8-2023

The Effect of Herbal Adaptogen on Feeding-Related Hypothalamic Neuropeptides in Heat-Stressed Broilers

Maryam Afkhami Ardakani
University of Arkansas-Fayetteville

The Effect of Herbal Adaptogen on Feeding-Related Hypothalamic Neuropeptides in Heat-Stressed Broilers

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Poultry Science

by

Maryam Afkhami Ardakani
University of Isfahan
Bachelor of Science in Cellular and Molecular Biology, 2011

August 2023
University of Arkansas

This thesis is approved for recommendation to the Graduate Council

Sami Dridi, Ph.D.
Dissertation Director

Michael T. Kidd, Ph.D.
Committee Member

Sun-Ok Lee, Ph.D.
Committee Member

Wayne J. Kuenzel, Ph.D.
Committee Member

Abstract

Heat stress is a major issue in the poultry industry, having numerous negative consequences for chicken productivity. Decreasing feed intake is one of these issues, which results in higher mortality and poor growth performance. Moreover, Herbal adaptogens, plant extracts that are also known as stress response modifiers, are metabolic regulators that improve an organism's ability to adapt to environmental stresses and minimize damage from such stressors. Previously, we published data indicating that herbal adaptogen supplementation increased feed intake under heat stress conditions. Therefore, we hypothesized that dietary herbal adaptogen supplementation might exert these effects through alteration of the expression of hypothalamic neuropeptides involved in appetite and feed intake in heat-stressed broilers. A total of 720 male broiler cobb 500 chicks were randomized to 12 chambers for three different diet treatments. As a control group, broilers in treatment 1 were fed a corn-soybean diet. The broilers in treatments 2 and 3 were supplemented with the herbal adaptogen in two different doses in control diets 500 g/1000 kg (NRPHY-500) and 1 kg/1000 kg (NR-PHY-1000), respectively. Broilers in 9 chambers were exposed to 35 °C for 8 hours (a cyclic heat stress condition) from d29 to d42, while 3 chambers were maintained at 24 °C (thermoneutral condition) for all 42 days. The real-time qPCR was performed to evaluate the gene expression of 17 hypothalamic neuropeptides that play a vital role in feed intake and appetite, and the data were analyzed using ANOVA. Only the gene expression of visfatin changed significantly ($P = 0.0105$) among the 17 genes studied, while the expression of the other neuropeptides remained unchanged. We concluded these findings suggested that adding adaptogen supplementation to broilers' diets could be a promising way to reduce the negative effects of heat stress by modulating hypothalamic genes that control appetite and feed intake.

Keywords: broilers, heat stress, herbal adaptogen, feed intake, hypothalamic neuropeptides

Table of Contents

List of Figures

List of Tables

1. The importance of the broiler industry in the world

Poultry production has increased more quickly than any other meat since the 1960s, in both developing and developed countries. It is anticipated that this growth pattern would persist because the most effective and cost-effective source of protein is poultry, which is also considered to be very healthy. So, for billions of people, food security and availability are significantly impacted by poultry production (1).

1.1 Challenges Facing the poultry industry

The current primary objectives of the poultry industry have been disease control, high output, product quality, and affordable production costs (2).

Food safety is one of the challenges for the poultry industry. Through the food chain, numerous foodborne illnesses can spread. Many studies have shown that Salmonella and Campylobacter spp. are important bacteria in the poultry industry that cause many human foodborne illnesses (3).

Currently, antibiotic resistance in both humans and animals is another challenge that has become a widespread issue, and it is anticipated to constitute a persistent public health risk (4). Using antibiotics can improve broiler development performance by improving digestion and absorption, but it can also make bacteria more resistant to antibiotics. So, the use ofantibiotics to enhance and increase the growth of chicken has become a public concern (5,6).

Another significant challenge is climate change, which has seen severe changes recently. Due to the poor environmental adaptation of modern broilers the poultry industry has suffered significant financial losses on a global scale (7).

2. Stress Definition

The term “Stress” is straightforward but has several meanings. An animal is said to be under stress, according to Fraser if it must alter its behavior or physiology in an abnormal or excessive way to deal with unfavorable features of its environment and management (8). Koolhaas suggested the term “stress” should only be used to describe situations in which an organism's intrinsic capacity for regulation is exceeded by environmental demands, particularlythose that involve unpredictability and uncontrollability. Stress appears to be physiologically defined by either a diminished neuroendocrine response that is uncontrolled or the absence of an anticipatory response (unpredictable). The implications of these narrow criteria for stress research are examined, as well as how to interpret the findings in terms of the adaptive or maladaptive character of the reaction (9).

Stress, in Selye's words, is "the body's nonspecific response to any demand." Stress, then, is an animal's response to stimuli that upset its physiological homeostasis or normal physiological equilibrium (10). Stress is more frequently induced by unpleasant than by pleasant stimuli, implicating a complex network of psycho-physiological reactions that can be grouped together inwhat Pasternac and Talajic refer to as the " defense reaction," which enables the animal to either face a threatening situation or flee (11). Konstantinos Kagias claims that stress is a fundamental aspect of the natural environment that affects almost all biological systems. Any situation that pushes living systems away from a physiological steady state is referred to as biological stress, and the nature of the factors that influence live creatures strongly influences howit affects them. The term "stress" has been used in a wide variety of contexts thus far because it may be applied to a wide range of biological organization levels. The term “Physiological Stress”which is also known as the "basic biological stress," refers to any internal or external circumstance that compromises a cell's or an organism's ability to maintain homeostasis. We can envision threedistinct types of physiological stress, considering the various potential sources of biological stress:intrinsic developmental stress, environmental stress, and aging (12).

1.2 Types of stress

Numerous pressures, including those related to the environment, nutrition, and internal health, are encountered in the commercial production of poultry. These stresses have an impact on the health of poultry birds as well as their ability to produce and reproduce.

1.2.1 Cold Stress

Cold stress is one of the significant environmental stressors (CS). When the temperature drops below 18°C, cold stress develops. If this occurs, the animal may struggle to warm itself, which could lead to major cold-related illness, tissue damage, and final death (13). When chickens older than 21 days are exposed to a cold stress environment at 15.5°C, the feed intake, feed conversion ratio, and mortality considerably rise, while the body weight and productivity normally decrease as a result of the cold stress (14). Adult birds can resist cold stress, while neonatal chicks are vulnerable to cold temperatures because adult chickens can regulate their body temperature and generate heat by metabolism, but neonatal chicks are unable to regulate their body temperature in chilly surroundings until they develop the ability for thermogenesis and fully develop thermogenic organs (15,16).

1.2.2 Stocking density stress

Another crucial element, stocking density (SD), has been linked to physiological stress in several poultry species, which has become a major source of worry (17). The performance, welfare, and well-being of birds are directly impacted by stocking density. High stocking density is strongly linked to leg abnormalities such as lameness, footpad dermatitis, irregular gaits, and hock burns (18). High SD has been shown to increase eggshell lightness, breakage, decreased egg laying rates, shell color, strength, thickness, and relative weight in layers (19).

1.2.3 Heat Stress

Due to decreasing feed intake and a negative energy balance, heat stress is a significant role in the reduction of animal productivity and reproductive efficiency. The two main environmental parameters that affect the level of heat stress in animals are air temperature and humidity. Egg production, laying hen egg quality, and broiler growth rate are all negatively impacted by heat stress. Unwanted meat quality has been linked to broiler exposure to environmental stress throughout their growth stage (20).

The symptoms of heat stress in poultry include panting, increased respiratory rate increased water intake, decreased appetite, decreased egg production, smaller eggs, poorer egg quality, reduced body weight, and increased cannibalism (21). When birds are experiencing heat stress conditions; they spend less time eating and walking, and more time drinking, and panting.

2. Heat Stress

2.1 Effect of Heat Stress on the Poultry Industry

One of the most important environmental stressors is heat stress, which is posing problemsfor the global poultry industry. Due to heat stress, the commercial poultry industries are seriously threatened, because heat stress has an impact on both broilers and laying hens in wide-ranging, from reduced growth and egg production to decreased flesh and egg quality. So, industries are looking for reasonable and practical strategies to adapt to global warming.

2.2 Effect of heat stress on birds

2.2.1 Behavioral and Physiological Effects of Heat Stress

Birds adjust their behavior and physiological homeostasis to seek thermoregulation in hot weather, which lowers body temperature. Different species of birds generally respond to heat stressin a similar manner, displaying some individual variation in the strength and length of their responses. According to a recent study, when birds are under heat stress, they spend less time eating, more time drinking and more time panting (23,24).

Air sacs are an additional mechanism used by birds to facilitate heat transfer between their bodies and the environment. In order to boost gas exchanges with the air and reduce the evaporative loss of heat, air sacs are highly helpful while panting. It is important to note that increased panting under heat stress conditions raises blood pH and carbon dioxide levels, which in turn reduces the blood's availability of bicarbonate for the mineralization of eggshells and raises the availability of organic acids while also lowering the blood's free calcium levels. Due to its impact on the quality of the eggshell, this process is crucial in breeders and laying hens (25).

The reproductive system of chickens can be impacted by heat stress in a variety of ways. Heat stress can alter the normal condition of the ovary and hypothalamus where reproductive hormones are produced in females, which results in decreased systemic levels and functioning. Additionally, studies have demonstrated the harmful effects of heat stress in males. When males were exposed to heat stress, their sperm volume, concentration, number of viable sperm cells, and motility all reduced (26,27).

1.1.1 Effect of Heat Stress on the Immune Response

The impact of heat stress on the immunological response has recently been the subject of numerous investigations in chickens. Under the HS scenario, significant organ weights (such as the liver and muscle) do not increase as anticipated, along with performance degrading. Thymus, bursa of fabricius, and spleen weight are three lymphoid tissues that are also lighter. Reduced macrophage activity altered splenic and cecal tonsil inflammatory cytokine expression, and decreased antibody production against immunized antigens are all other effects of HS (28, 29). The production of T and B lymphocytes is reduced at high temperatures, and blood leukocyte phagocytic activity is suppressed (22). In broilers exposed to heat stress, Bartlett and Smith discovered lower levels of circulating antibodies overall as well as lower levels of IgM and IgG (23). Following heat exposure, total WBC and leukocyte activity were shown to decrease. It was further substantiated by Zulkifi that the synthesis of antibodies was greatly reduced by heat stress. A complex network of the immunological, endocrine, and neurological systems is responsible for the central nervous system's (CNS) inflection of the immune response (24). According to Ryota Hirakawa's research, HS affects T and B cell maturation and development in primary and secondary lymphoid tissues, leading to a variety of immunological disorders in broilerchickens(25).

