamily ABC transporters F and C were upregulated while subfamily ABC1 was downregulated. Additionally, a gene similar to ATP-binding cassette transporter 13 was found to be downregulated in high-weight broilers compared to low-weight ones. The ABCA subfamily consists of 12 functional transporters, from ABCA1 to ABCA13. ABCA1 plays a crucial role in cholesterol homeostasis by exporting excess cellular cholesterol and phospholipids to apolipoproteins, particularly in the brain where cholesterol synthesis in astrocytes supports neuronal cholesterol distribution (Tall and Wang, 2000; Wahrle et al., 2004). Furthermore, ABC sub-family F exhibits anti-inflammatory properties, reducing proinflammatory cytokines (Powell et al., 2013), while sub-family C, including CFTR/MRP members, functions as essential ATPase transporters with roles in heavy metal efflux, protecting cells from toxicity (Toyama et al., 2011).

AAA+ proteins are characterized by possessing a conserved ATPase domain responsible for converting ATP hydrolysis into mechanical

energy. These enzymes, known as AAA+ enzymes, play a crucial role in targeting protein substrates rather than nucleic acids, acting as essential regulators that drive diverse biological processes such as protein quality control, cytoskeleton rearrangement, and membrane fusion (Zhang and Mao, 2020). In study involving seven-week-old high-weight broilers, it was observed that this gene exhibits significantly higher expression levels in comparison to low-weight broilers. The specific AAA ATPase mentioned is classified as a Type I ATPase and is particularly important for meiosis. This enzyme plays a critical role in cytoskeleton remodeling, a function that is essential for membrane remodeling in various biological contexts (Puchades et al., 2020). The ATP synthase located in the inner mitochondrial membrane utilizes the energy from a gradient of H+ ions, which is created by electron transport, to link the translocation of H+ ions with the production of ATP. In high-weight broilers, there is a higher expression of genes related to this ATP synthase in the pituitary gland compared to low-weight broilers. F1Fo-type ATPases, also known as F-ATP synthases, are responsible for generating ATP during oxidative phosphorylation. Within animal cells, this enzyme spans the inner mitochondrial membrane and harnesses the energy from an electrochemical gradient of H+ ions produced by an electron transport system that pumps H+ ions (Brandt and Müller, 2015).

The pH levels within different intracellular compartments exhibit significant variation: mitochondria tend to be basic, while lysosomes are notably acidic. This distinct pH environment within each organelle plays a critical role in its functionality, influencing a wide array of biological processes such as protein processing and breakdown, intracellular transportation, synaptic communication, and viral replication. The vacuolar H+-ATPase (V-ATPase) located on the organelle membranes is primarily responsible for creating and sustaining the acidic pH within endocytic and secretory organelles by actively moving hydrogen ions (H+) from the cytoplasm into their interior in an energy-dependent manner using ATP (Forgac, 2007).

V-ATPase plays a significant role in the buildup of hydrogen ions (H+) within these vesicles. Maintaining an acidic environment within endosomes is crucial for separating receptors from their ligands and ensuring their proper targeting (Saftig and Klumperman, 2009; Zoncu et al., 2011).

The V-ATPase consists of two complex domains, V1 and V0, which play a crucial role in cellular processes (Benlekbir et al., 2012). Research indicates that these two subunits are significantly increased in high-weight broilers at different ages compared to low-weight broilers. This suggests that in high-weight broilers, the pituitary gland requires more active lysosomes during periods of rapid growth. Lysosomes are essential organelles responsible for breaking down materials within the cell through processes like endocytosis and autophagy.

The adenine nucleotide translocase, a key protein found within the inner mitochondrial membrane and encoded by nuclear DNA, facilitates the exchange of ADP and ATP molecules between the cytosol and the mitochondrial matrix. This electrogenic process, involving the most abundant protein in mitochondria, plays a crucial role in cellular energy metabolism and the generation of mitochondrial energy. The expression of genes related to the ADP/ATP translocase is tightly regulated in response to various functional and developmental cues (Ruprecht and Kunji, 2019). Studies have shown that in cell cultures, the expression of these genes is linked to the metabolic and energetic state of the cells, with fluctuations occurring during different growth phases (Lunardi and Attardi, 1991). Inhibition of ADP/ATP translocase gene expression has been associated with a delay in cell growth due to its pivotal role in the respiratory capacity of the cell, acting as a rate-limiting factor in the assembly of respiratory complexes (Lunardi et al., 1992).