3.3 Molecular Mechanisms involved in Feed Intake

A variety of hormones, neurotransmitters, released substances, and cytokines are part of the intricate, extensive, and elaborate network that makes up the feeding response in poultry (34). Like all animals, poultry species regulate their feed intake and energy expenditure to maintain body weight (BW) over the course of their lives. Complex and interconnected neural and endocrine networks are in charge of both of these processes, which are used to maintain BW and achieve energy balance. To detect dietary (energy) status, specific systems have evolved. They work in conjunction with distinctive signaling pathways to connect peripheral tissues to the central nervous system (CNS). Numerous signaling molecules that provide details about the nutritional status of the entire body have been discovered and researched. Neuropeptides are among them. These signaling molecules work at a peripheral, central nervous system (CNS), or both locations to activate particular neuronal circuits or operate as endocrine and metabolic regulators that eventually influence feed intake and energy homeostasis (35).

3.3.1 NPY (Neuropeptide Y)

Over 40 years ago, Tatemoto & Mutt made the discovery of neuropeptide Y (NPY) (26). A member of the pancreatic polypeptide family, it is a 36 amino acid peptide. Since it contains a number of tyrosine residues, including an amidated C-terminal tyrosineresidue, its name is derived from the single-letter code (Y) for the amino acid tyrosine. One of thepeptides most frequently discovered in the brain is this one. In the course of evolution, it has been one of the most conserved peptides. These characteristics imply that it is crucial for the regulationof vital physiological processes. In both birds and mammals, neuropeptide Y (NPY) regulates foodintake and body weight in a crucial way. Only two amino acids separate chicken- and porcine- NPY (94% identity). In chickens, six NPY receptors, including Y1, Y2, Y4, Y5, Y6, and Y7, have been discovered and studied. The orexigenic impact of NPY in mammals is mediated via the Y1 and Y5 receptors, both of which exhibit high levels of mRNA expression in the chicken hypothalamus (37). Comparing the NPY Y4 receptor to the NPY Y1 receptor, Y2 receptor, and Y5 receptor, the NPY Y4 receptor exhibits the lowest degree of commonality between species. Chicken NPY Y4 receptors only share 56–60% of their overall amino acid composition with mammalian NPY Y4 receptors. Based on study done by Lundell in 2000, a partial chicken NPY Y6 receptor sequence derived from polymerase chain reaction shares 65% similarity with Y6 from mice and rabbits (human NPY Y6 receptor is a pseudogene) (38). When the transmembrane portions of the NPY Y1, Y2, and Y5 receptors were compared to their mammalian orthologues, the amino acid identity was 85%, 85%, and 77%, respectively (37).

3.3.2 CART (Cocaine and Amphetamine Regulated Transcript)

A specific mRNA was reported to be increased by the acute ingestion of cocaine or amphetamine by Douglass. They called this transcript the term "cocaine- and amphetamine- regulated transcript" (CART) (39). CART (cocaine and amphetamine-regulated transcript) peptides‘’ anorectic peptides of 41 or 48 amino acids and three intramolecular disulfide links’’ are important neurotransmitters and hormones that are widely distributed in the central nervous system (CNS) and are involved in controlling a variety of processes, such as controlling food intake, body weight maintenance, and endocrine functions (40).

3.3.3 CCK (cholecystokinin)

One of the most prevalent neuropeptides in the brains of mammals and chickens is cholecystokinin (CCK), which is well-recognized as a hormone that regulates digestion (27). Cholecystokinin (CCK) is a peptide that operates as a neurotransmitter in the brain and enteric nervous system via CCK-B and CCK-A receptors as well as a peripheral hormone via CCK-A receptors (28). Sequencing has shown that the CCK-A and -B receptors are unique from one another, although the gastrin receptor is identical to the CCK-B receptor. Animals of different species were used to study how CCK affected feeding. It has been shown that CCK has the power to block animal feeding both through the central and peripheral nervous systems (43).

3.3.4 Ob-R (Leptin Receptor)

Ob-R, also known as leptin, belongs to the class I cytokine receptor family. The isoforms of the receptor can be categorized into three groups based on structural differences: long, short, and secretory isoforms. The hypothalamus is the primary site of expression for a long, fully active isoform of Ob-Rb, which participates in energy balance and the control of secretory organ function (44). Leptin is secreted by adipose tissue in mammals; however, it is largely produced in the brain and pituitary (non-adipose tissues) in chickens. It is crucial in feeding control, energy homeostasis, and metabolism (45).

3.3.5 Corticotropin-releasing hormone (CRH)

The hypothalamic-pituitary-adrenal (HPA) axis is activated by CRH, which can stimulate anterior pituitary corticotrophs to release adrenocorticotropic hormone (ACTH). The release of corticotropin and glucocorticoids "a member of the compound family known as corticosteroids (steroids)" is stimulated by corticotropin-releasing hormone (CRH), a key regulator of the hormonal stress response. The fact that CRH is also frequently thought to mediate stress-related behaviors suggests that the hormone has a larger, integrative role in the psychological stress response (46). This hormone increases feed intake in the broiler (47).

3.3.6 Visfatin

Visfatin as a protein that is produced by adipose tissue is a novel adipocyte-secreted factor has actions similar to those of insulin and has a strong correlation with visceral obesity (29). It plays a role in various biological processes, including metabolism, inflammation, and aging. Visfatin was first identified as a novel adipokine that was secreted by visceral adipose tissue and was thought to be involved in insulin resistance and type 2 diabetes (30). According to Cline's research,broiler appetites can be greatly increased by intracerebroventricular (ICV) injections of human recombinant visfatin (31). According to studies, this surge may be caused by a decrease in the hypothalamic activities of dopamine (DA), corticotropin- releasing hormone (CRH), and CART. These data show that visfatin may have a role in feeding promotion (34).

3.3.7 AgPR (Agouti-Related Protein)

Parallel to Shutter and coworkers, Ollmann and colleagues discovered a hypothalamus protein in 1997, which they named agouti-related transcript and then agouti-related protein because of its great sequence similarities to agouti (AgRP). Because of its pattern of expression and physiological effects, AgRP has been hypothesized to play a crucial role in the regulation of energy balance. In fact, transgenic mice that overexpress AgRP everywhere are hyperphagic and obese, while obese and diabetic mice have higher hypothalamus AgRP levels. It is recognized as a crucial modulator of energy balance. Due to its physiological effects and pattern of expression, AgRP has been hypothesized to play a crucial role in the regulation of energy balance. It is a potent orexigenic peptide that boosts food intake in both chickens and mammals (53).

3.3.8 Orexin

The posterior and lateral hypothalamus of the rat brain served as the initial sites of the orexins (orexin A and orexin B) discovery. These neuropeptides bind to the orexin receptor 1(ORXR1) and orexin receptor 2 which are located in the brain. Additionally, in mammals play an important role in many functions such as feed intake, sleeping, and glucose homeostasis. Orexin and its receptors are found in many different regions of the brain such as the pituitary gland, adrenal gland, gut, basal hypothalamus, and anterior and posterior eminence species in avian. Although orexin increases feed intake in a mammal, its function in birds is not determined (54,55).

3.3.9 Melanocortins

Melanocortin receptors are seven-transmembrane domain receptor proteins connected to G-proteins that primarily transmit signals via intracellular cyclic adenosine monophosphate. A single polypeptide with seven -helical TM domains, an external N-terminus, and an intracellular C-terminus makes up melanocortin receptors (56).

The melanocortin system is critical in animals for regulating food intake and energy metabolism. Melanocortin MC1 receptor is a melanocyte-MSH receptor that is expressed on cutaneous melanocytes and plays an important role in the pigmentation of the skin and hair. Melanocortin

MC1 receptor is also found on leukocytes, where it may play a role in anti- inflammatory activity. Melanocortin MC2 receptor is an adrenocortical ACTH receptor found in the adrenal cortex's zona reticularis and zona fasciculate, where it acts as a mediator between ACTH and steroid release. Melanocortin MC3 receptors have been found in various parts of the central nervous system and peripheral tissues, where they play a role in energy regulation. Melanocortin MC4 receptor is mostly expressed in the central nervous system and governs both food intake and sexual function. Melanocortin MC5 receptor is expressed in a variety of human peripheral tissues and is primarily engaged in exocrine function, most notably sebaceous gland secretion, as revealed by targeted deletion of that receptor (56).

3.3.10 NPGL (Neurosecretory Protein GL)

This tiny protein was termed neurosecretory protein GL because it includes Gly-Leu-NH2 at its C-terminus (NPGL). In situ hybridization indicated that the NPGL mRNA was expressed in the hypothalamic infundibular nucleus (IN) and medial mammillary nucleus (MM). Subcutaneous NPGL infusion boosted body mass gain in chickens, indicating that NPGL is involved in growth processes such as energy balance (57). Human, rat, and mouse have similar Npgl genes; the basic structure of NPGL is well conserved throughout mammals and avian species (58).

3.3.11 POMC (pro-opiomelanocortin)

POMC is a polyprotein hormone precursor that includes sequences of several peptide hormones that have separate biological action, such as melanocortin, lipotropins, and B-endorphin. The chicken POMC gene was the first to be isolated among avian species. It was discovered to be a single-copy gene and displayed a similar structural organization to that of other species across different classes (59). POMC neurons are activated by various peripheral and central signals, such as leptin and insulin, that reflect the energy status of the body. When energy levels are high, these signals activate POMC neurons, leading to the release of α-MSH, which in turn reduces feeding and maintains energy balance. Conversely, when energy levels are low, the absence of these signals leads to decreased POMC activity, resulting in increased feeding and the replenishment of energy stores. Therefore, POMC plays a crucial role in regulating food intake and energy balance (60).

4. Strategies for Dealing with Heat Stress

There are various methods for preventing heat stress in the poultry industry, but it's crucial to remember that these methods work best when they're used in tandem to prevent the harmful consequences of heat stress.

4.1 Environmental Strategies

The key to eliminating bird heat is airflow at the birds' level. Increasing ventilation is one of the most efficient ways to remove heat from the birds. It is necessary to make sure that outside air is able readily to flow into and out of the house. It is advised to employ circulation fans for proper ventilation. In a naturally ventilated building, circulation fans' main function is to create air movement over the birds to improve convective cooling, not to draw air into the building. Maintaining a clean, shining roof is another technique to prevent heat buildup because a shiny roof reflects heat and light (32).

4.2 Genetic Selection Strategies

Applying genetic engineering to produce species that are more heat adapted is another strategy. The dominant genes bare neck (Na) and frizzle (F) are examples of those that can affectthe appearance of feathers through selection and/or mutation. For example, in a study, the three genotypes of the frizzle gene were evaluated in terms of egg production, egg quality, and feed efficiency. It is concluded that the homozygous F mutation reduces heat stress in poultry production, which may be especially helpful in tropical nations where average temperatures are never low enough to adversely affect feed efficiency (33).

4.3 Feeding Strategies

Interest in nutritional alterations has grown recently because it is expensive and impossible to cool animal housing. As the use of synthetic compounds in feed is being curtailed globally, there is an increasing preference for natural anti-stressors. So, the development of innovative dietary strategies such as the addition of stress-relieving vitamins, minerals, and other nutrients may be helpful (34).

One of the most popular methods for reducing the effects of heat stress on avian performance is dietary modification, which can be performed with conventional antioxidants like vitamin C, vitamin E, and acetylsalicylic acid. Additionally, among all the vitamins, vitamin C dosage is the most advantageous during the heat stress period. Since chickens already have the gluconolactone oxidase enzyme needed for its manufacture in the kidneys, vitamin C, or L- ascorbic acid, is not necessary for poultry diets. However, under conditions of heat stress, this ability to produce vitamin C is insufficient (35).

5 Herbal Adaptogen

The term "adaptogen" was first used by the Russian scientist Lazarev in 1947 while researching the synthetic substance danazol (2 benzyl benzimidazole), which was discovered to increase organisms' nonspecific resistance (36). According to Lazarev, "adaptogens" are substances that enable an organism to overcome any harmful physical, chemical, or biological stressor by producing non-specific resistance and therefore "adapting" to various demands placed on it (37). Three requirements must be met by these substances in order for them to be considered adaptogens: To begin with, adaptogens must be non-specific and aid the body in resisting a wide range of unfavorable conditions, such as physical, chemical, or biological stress. These could be radiation, infectious illnesses, climatic change, and interpersonal conflict. Second, adaptogens must keep humans' homeostasis intact; in other words, they must be able to counteract or resist the physical ill effects of stress on the body. Third, adaptogens must not interfere with how the body normally functions. Nearly 20 years later, Brekhman and Dardymov(1969) proposed specified requirements that must be met for a substance to qualify as an adaptogen, further defining the term "adaptogen." In order to increase resistance to multiple (physical, chemical, or biological) stressors, an adaptogen must: i. produce a nonspecific response. ii. have a normalizing effect, regardless of the direction of departure from physiological norms caused by the stressor, and iii. be harmless and not interfere with normal body functions more than is necessary (36). A team of researchers led by George Wikman, Hildebert Wagner, and Alexander Panossian conducted a number of investigations on adaptogens in the 1990s and offered the following definition: natural bioregulators which are called adaptogens to help organisms better adapt to their environment and lessen the harm that comes with it. Actually, adaptogens have the advantage of minimizing the body's reaction to stress by minimizing adverse reactions during the alarm phase and preventing, or at least delaying, the development of the exhaustion phase, which is a component of the so-called general adaption syndrome (38).

5.1 Components

The herbal adaptogen utilized in this study is made by the Natural Remedies company in Karnataka, India, and contains three plants including holy basil (Ocimum sanctum), gooseberry (Emblica officinalis), and winter cherry (Withania somnifera) (39).

5.1.1 Emblica Officinalis

It is also known as Indian gooseberry, Amla, and Phyllanthus Emblica. Throughout south and east Asia, China, Malaysia, Pakistan, Uzbekistan, Sri Lanka, and South Africa, amla trees, which are small to medium-sized deciduous trees, can be found. It is between 8 and 18 meters tall. Greenish yellow flowers, simple, sub-sessile leaves that appear to be pinnately divided, a thin, light grey bark, globose, fleshy, and pale-yellow fruits with six oblique vertical furrows containing six trigonous seeds in two-seeded, three-crustacean cocci, and greenish-yellow flowers are all features of this plant.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malpighiales
Family	Phyllantheae
Genus	Emblica
Species	E. officinalis

Table 1: Taxonomical Classification of Emblica officinalis

This plant has 13 chemical constituents including tanins, alkalods, phenolic compounds, amino acids, carbohydrates, vitamin C, flavanoid, ellagic acid, chebulinic acid, quercetin, chebulagic acid, emblicanin-A, gallic acid, emblicanin-B, punigluconin, pedunculagin, citric acid, ellagotannin, trigallayl glucose, pectin. In addition, it contains moisture 81.2%, protein 0.5%, fat 0.1%, mineral matter 0.7%, fiber 3.4%, carbohydrate 14.1%, calcium 0.05%, phosphorous 0.02%, iron 1,2mg/100gm, nicotinic acid 0.2mg/100gm, vitamin C 600 mg/100 gm. It also contains a variety of phenolic compounds and is a strong nutritional source of minerals, amino acids, and vitamin C. One of the best sources of ascorbic acid, minerals, amino acids, tannins, and phenolic compounds is *Emblica Officinalis* (amla). Due to enhanced free radical production, commercial broilers with rapid growth rates have faster metabolic rates and are more susceptible to oxidative stress. In addition to ascorbic acid, the phenolic acids gallic and tannic acids found in E. Officinalis also contribute to antioxidant action (40).

5.1.2 Ocimum Sanctum

This genus contains numerous species and varieties. There are between 60 and 150 species in the genus. Every species in this genus (family Lamiaceae) is described based on its habitat and leaves (41). The shape of the leaves in Ocimum Sanctum and its close relatives vary depending on the size of the leaves, veins, and petioles. The leaves' hues range from brilliant green to dark green and occasionally practically black (42).

Over Asia, Africa, and Central and Southern America, the genus Ocimum (sometimes known as tulsi or holy basil) is widely distributed (42). The Ocimum genus is grown for its exceptional essential oil (eugenol), which has a wide range of applications in food, medicine, aromatherapy, and other fields. Eugenol has antifungal, antibacterial, and antiviral effects. It is also used as a flavoring ingredient and in fragrances for herbal products (43).

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	Ocimum
Species	O.Sanctum

Table 2: Taxonomical Classification of Ocimum Sanctum (44)

Ocimum sanctum L. has a very intricate chemical composition that includes a variety of nutrients and other biologically active substances such as urosolic acid, linalool, methyl eugenol, sesquiterpine, limatrol, caryophyllene, carvacrol, estragol saponins, flavonoids, triterpenoids and tannins (45,46). In addition to calcium, zinc, iron, vitamin C, and vitamin A, Ocimum sanctum L. also has a lot of phytonutrients including chlorophyll (47).

It has a wide range of pharmacological effects, including antimicrobial, immunomodulatory, stress-relieving, anti-inflammatory, antiulcer, antidiabetic, hepatoprotective, chemoprotective, cardioprotective, antioxidant, antitussive, radioprotective, memory-improving, antiarthritic, antifertility, antihypertensive, anticoagulant, anticataract, anthelmintic, and antinociceptive. O. sanctum leaf ethanolic extract has been shown to have anti-stress properties in both acute and chronic stress situations (48).

In one study that the effect of Ocimum sanctum (OS) on noise stress in rats was done. This study emphasizes the anti-stressor potential of this plant by showing that administration of the 70% ethanolic extract of OS had a normalizing effect on specific brain areas and reduced the alteration in neurotransmitter levels caused by noise stress (49).

A team of researchers employed this plant's extraction in 2000 to study mice's swimming abilities. The swimming test is frequently used to assess how different substances, including CNS depressants, antidepressants, sedative-hypnotics, psychostimulants, euphorics, nootropics, and adaptogens, affect the central nervous system (CNS). Because rodents' immobility during swimming is a reflection of the behavioral hopelessness present in depression in humans. The antistress activity of plant medicines has also been widely evaluated in mice and rats using the swimming test. Based on their observations, a high dose (400 mg/kg, i.p.) of the Ocimum sanctum extracts prolonged swimming, indicating a stimulant and anti-stress effect on the central nervous system.

5.1.3 Withania Somnifera

W. Somnifera is a xerophytic plant that grows in subtropical dry zones such as Africa, the Mediterranean, Sri Lanka, Pakistan, and India. It is also known by the alternate names ashwagandha, suranjan, winter cherry, and Indian ginseng (50).

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Angiosperma
Class	Dicotyledons
Order	Tubiflorae
Family	Solanaceae
Genus	Withania
Species	W.somnifera

Table 3: Taxonomical Classification of Withania Somnifera (51)

In general, W. Somnifera is helpful for a range of neurological conditions, but it is especially effective for tardive dyskinesia, cerebral ischemia, epilepsy, Alzheimer's disease, and Parkinson's disorders (52). Apart from its neurological effects, numerous studies have been conducted on the pharmacological effects of W. somnifera extract. It has demonstrated anticancer, anti-inflammatory, immunomodulatory, anti-stress, and analgesic properties (34–36).

Alkaloids and steroidal lactones, also referred to as withanolides, are the primary metabolites of

Withania Somnifera and the most significant elements in charge of its therapeutic activities (53). It has been claimed that the herb also includes additional chemical metabolites, such as acylsteryl glucosides, starch, reducing sugars, hantreacotane, and different amino acids (37,38). One of the most prevalent withanolides identified in W. somnifera, withaferin A, has been shown to have anti-tumor properties, including pro-apoptotic and anti-angiogenesis action (39).