Conclusion

The findings suggest that ATP-related genes are critical in the development of high-weight-gain (HWS) broilers, with higher transcription levels observed in the anterior pituitary compared to low-weight-gain (LWS) broilers. In contrast, ubiquitin-related genes have higher transcription levels in LWS broilers, indicating that ubiquitination is more activated in this strain during selection and development. Finally, SLC genes have shown equal up or down-regulation but with different distributions throughout the weeks of broiler age. These results provide valuable insights into the genetic differences between HWS and LWS

broilers, which could inform future breeding strategies for improved poultry production.

In conclusion, the long-term selection for juvenile body weight in chickens has resulted in the emergence of two distinct lines: the low-weight selected line (LWS) and the high-weight selected line (HWS). This study has elucidated the differential expression of pathway and system genes between these broiler lines, providing significant insights into the molecular mechanisms underlying weight regulation. The analysis of microarray data revealed notable variations in the expression of solute carrier (SLC) family genes, ubiquitination-related genes, and ATP pump-associated genes across different age stages.

Specifically, the research highlighted a marked reduction in SLC7A2 expression in HWS broilers during the first week, along with distinct patterns of upregulation and downregulation in various SLC genes over subsequent weeks. The LWS line exhibited a pronounced upregulation in genes related to ubiquitination, such as UBAP2L, USP16, and HERPUD1, indicating heightened ubiquitination activity. In contrast, the HWS line demonstrated significant changes in ATP pump gene expression, particularly during the seventh week of age.

These findings underscore the intricate roles of transport proteins, ubiquitination processes, and ATP-related genes in maintaining cellular energy balance and regulating body weight. The differential gene expression patterns between the LWS and HWS lines suggest potential targets for genetic and breeding interventions aimed at optimizing broiler production. Future research building on these results can further enhance our understanding of the genetic and molecular bases of weight regulation, ultimately contributing to improved breeding strategies and greater efficiency in poultry production.

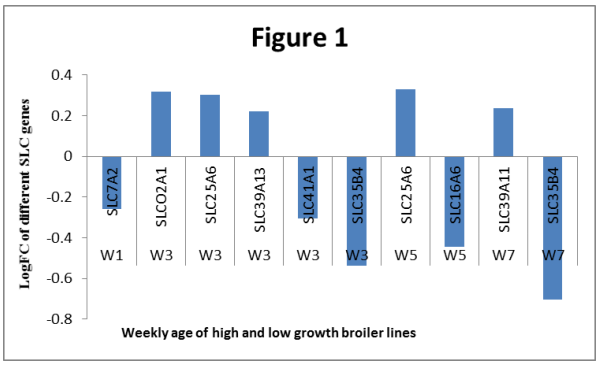


Figure 1: The expression of SLC genes in the anterior pituitary of the high-weight broiler line compared to the low-weight line, with all genes showing a significant difference at a level of P<0.01. SLC7A2: Solute carrier 7A2, SLCO2A1: Solute Carrier Organic Anion Transporter Family Member 2A1, SLC25A6: Solute Carrier Family 25 Member 6, SLC39A13: Solute Carrier Family 39 Member 13, SLC41A1: Solute Carrier Family 41 Member 1, SLC35B4: Solute carrier family 35 member B4, SLC16A6: Solute Carrier Family 16 Member 6, SLC39A11: Solute Carrier Family 39 Member 11, SLC35B4: Solute carrier family 35 member B4.

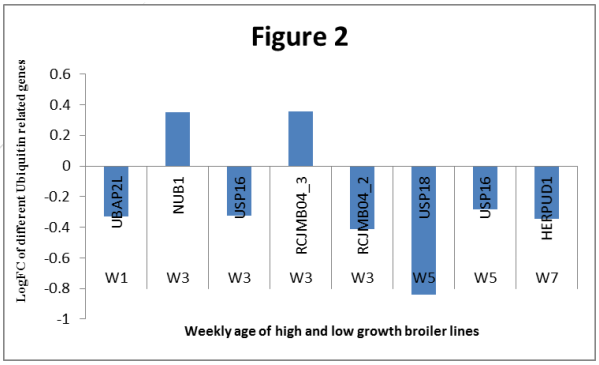


Figure 2: The expression of Ubiquitin genes in the anterior pituitary of the high-weight broiler line compared to the low-weight line, with all genes showing a significant difference at a level of P<0.01. UBAP2L: Ubiquitin Binding Protein 2-like, NUB1: NEDD8 ultimate buster 1, USP16: Ubiquitin Specific Peptidase 16, RCIMB04: RNA binding motif protein 4B, USP18: Ubiquitin Specific Peptidase 18, HERPUD1: Homocysteine Inducible ER Protein With Ubiquitin Like Domain 1.