5.2 Effect of herbal adaptogen on feed intake

Herbal adaptogens, also known as phytobiotics, are compounds made from plants (such as herbs, botanicals, essential oils, and oleoresins) that are added to feed to enhance broiler performance. Numerous studies have been conducted in this area, some of which are included here.

An investigation into the effects of adding powdered Withania somnifera root to feed on broiler performance and the hemo-immune response was carried out over the course of six weeks. They applied five different percentages of Withania somnifera powder at rates of 0%, 0.5%, 0.75%, 1.0%, and 1.25%, respectively. According to the study, adding 1% Ashwagandha (Withania somnifera) root powder to the diet of birds dramatically increased body weights, feed effectiveness, and immune status (54).

Ansari conducted a study with the goal of comparing the growth-promoting abilities of six different medicinal plants, including Corylus avellena, Nigella sativa, Boerhavia diffusa, Withania somnifera, Ipomea digitata, and Azadirachta indica. This study showed that the use of medicinal plants, particularly Withania somnifera, Nigella sativa, and Azadirachta indica, as growth promoters in poultry diets can improve production results (55).

The impact of Withania Somnifera (Ashwagandha powder) alone or in combination with ascorbic acid on broiler production efficiency was researched by Biswas in 2012. There were four treatment groups: control (C), T1 (1% W. Somnifera powder), T2 (0.05% ascorbic acid), and T3 (0.5) W. Somnifera powder and 0.025% ascorbic acid). Supplementing broiler diets with ashwagandha, ascorbic acid, or both had a substantial impact on feed intake and feed conversion ratio compared to the control group. T1 experienced the greatest increase in body weight, rather than T2, T3, and the control group (56).

According to another research done by Kumar, ashwagandha root extract and powder are useful for reducing heat stress in broilers during the summer (57). Gaikwad studied the impact of adding amla (Emblica officinalis) supplements on broiler growth performance and came to the conclusion that adding 1% amla powder supplements improved broiler growth and increased economic returns without causing any observable side effects (58). Rathod investigated the effect of two herbs, Coriander powder (CP) and Tulsi powder (TP) at various dosages (T1 stands for control, T2 for 1% CP, T3 for 2% CP, T4 for 1% TP, and T5 for 2% TP.) The birds in treatment 5 with 2 percent Tulsi had the highest cumulative feed consumption, the researchers discovered (59).

The aim of the present study was to investigate the impact of herbal adaptogens on heat stressed broilers hypothalamus neuropeptide expression and in order to specify the most likely underlying molecular pathways. In the presence of herbal adaptogens, there is an anticipation that anorexigenic neuropeptides may demonstrate a decrease in gene expression, or orexigenic neuropeptides might undergo a reduction in functionality.

Materials and Methods:

2.1 Ethic Statement

The present study was conducted in accordance with the recommendations in the guide for the care and use of laboratory animals of the National Institute of Health and the protocol was approved by the University of Arkansas Animal Care and use committee under protocols 13039 and 10025.

2.2 Birds, Diet treatments and Experimental design

At the University of Arkansas research farm's poultry environmental research laboratory, 672 male Cobb 500 broiler chicks that were one day old were dispersed among twelve climate-controlled chambers. Each chamber was divided into two floor pens, each of which had a separate feeder and drinker. There were 28 birds in each pen.

A three-phase corn-and-soybean diet (Table 1) fed as (Control C) or supplemented with the herbal adaptogen NR-PHY-30 (Natural Remedies, Karnataka, India) at 500 g/1,000 kg control diet (NR-PHY-500) or at 1 kg/1,000 kg control diet (NR-PHY-1000) in accordance with the manufacturer's recommendations were used in this experiment.

According to the manufacturer's recommendations, the adaptogen was introduced to the diet in the pelleted grower (d15-28) and finisher (d29-42) as well as the crumble starter (d0-14).

The lighting program was 24 hours of light for the first three days, 23 hours of light and 1 hour of dark from days four to seven, and then 18 hours of light and 6 hours of dark the remaining days.

The ambient temperature was kept at 32°C for the first three days, then dropped by roughly 3°C every week until it reached 24°C on day 21. In order to produce a cyclic heat stress pattern and replicate the summer season in the United States until day 42, the temperature was raised to 35°C for 8 hours each day (9:30 am to 5:30 pm) in nine chambers starting on day 28. After the temperature was adjusted, these chambers reached 35°C in 15 minutes. Six pens were kept in the three remaining rooms, which were kept at a thermoneutral 24 degrees (TN).

As a result, there are four experimental groups (6 pens each, 168 birds each): consisting of birds fed the C diet and kept at TN, birds given the C diet and subjected to heat stress, birds fed the NR-

PHY-500 and subjected to heat stress, and birds fed the NR-PHY-1000 and subjected to heat stress (NR-PHY-1000HS).

Following the collection of blood and tissue samples on day 41, these samples were straightly frozen in liquid nitrogen and stored at -80°C.

2.3 RNA Extraction

RNA isolation and extraction for hypothalamus tissue have two steps. The first step is specific for this kind of tissue, during this step, 1.7 – 2 mL lysis buffer was added to 5ml tube that already contained a small piece tissue, then was homogenized. The lysis buffer included Tris-HCL, NaCl, MgCl2, NP-40, Ribonucleoside Vanadyl Complex, ultra-pure water, A, P, L, NaF, Na3Vo4, and PMSF.

The second step is the usual RNA extraction method for other tissues was done. Briefly, 1 mL of Trizol was added to a 1.5mL tube that already contained 300 – 400 ul of the homogenized solution, 200 ul chloroform was added, incubated at room temperature for 5 minutes, and then centrifuged (12000 g, 15min, 4 C), and the upper phase that was colorless carefully removed, then 500 ul of isopropanol was added, incubated at room temperature for 10 minutes, and then centrifuged (12000g, 10min, 4 C), and then DEPC-EtOH was added to supernatant collected, and then centrifuged (12000g, 3 min, 4 C) to get the RNA pellet. Then decanted the supernatant and allowed the pellet to dry for approximately 5min. The RNA was suspended in an appropriate volume of DEPC-H2O that are usually between 20 – 40 ul. The RNA quantity and integrity were determined at 260/280 using Synergy HT multi-mode microplate reader (BioTek) and by electrophoresis.

2.4 Reverse Transcription and real-time Quantitative PCR (qPCR)

1 ul of total RNA, 15 ul of ultra-pure water (Eup H2O), and 4ul of cDNA Super Mix (5X) [Quanta](Biosciences) were added into 0.5 tubes in order to make cDNA. The cDNA was then subjected to qPCR using syber green and chicken specific primers (see Table 1). The qPCR was run using Applied Biosystems 7500 machine (Life Technologies). The relative expressions of target genes were determined by the $2^{-\Delta\Delta Ct}$ method.

Table 4: List of qPCR chicken-specific oligonucleotide primers.

Gene	Accession number	Primer sequence (5'→3')	Orientation	Product size (bp)
NPY	NM_205473	CATGCAGGGCACCATGAG CAGCGACAAGGCGAAAGTC	F R	55
AgRp	AB029443	GCGGGAGCTTTCACAGAACA CGACAGGATTGACCCCAAAA	F R	58
POMC	AB019555	GCCAGACCCCGCTGATG CTTGTAGGCGCTTTTGACGAT	F R	56
CART	KC249966	GCTGGAGAAGCTGAAGAGCAA GGCACCTGCCCGAACTT	F R	60
ORX	AB056748	CCAGGAGCACGCTGAGAAG CCCATCTCAGTAAAAGCTCTTTGC	F R	67
ORX1	AB110634	TGCGCTACCTCTGGAAGGA GCGATCAGCGCCCATTC	F R	58

ORX2	XM_004945362	AAGTGCTGAAGCAACCATTGC AAGGCCACACTCTCCCTTCTG	F R	61
CRH	NM_001123031	TCAGCACCAGAGCCATCACA GCTCTATAAAAATAAAGAGGTGA CATCAGA	F R	74
Ghrelin	AY303688	CACTCCTGCTCACATACAAGTTCA TCATATGTACACCTGTGGCAGAAA	F R	75
GHSR	NM_204394	GCACAAATCGGCAAGGAAA GTGACATCTCCCAGCAAATCC	F R	61
MC1R	NM_001031462	GCTCTGCCTCATTGGCTTCT TGCCAGCGCGAACATGT	F R	76
MC2R	NM_001031515	GCTGTTGGGCCCCCTTT AAGGGTTGTGTGGGCAAAAC	F R	60
MC3R	AB017137	GCCTCCCTTTACGTTCACATGT GCTGCGATGCGCTTCAC	F R	59
MC4R	NM_001031514	CCTCGGGAGGCTGCTATGA GATGCCCAGAGTCACAAACACTT	F R	62
MC5R	NM_001031015	GCCCTGCGTTACCACAACAT CCAAATGCATGCAATGATAAGC	F R	63
Ob-R	NM_204323	GCAAGACCCTCTCCCTTATCTCT TCTGTGAAAGCATCATCCTGATCT	F R	70
18s	AF173612	TCCCCTCCCGTTACTTGGAT GCGCTCGTCGGCATGTA	F R	60

Accession number refers to GenBank (National Center for Biotechnology Information—NCBI). F, forward; R, reverse. NPY, neuropeptide Y; AgRP, agouti-related peptide; POMC, proopiomelanocortin; CART, cocaine and amphetamine-regulated transcript; ORX, orexin; ORXR1, orexin receptor 1; ORXR2, orexin receptor 2; CRH, corticotropin-releasing hormone; GHSR, growth hormone secretagogue receptor; MC1R, melanocortin receptor 1; MC2R, melanocortin receptor 2; MC3R, melanocortin receptor 3; MC4R, melanocortin receptor 4; MC5R, melanocortin receptor 5; Ob-R, leptin receptor, NPGL, neurosecretory protein.

2.5 Statistical Analysis

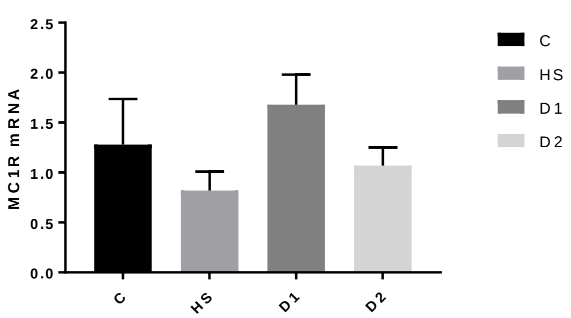
One-way ANOVA with full randomization was used to assess the data on gene expression. Graph Pad Prism version 6.00 for Windows (Graph Pad Software, La Jolla, California, USA) was used to compare the means when ANOVA revealed significant effects. Differences were deemed significant at P = 0.05.

3. Results & Discussion

Results for herbal feed additive supplementation on gene expressions are presented in figures 3.1A-3.4E.

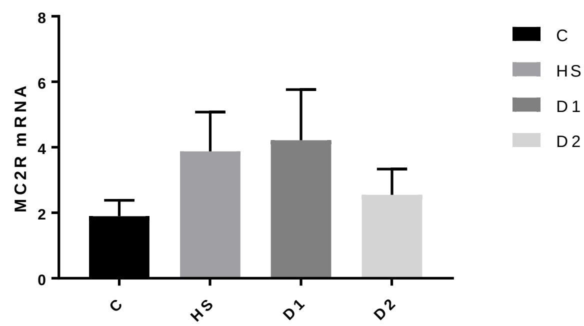
3.1.A

P=0.2607



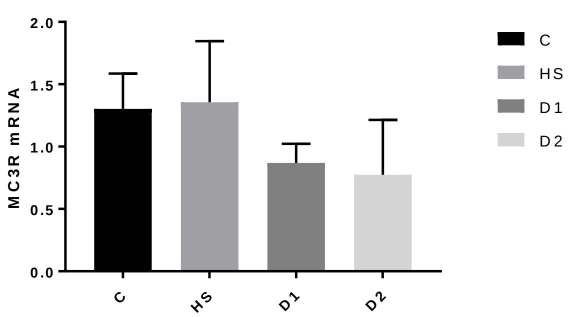
3.1.B

P=0.4191



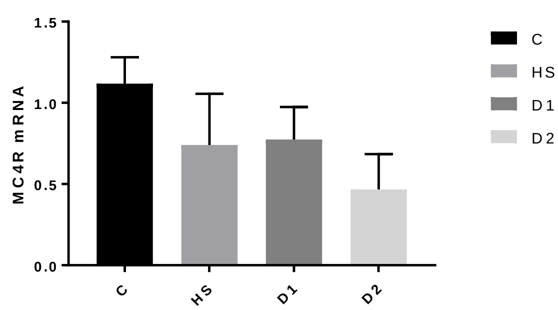
3.1.C

P=0.5679



3.1.D

P=0.2902



3.1.E

P=0.1283

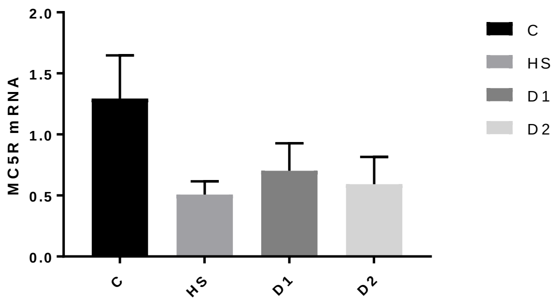
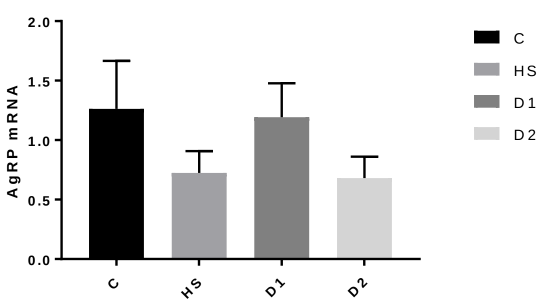


Figure 3.1 Effect of herbal adaptogen on the hypothalamic expression of melanocortin receptors (MC1-5R) in heat stressed-broilers. The expression of MC1R (A), MC2R (B), MC3R (C), MC4R (D), and MC5R gene (E) wasdetermined by qPCR using the $2^{-\Delta\Delta Ct}$ method. Data are mean ± SEM (n = 6 birds/group). C, control; HS, heat stress; D1, diet 1; D2, diet 2.

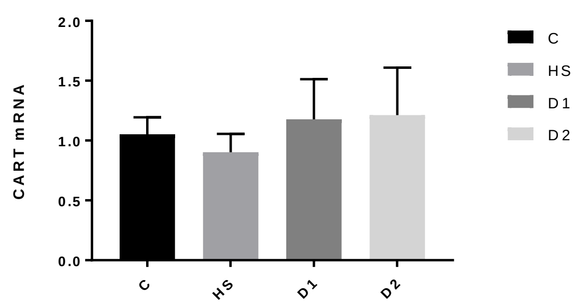
3.2.A

P=0.3304



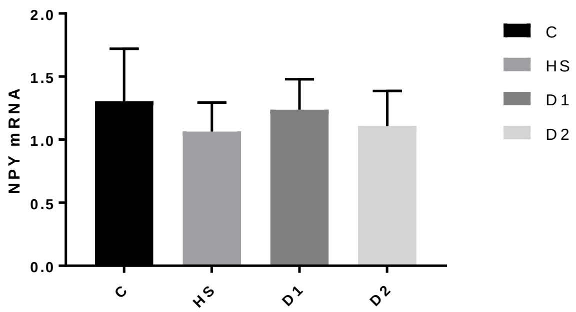
3.2.B

P=0.8581



3.2.C

P=0.9374



3.2.D

P=0.7971

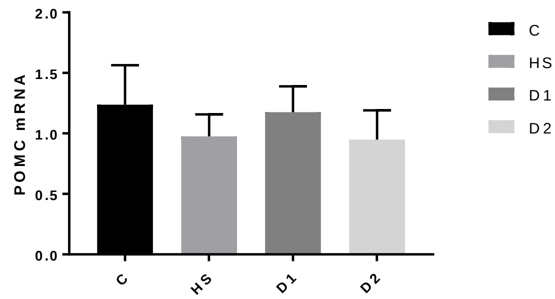
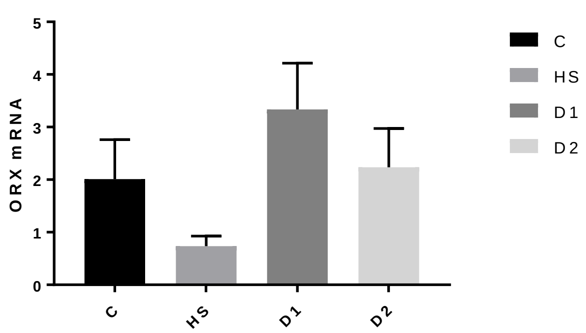


Figure 3.2 Effect of herbal adaptogen on the hypothalamic expression of AgRP (A), CART (B), NPY (C), and POMC (D) in heat stressed broilers. The mRNA abundances were determined by qPCR using $2^{-\Delta\Delta Ct}$ method. Dataare mean ± SEM (n = 6 birds/group). C, control; HS, heat stress; D1, diet 1; D2, diet 2.

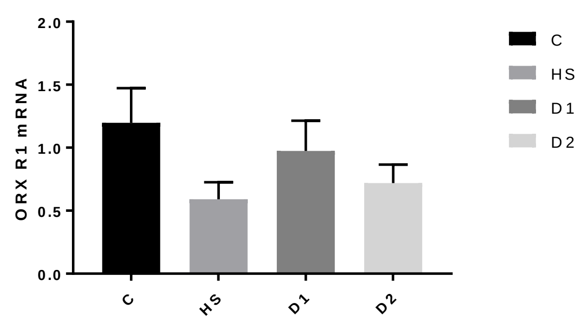
3.3.A

P=0.1000



3.3.B

P=0.2008



3.3.C

P=0.2000

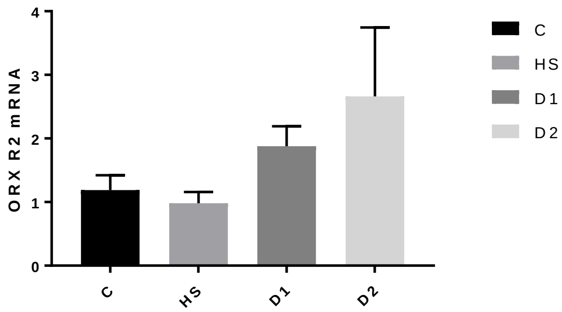
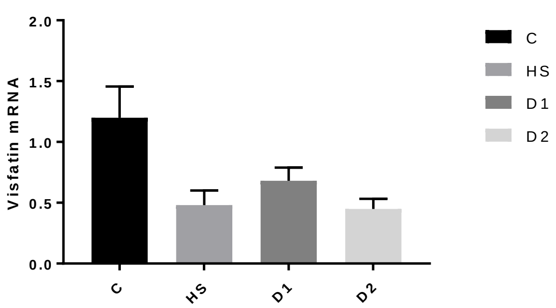


Figure 3.3 Effect of herbal adaptogen on the hypothalamic expression of ORX and its receptors in heat- stressed broilers. The expression of ORX (A), ORXR1(B), and ORXR2 (C) was determined by qPCR using the 2−ΔΔCt method. Data are mean ± SEM (n = 6 birds/group). C, control; HS, heat stress; D1, diet 1; D2, diet 2.