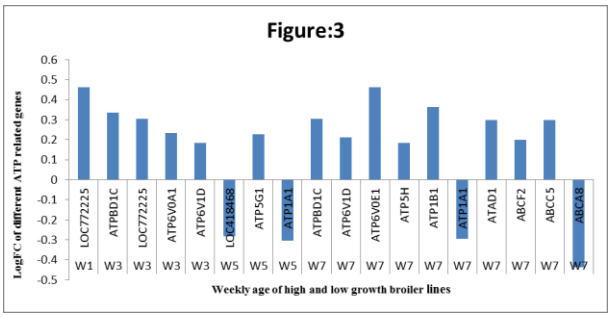


Figure 3: The expression of ATP related genes in the anterior pituitary of the high-weight broiler line compared to the low-weight line, with all genes showing a significant difference at a level of P<0.01. ATPBD1:ATP-binding cassette B1, ATP6V1D: V-type proton ATPase subunit D, ATP5G1: subunit of mitochondrial ATP synthase, ATP1A1: ATPase Na+/K+ Transporting Subunit Alpha 1, ATP5H: Mitochondrial ATP synthase catalyze, ABCF2: ATP-binding cassette sub-family F number 2, ABCC5: ATP Binding Cassette Subfamily C Member 5, ABCA8: ATP Binding Cassette Subfamily A Member 8.

Table 1. Gene ontology of genes expressed differently in anterior- pituitary of high and low weight broiler strains.

Gene term	P value	Log Fc	Gene ontology
SLC7A2	0.04657	-0.2572	Organic acid transmembrane transport
SLCO2A1	0.0413642	0.316944	Organic substance transport
SLC39A13	0.060938	0.222306	Zinc ion transport
SLC41A1	0.0270626	-0.30516	Cation transmembrane transporter activity
SLC35B4	0.0186049	-0.53598	Ion transmembrane transporter activity
SLC25A6	0.026163	0.329619	Mitochondrial transport
SLC16A6	0.012216	-0.44323	Ion transport, Organic anion transport
SLC39A11	0.0446561 5	0.23598 7	Inorganic cation transmembrane transport
ATP6V1D	0.0417946 2	0.21349	Insulin receptor recycling
ATP6V0E1	0.0129896 6	0.46352 6	ATPase-coupled cation transmembrane transporter activity
ATP1B1	0.0132175 1	0.36309	Cation homeostasis
ATP1A1	0.0333217 3	-0.29546	Potassium ion transport
ATAD1	0.0245823 1	0.29777 9	ATP binding
ABCF2	0.0475834 8	0.20074 8	ABC-transporter extension domain
ABCC5	0.0097576 1	0.29831 5	Peptide transport
ABCA8	0.0020937 7	-0.43666	Lipid transport
UBAP2L	0.03343	-0.33124	Intracellular membrane-bounded organelle

NUB1	0.0070865	0.352555	Protein ubiquitination
USP16	0.0133493	-0.32316	Ubiquitin-dependent protein catabolic process
HERPUD1	0.00866142	-0.34343	Regulation of programmed cell death

SLC7A2: Solute carrier 7A2, SLCO2A1: Solute Carrier Organic Anion Transporter Family Member 2A1, SLC25A6: Solute Carrier Family 25 Member 6, SLC39A13: Solute Carrier Family 39 Member 13, SLC41A1: Solute Carrier Family 41 Member 1, SLC35B4: Solute carrier family 35 member B4, SLC16A6: Solute Carrier Family 16 Member 6, SLC39A11: Solute Carrier Family 39 Member 11, SLC35B4: Solute carrier family 35 member B4. UBAP2L: Ubiquitin Binding Protein 2-like, NUB1: NEDD8 ultimate buster 1, USP16: Ubiquitin Specific Peptidase 16, RCIMB04: RNA binding motif protein 4B, USP18: Ubiquitin Specific Peptidase 18, HERPUD1: Homocysteine Inducible ER Protein With Ubiquitin Like Domain 1. ATPBD1:ATP-binding cassette B1, ATP6V1D: V-type proton ATPase subunit D, ATP5G1: subunit of mitochondrial ATP synthase, ATP1A1: ATPase Na+/K+ Transporting Subunit Alpha 1, ATP5H: Mitochondrial ATP synthase catalyze, ABCF2: ATP-binding cassette sub-family F number 2, ABCC5: ATP Binding Cassette Subfamily C Member 5, ABCA8: ATP Binding Cassette Subfamily A Member 8.

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