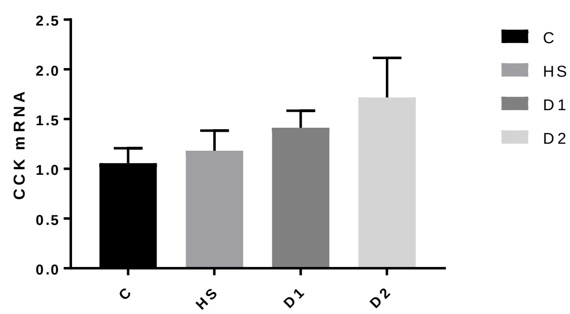
3.4.A

P=0.0105



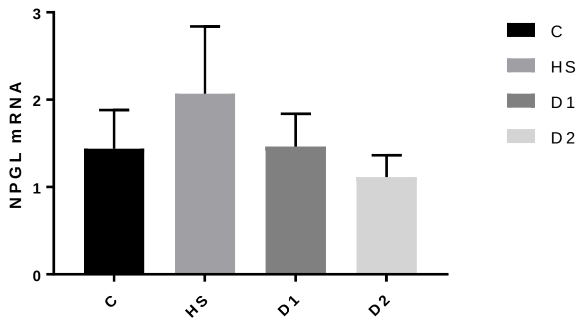
3.4.B

P=0.2906



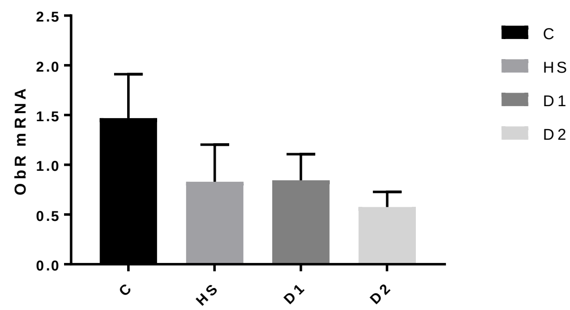
3.4.C

P=0.5981



3.4.D

P=0.2816



3.4.E

P=0.2300

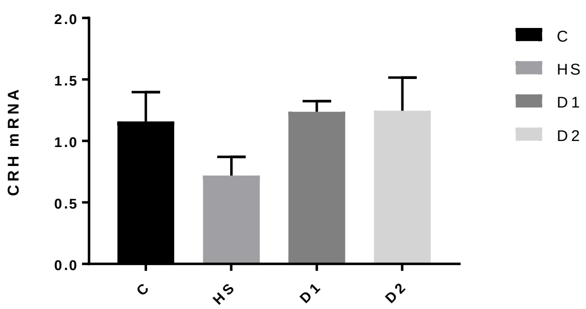


Figure 3.4 Effect of herbal adaptogen on the hypothalamic expression of Visfatin (A), CCK (B), NPGL (C), ObR (D), and CRH (E) in heat-stressed broilers. The mRNA abundances were determined by qPCR using the $2^{-\Delta\Delta Ct}$ method. Data are mean ± SEM (n = 6 birds/group). C, control; HS, heat stress; D1, diet 1; D2, diet 2.

Results reported in our previously published paper, supplementation of the herbal adaptogen NR-PHY-30 stimulated appetite and feed intake, and inturn improved growth performance in a dose-dependent manner (average 66–83 gain body weightand 5–10 points better FCR) in cyclic heat-stressed broilers, which make it a promising nutritional strategy (40). In this study, we are investigating deeply to define its mode of action and its underlyingmolecular mechanisms.

A variety of hormones, neurotransmitters, released substances, and cytokines are all involved in the complicated, extensive, and intricate network that makes up the feeding response in poultry. The peripheral and central neural systems of chickens primarily regulate their feeding behavior. A number of peptides and steroid hormones are secreted by peripheral tissues to control hunger and sense the body's nutritional metabolism through sensors. Mammals' and birds' feeding behaviors and energy homeostasis are both influenced by the central nervous system, and the hypothalamus' intricate neural network controls hunger and energy balance. By synthesizing a variety of feeding-promotional and feeding-suppressive neuropeptides, the hypothalamus, the brain's control center, may manage food intake.

The brain and endocrine networks that regulate food intake are largely conserved across animals, and as a result, so are the networks that regulate this behavior. The primary location for food intake has proven to be the hypothalamus. The intestines, pancreas, liver, and adipose tissue send signals to the hypothalamus, which integrates these signalswith those from other regions of the brain (41).

The aim of the present study was to investigate the impact of herbal adaptogens on heat- stressed broilers hypothalamus neuropeptide expression and in order to specify the most likely underlying molecular pathways.

Feeding-related hypothalamic neuropeptides (FRHN) are a group of neuropeptides produced in the hypothalamus region of the brain that play a critical role in regulating feeding behavior and energy balance. The most well-known feeding-related hypothalamic neuropeptides (FRHN) include NPY, AgRP, POMC, and CART. In the infundibular nucleus (IN) of the avian hypothalamus, which is analogous to the mammalian arcuate nucleus (ARC), NPY and AgRP are orexigenic peptides (stimulate food intake and reduces energy expenditure) that are co-expressed by a subpopulation of neurons (42), whereas POMCand CART, generated by a different subgroup of IN neurons, function as anorexigenic peptides that means they reduces food intake and increases energy expenditure. These FRHN work in a complex and coordinated manner to regulate feeding behavior and energy expenditure, and dysregulation of these neuropeptides can lead to obesity and other metabolic disorders.

In chickens, neuropeptide Y (NPY) plays a role in regulating feeding behavior and energy balance, similar to its role in other animals. NPY is expressed in the hypothalamus region of the chicken brain, where it interacts with other neuropeptides and hormones to modulate feeding behavior. Studies have shown that NPY injection in chickens increases food intake, while blocking NPY signaling reduces food intake. Additionally, NPY has been shown to stimulate the release of the hormone ghrelin, which further stimulates food intake. Therefore, NPY appears to play an orexigenic role in chickens, promoting food intake and regulating energy balance (43–45).

Based on the information presented in Figure 3.2.C, the expression of the NPY gene is found to be reduced in the heat stress group as compared to the control group. While the D1 group shows some potential in increasing the expression of this gene, the increase is not statistically significant.

POMC (Pro-opiomelanocortin) is a prohormone that is processed in the body to produce several neuropeptides, including alpha-melanocyte-stimulating hormone (α-MSH), which is known to play a critical role in the regulation of feeding behavior. POMC plays a similar role in regulating feed intake in broiler chickens as it does in other species, by responding to and integrating various peripheral and central signals to control feeding behavior and energy balance (46). Based on the results presented in Figure 3.2.D, the addition of herbal adaptogen in the presence of heat stress does not appear to have a significant effect on the expression of this gene being studied. This suggests that the adaptogen may not have a significant impact on the regulation of POMC gene expression under these experimental conditions.

AgRP is primarily produced in the arcuate nucleus, where it is involved in hypothalamic regulation of feeding and energy balance (47). According to our result (Figure 3.2.A), although the addition of 500 grams of herbal adaptogen to the chicken diet in the D1 group shows an increase in the expression of this orexigenic neuropeptide compared to the heat stress group, the difference is not statistically significant.

CART (cocaine- and amphetamine-regulated transcript) is a neuropeptide that is involved in regulating feeding behavior and energy metabolism in animals, including broiler chickens. In broilers, CART is expressed in various regions of the brain, including the hypothalamus, which is responsible for regulating feeding and energy expenditure. CART has been shown to decrease food intake and increase energy expenditure in broilers (48). Based on the information presented in figure 3.2B, this particular herbal adaptogen is unable to modify gene expression of CART under conditions of heat stress.

Overall, because no change in the expression of these key FHRNs was observed in this investigation, it is possible to assume that utilizing this herbal adaptogen has no influence on their lowering or rising expression.

Melanocortin receptors (MCRs) play an important role in broilers, as they are involved in regulating various physiological processes such as food intake, energy homeostasis, and immune function. Specifically, MCRs are a class of G protein-coupled receptors that are activated by melanocortin peptides, including alpha-melanocyte stimulating hormone (α-MSH) (49,50). Melanocortin receptor 1 is connected to the color of feathers and plays a role in melanogenesis.

Although it has been demonstrated that MC5R is widely distributed in exocrine organs and plays significant roles in lipolysis, fatty acid oxidation, and lipid synthesis in humans, no specific function for MC5R in chicken has yet been identified. However, it has been hypothesized that the chicken melanocortin receptor 2 controls steroidogenesis. The regulation of feedand water intake is thought to be influenced by melanocortin receptor 3 and MC4R (51).

The melanocortin-4 receptor (MC4-R) is a seven-transmembrane G protein-coupled receptor found in the brain. When this receptor is inactivated through gene targeting, mice develop a maturity-onset obesity syndrome characterized by hyperphagia, hyperinsulinemia, and hyperglycemia. This syndrome resembles the agouti obesity syndrome, which is caused by ectopic expression of agouti protein, a pigmentation factor that is normally expressed in the skin. Their findings establish a unique signaling route in mice for body weight regulation and support a paradigm in which persistent antagonism of the MC4-R is the principal mechanism through which agouti produces obesity (52). In 1986, Poggioli and her colleagues concluded that melanocortin peptides have an inhibitory function in the complicated regulation of food intake, and they support and extend the idea of a melanocortin-opioid homeostatic system (52).

According to the result, although the high dose of herbal adaptogen can decrease the expression of these genes compared to heat stress in all five melanocortin receptors, but this particular herbal adaptogen is unable to modify gene expression of them significantly.

Research into orexin's peripheral effects is still restricted in avian species, despite extensive research in other animals, particularly mammals. Ozdemir investigated the effect of supplementary organic chromium (Cr) forms on the expression of ovarian orexin (hypocretin) in heat-stressed laying hens in 2017. They came to the conclusion that dietary chromium supplementation (CrPic-CrHis) boosted orexin levels (53). According to Greene and her colleagues, the hepatic orexin system might represent a molecular signature in the heat and oxidative stress response (54). Sakurai et al. published a report on orexins (orexin-A and -B), which strongly enhance rat food intake. Orexin-A is a peptide made upof 33 amino acid residues, while orexin-B only has 28. According to Sakarai's publication, orexin- B and orexin-A share a 46% sequence identity. Orexin-amino A's acid sequence is identical in allfive species—human, bovine, rat, and mouse—but human orexin-amino B's acid sequence differsfrom rodents' (rat and mouse's) at two points (55). The question of whether central injection of mammalian orexin-A or -B enhances food intake in the chick was investigated by furuse in 1999. When given free access to food, orexin-A had no effect on the amount of food thatchicks consumed, whereas orexin-B drastically reduced the amount of food consumed over time. To verify its suppressive action, the orexin-B was then given to chicks that had been starved for three hours. Food intake was not significantly affected by orexin-B. When hunger was stimulatedby fasting, central injection of orexin-B did not change food intake. It appears that neither of theseorexins encourages chicks to eat (56).

In this project, we determined the gene expression of Orexin and its receptors (ORX 1 & ORX 2). According to Figure 3.3.A, the herbal adaptogen at both doses has the potential to increase the gene expression of orexin compared to the heat stress group, although the difference is not statistically significant. Furthermore, the increase in gene expression was observed in both orexin receptors in comparison to the heat stress group. In ORX and ORX1, the lower dose of the herbal adaptogen has a greater potential to increase gene expression than the higher dose. However, in ORX2, the opposite effect was observed, with the D2 group showing a greater increase in gene expression compared to the D1 group.

This project evaluated several other anorexigenic neuropeptides, including CCK, CRH, and OBR. But herbal adaptogen did not have any significant effect on their expression.

Another new hypothalamic neuropeptide, NPGL, has just been found in chickens (57,58). Shikano discovered that NPGL is orexigenic (58). Although herbal extraction had no effect on the expression of this hypothalamus neuropeptide.

The visfatin, which is also known as the nicotinamide phosphoribosyl transferase (NAMPT), is a relatively novel adipocytokine with a variety of regulating and metabolic functions (59). The primary pathway activated by visfatin is the NAD+ biosynthesis pathway, which involves the conversion of nicotinamide adenine dinucleotide (NAD+) from nicotinamide. This pathway is critical for energy metabolism and cellular function, as NAD+ is required for several key cellular processes, including oxidative phosphorylation, DNA repair, and cellular signaling (60).

Visfatin also activates the phosphatidylinositol 3-kinases (PI3K)/ protein kinase B (Akt) pathway, which is involved in the regulation of cell survival, growth, and metabolism. Activation of this pathway by visfatin leads to increased glucose uptake and metabolism in cells, which can improve insulin sensitivity and glucose homeostasis (61,62).

Additionally, visfatin has been found to activate the NF-κB pathway, which is involved in regulating inflammation and immune responses. Activation of this pathway by visfatin leads to the production of pro-inflammatory cytokines, such as TNF-α and IL-6, which can contribute to the development of chronic inflammation and related diseases (63).

Most of the research on vasfatin to date has examined its effects on humans and other animals; very few studies, however, have looked at how visfatin affects birds. Visfatin promotes chicken appetite, unlike mammals. Since visfatin gene expression is higher in muscle than visceral fat in chickens, it has been hypothesized that visfatin is a myokine rather than an adipokine. Additionally, visfatin is probably essential for the control of food intake, maintenance of energy balance, lipid metabolism, and muscular growth in avian species (64).

In 2008, Cline and his colleagues conducted a study to investigate the impact of central visfatin on the intake of feed and water. The experiment showed that visfatin was more effective than other central orexigenic factors in increasing feed intake, as a lower dose of visfatin was sufficient to produce the same effect compared to NPY, N/OFQ, and beta-endorphin (31). The rapid stimulation of feed intake within 30 minutes post-injection suggests that visfatin acts as a quick-acting hunger signal in the central nervous system, which is comparable to the effects of NPY and N/OFQ in chicks (63,64), and beta-endorphin in chickens (65).

Based on our findings, the expression of only one neuropeptide, visfatin, was significantly altered by the herbal adaptogen. The expression of this gene was decreased under heat stress conditions significantly in comparison to the control group. The addition of a low dose of herbal adaptogen has the potential to enhance the expression of this appetite-inducing gene. Furthermore, based on the graph and the comparison between low and high doses of herbal adaptogen, it can be inferred that increasing the amount of herbal adaptogen does not result in a proportionate increase in appetite. However, it is possible that the effect of this adaptogen was not strong enough to modify the expression of other orexigenic neuropeptides to the same extent as visfatin. Perhaps due to the superior effectiveness of vifatine compared to other orexigenic neuropeptides (31) , the herbal adaptogen may have the ability to modify its expression. It can be inferred that the observed increase in broiler weight, as documented in our previous paper, can be attributed to the upregulation of this gene's expression. To achieve better results, it may be necessary to use a different dosage of the herbal adaptogen, particularly one that exceeds the amount utilized in this study.

4. Conclusion

Heat stress (HS) is one of the major new environmental concerns facing the poultry industry due to the increased worldwide demand for animal protein and rising temperatures brought on by climate change. Since birds raised for commercial purposes are especially susceptible to heat, establishing production practices that lessen the negative effects of HS on bird performance is crucial and necessitates a comprehensive strategy. In order to reduce the heat burden on birds, feeding, and nutrition can be crucial.

One of the primary behavioral alterations brought on by hot weather is a decrease in FI as birds try to reduce the amount of heat produced by the digestion, absorption, and metabolism of nutrients. Reduced FI accounts for the majority of the performance degradation seen in HS broilers, while reduced nutrient digestibility appears to only account for a small portion of the reduced feed efficiency after HS, even though the intensity and length of the heat period influence the type and magnitude of responses.

Herbal adaptogens are stress-response substances that alter and regulate an organism's ability to adapt to external stresses like heat stress. Although herbal adaptogens alleviate the adverse effects of heat stress, their modes of action of them are not well defined. As a result, we concluded that dietary herbal adaptogen supplementation in heat-stressed broilers improves feed consumption by altering the expression of hypothalamic neuropeptides such as visfatin implicated in appetite and feed intake, but molecular research needs further work.

5. References

1. Taha, F. A. (2003). The poultry sector in middle-income countries and its feed requirements. Agriculture and trade reports: USDA: WRS-03-02.

2. Samad, A., Hamza, M., Muazzam, A., Ahmer, A., Tariq, S., Ahmad, S., & Mumtaz, M. T. (2022). Current perspectives on the strategic future of the poultry industry after the COVID-19 outbreak. Brilliance: Research of artificial intelligence, 2(3), 90-96.

3. Hafez, H. M., Schroth, S., Stadler, A., & Schulze, D. (2001). Detection of Salmonella, Campylobacter, and verotoxin producing E. coli in turkey flocks during rearing and processing. Archiv fur Geflugelkunde, 65(3), 130-136.

4. Hafez, H. M., & Attia, Y. A. (2020). Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak. Frontiers in veterinary science, 7, 516.

5. Andrew Selaledi, L., Mohammed Hassan, Z., Manyelo, T. G., & Mabelebele, M. (2020). The current status of the alternative use to antibiotics in poultry production: An African perspective. Antibiotics, 9(9), 594.

6. Mehdi, Y., Létourneau-Montminy, M. P., Gaucher, M. L., Chorfi, Y., Suresh, G., Rouissi, T., ... & Godbout, S. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. Animal nutrition, 4(2), 170-178.

7. Mengesha, M. (2011). Climate Change and the Preference of Rearing Poultry for the. Asian Journal of Poultry Science, 5(4), 135-143.

8. Fraser, D., Fraser, A. F., & Ritchie, J. S. D. (1975). The term “stress” in a veterinary context. British Veterinary Journal, 131(6), 653-662.

9. Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flügge, G., Korte, S. M., ... & Fuchs, E. (2011). Stress revisited: a critical evaluation of the stress concept. Neuroscience & Biobehavioral Reviews, 35(5), 1291-1301.

10. Selye, H. (1976). Forty years of stress research: principal remaining problems and misconceptions. Canadian Medical Association Journal, 115(1), 53.

11. Pasternac, A., & Talajic, M. (1991). The effects of stress, emotion, and behavior on the heart. Methods and Achievements in Experimental Pathology, 15, 47-57.

12. Kagias, K., Nehammer, C., & Pocock, R. (2012). Neuronal responses to physiological stress. Frontiers in genetics, 3, 222.

13. Dhanalakshmi, S., Devi, R. S., Srikumar, R., Manikandan, S., & Thangaraj, R. (2007). Protective effect of Triphala on cold stress-induced behavioral and biochemical abnormalities in rats. Yakugaku Zasshi, 127(11), 1863-1867.

14. Mendes, A. A., Watkins, S. E., England, J. A., Saleh, E. A., Waldroup, A. L., & Waldroup, P. W. (1997). Influence of dietary lysine levels and arginine: lysine ratios on performance of broilers exposed to heat or cold stress during the period of three to six weeks of age. Poultry science, 76(3), 472-481.

15. Mozo, J., Emre, Y., Bouillaud, F., Ricquier, D., & Criscuolo, F. (2005). Thermoregulation: what role for UCPs in mammals and birds? Bioscience reports, 25(3-4), 227-249.

16. Mujahid, A., & Furuse, M. (2009). Oxidative damage in different tissues of neonatal chicks exposed to low environmental temperature. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 152(4), 604-608.

17. Li, X. M., Zhang, M. H., Liu, S. M., Feng, J. H., Ma, D. D., Liu, Q. X., ... & Xing, S. (2019). Effects of stocking density on growth performance, growth regulatory factors, and endocrine hormones in broilers under appropriate environments. Poultry Science, 98(12), 6611-6617.

18. Bilal, R. M., Hassan, F. U., Farag, M. R., Nasir, T. A., Ragni, M., Mahgoub, H. A., & Alagawany, M. (2021). Thermal stress and high stocking densities in poultry farms: Potential effects and mitigation strategies. Journal of Thermal Biology, 99, 102944.

19. Wang, J., Qiu, L., Gong, H., Celi, P., Yan, L., Ding, X., ... & Zhang, K. (2020). Effect of dietary 25-hydroxycholecalciferol supplementation and high stocking density on performance, egg quality, and tibia quality in laying hens. Poultry science, 99(5), 2608-2615.

20. Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M. S., & Bernabucci, U. (2010). Effects of climate changes on animal production and sustainability of livestock systems. Livestock Science, 130(1-3), 57-69.

21. Mack, L. A., Felver-Gant, J. N., Dennis, R. L., & Cheng, H. W. (2013). Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. Poultry science, 92(2), 285-294.

22. Kadymov, R. A., & Aleskerov, Z. A. (1988). Immunological reactivity of poultry organism under high temperature conditions. Soviet agricultural sciences (USA).

23. Bartlett, J. R., & Smith, M. O. (2003). Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poultry science, 82(10), 1580-1588.

24. Zulkifli, I., Norma, M. C., Israf, D. A., & Omar, A. R. (2000). The effect of early age feed restriction on subsequent response to high environmental temperatures in female broiler chickens. Poultry Science, 79(10), 1401-1407.

25. Hirakawa, R., Nurjanah, S., Furukawa, K., Murai, A., Kikusato, M., Nochi, T., & Toyomizu, M. (2020). Heat stress causes immune abnormalities via massive damage to effect proliferation and differentiation of lymphocytes in broiler chickens. Frontiers in veterinary science, 7, 46.

26. Tatemoto, K., Carlquist, M., & Mutt, V. (1982). Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature, 296, 659-660.

27. Jelokhani, M., Vazir, B., Zendehdel, M., & Jahandideh, A. (2022). Interactions of Cholecystokinin and Glutamatergic Systems in Feeding Behavior of Neonatal Chickens. Archives of Razi Institute, 77(2), 681-688.

28. Niederau, C., Meereis-Schwanke, K., Klonowski-Stumpe, H., & Herberg, L. (1997). CCK-resistance in Zucker obese versus lean rats. Regulatory peptides, 70(2-3), 97-104.

29. Kralisch, S., Klein, J., Lossner, U., Bluher, M., Paschke, R., Stumvoll, M., & Fasshauer, M. (2005). Interleukin-6 is a negative regulator of visfatin gene expression in 3T3-L1 adipocytes. American Journal of Physiology-Endocrinology And Metabolism, 289(4), E586-E590.

30. Hug, C., & Lodish, H. F. (2005). Visfatin: a new adipokine. Science, 307(5708), 366-367.

31. Cline, M. A., Nandar, W., Prall, B. C., Bowden, C. N., & Denbow, D. M. (2008). Central visfatin causes orexigenic effects in chicks. Behavioural brain research, 186(2), 293-297.

32. Pawar, S. S., Sajjanar, B., Lonkar, V. D., Kurade, N. P., Kadam, A. S., Nirmal, A. V., ... & Bal, S. K. (2016). Assessing and mitigating the impact of heat stress in poultry. Adv. Anim. Vet. Sci, 4(6), 332-341.

33. Zerjal, T., Gourichon, D., Rivet, B., & Bordas, A. (2013). Performance comparison of laying hens segregating for the frizzle gene under thermoneutral and high ambient temperatures. Poultry Science, 92(6), 1474-1485.

34. Li, Z., Liu, X., Zhang, P., Han, R., Sun, G., Jiang, R., ... & Tian, Y. (2018). Comparative transcriptome analysis of hypothalamus-regulated feed intake induced by exogenous visfatin in chicks. BMC genomics, 19, 1-17.

35. Ziauddin, M., Phansalkar, N., Patki, P., Diwanay, S., & Patwardhan, B. (1996). Studies on the immunomodulatory effects of Ashwagandha. Journal of ethnopharmacology, 50(2), 69-76.

36. Archana, R., & Namasivayam, A. (1998). Antistressor effect of Withania somnifera. Journal of ethnopharmacology, 64(1), 91-93.

37. Ganzera, M., Choudhary, M. I., & Khan, I. A. (2003). Quantitative HPLC analysis of withanolides in Withania somnifera. Fitoterapia, 74(1-2), 68-76.

38. Matsuda, H., Murakami, T., Kishi, A., & Yoshikawa, M. (2001). Structures of withanosides I, II, III, IV, V, VI, and VII, new withanolide glycosides, from the roots of Indian Withania somnifera DUNAL. and inhibitory activity for tachyphylaxis to clonidine in isolated guinea-pig ileum. Bioorganic & medicinal chemistry, 9(6), 1499-1507.

39. Park, H. J., Rayalam, S., Della-Fera, M. A., Ambati, S., Yang, J. Y., & Baile, C. A. (2008). Withaferin A induces apoptosis and inhibits adipogenesis in 3T3-L1 adipocytes. Biofactors, 33(2), 137-148.

40. Greene, E. S., Maynard, C., Owens, C. M., Meullenet, J. F., & Dridi, S. (2021). Effects of herbal adaptogen feed-additive on growth performance, carcass parameters, and muscle amino

acid profile in heat-stressed modern broilers. Frontiers in Physiology, 12, 784952.

41. Hussain, S. S., & Bloom, S. R. (2013). The regulation of food intake by the gut-brain axis: implications for obesity. International journal of obesity, 37(5), 625-633.

42. Brugaletta, G., Greene, E., Tabler, T., Orlowski, S., Sirri, F., & Dridi, S. (2021). Effect of cyclic heat stress on feeding-related hypothalamic neuropeptides of three broiler populations and their ancestor jungle fowl. Frontiers in Physiology, 12, 2383.

43. Clark, J. T., Kalra, P. S., Crowley, W. R., & Kalra, S. P. (1984). Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology, 115(1), 427-429.

44. Sartsoongnoen, N., Kamkrathok, B., Songserm, T., & Chaiseha, Y. (2021). Distribution and variation of neuropeptide Y in the brain of native Thai chicken. Avian Biology Research, 14(1), 27-36.

45. Kuenzel, W. J., & McMurtry, J. (1988). Neuropeptide Y: brain localization and central effects on plasma insulin levels in chicks. Physiology & Behavior, 44(4-5), 669-678.

46. Richards, M. P. (2003). Genetic regulation of feed intake and energy balance in poultry. Poultry science, 82(6), 907-916.

47. Song, Z., Yuan, L., Jiao, H., & Lin, H. (2011). Effect of corticosterone on hypothalamic corticotropin-releasing hormone expression in broiler chicks (Gallus gallus domesticus) fed a high energy diet. Asian-Australasian Journal of Animal Sciences, 24(12), 1736-1743.

48. Honda, K., Kamisoyama, H., Saneyasu, T., Sugahara, K., & Hasegawa, S. (2007). Central administration of insulin suppresses food intake in chicks. Neuroscience letters, 423(2), 153-157.

49. Balthasar, N., Dalgaard, L. T., Lee, C. E., Yu, J., Funahashi, H., Williams, T., ... & Lowell, B. B. (2005). Divergence of melanocortin pathways in the control of food intake and energy expenditure. Cell, 123(3), 493-505.

50. Mercer, A. J., Hentges, S. T., Meshul, C. K., & Low, M. J. (2013). Unraveling the central proopiomelanocortin neural circuits. Frontiers in neuroscience, 7, 19.

51. Zendehdel, M., Hamidi, F., Babapour, V., Mokhtarpouriani, K., & Fard, R. M. N. (2012). The effect of melanocortin (Mc3 and Mc4) antagonists on serotonin-induced food and water intake of broiler cockerels. Journal of veterinary science, 13(3), 229-234.

52. Poggioli, R., Vergoni, A. V., & Bertolini, A. (1986). ACTH-(1–24) and α-MSH antagonize feeding behavior stimulated by kappa opiate agonists. Peptides, 7(5), 843-848.

53. Ozdemir, O., Tuzcu, M., Sahin, N., Orhan, C., Tuzcu, Z., & Sahin, K. (2017). Organic chromium modifies the expression of orexin and glucose transporters of ovarian in heat-stressed laying hens. Cellular and Molecular Biology, 63(10), 93-98.

54. Greene, E., Khaldi, S., Ishola, P., Bottje, W., Ohkubo, T., Anthony, N., & Dridi, S. (2016). Heat and oxidative stress alter the expression of orexin and its related receptors in avian liver cells. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 191, 18-24.

55. Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., ... & Yanagisawa, M. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell, 92(4), 573-585.

56. Furuse, M., Ando, R., Bungo, T., Shimojo, M., & Masuda, Y. (1999). Intracerebroventricular injection of orexins does not stimulate food intake in neonatal chicks. British poultry science, 40(5), 698-700.

57. Ukena, K., Iwakoshi-Ukena, E., Taniuchi, S., Bessho, Y., Maejima, S., Masuda, K., ... & Tachibana, T. (2014). Identification of a cDNA encoding a novel small secretory protein, neurosecretory protein GL, in the chicken hypothalamic infundibulum. Biochemical and Biophysical Research Communications, 446(1), 298-303.

58. Shikano, K., Kato, M., Iwakoshi-Ukena, E., Furumitsu, M., Matsuura, D., Masuda, K., ... & Ukena, K. (2018). Effects of chronic intracerebroventricular infusion of neurosecretory protein GL on body mass and food and water intake in chicks. General and Comparative Endocrinology, 256, 37-42.

59. Sonoli, S. S., Shivprasad, S., Prasad, C. V., Patil, A. B., Desai, P. B., & Somannavar, M. S. (2011). Visfatin-a review. Eur Rev Med Pharmacol Sci, 15(1), 9-14.

60. Dakroub, A., A. Nasser, S., Younis, N., Bhagani, H., Al-Dhaheri, Y., Pintus, G., ... & Eid, A. H. (2020). Visfatin: A possible role in cardiovasculo-metabolic disorders. Cells, 9(11), 2444.

61. Savova, M. S., Mihaylova, L. V., Tews, D., Wabitsch, M., & Georgiev, M. I. (2023). Targeting PI3K/AKT signaling pathway in obesity. Biomedicine & Pharmacotherapy, 159, 114244. 2023.

62. Lee, B. C., Song, J., Lee, A., Cho, D., & Kim, T. S. (2018). Visfatin promotes wound healing through the activation of ERK1/2 and JNK1/2 pathway. International journal of molecular sciences, 19(11), 3642.

63. Heo, Y. J., Choi, S. E., Jeon, J. Y., Han, S. J., Kim, D. J., Kang, Y., ... & Kim, H. J. (2019). Visfatin induces inflammation and insulin resistance via the NF-κB and STAT3 signaling pathways in hepatocytes. Journal of diabetes research, 2019.

64. Ons, E., Gertler, A., Buyse, J., Lebihan-Duval, E., Bordas, A., Goddeeris, B., & Dridi, S. (2010). Visfatin gene expression in chickens is sex and tissue dependent. Domestic animal endocrinology, 38(2), 63-74.