

# Investigating the potential role of the pituitary adenylate cyclase-activating polypeptide (PACAP) in regulating the ubiquitin signaling pathway in poultry

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## ARTICLE INFO

### Keywords:

Poultry  
cAMP  
CRH  
PACAP  
Ubiquitin signaling pathway  
Glucagon family

## ABSTRACT

The physiological processes in animal production are regulated through biologically active molecules like peptides, proteins, and hormones identified through the development of the fundamental sciences and their application. One of the main polypeptides that plays an essential role in regulating physiological responses is the pituitary adenylate cyclase-activating polypeptide (PACAP). PACAP belongs to the glucagon/growth hormone-releasing hormone (GHRH)/vasoactive intestinal proteins (VIP) family and regulates feed intake, stress, and immune response in birds. Most of these regulations occur after PACAP stimulates the cAMP signaling pathway, which can regulate the expression of genes like MuRF1, FOXO1, Atrogin 1, and other ligases that are essential members of the ubiquitin system. On the other hand, PACAP stimulates the secretion of CRH in response to stress, activating the ubiquitin signaling pathway that plays a vital role in protein degradation and regulates oxidative stress and immune responses. Many studies conducted on rodents, mammals, and other models confirm the regulatory effects of PACAP, cAMP, and the ubiquitin pathway; however, there are no studies testing whether PACAP-induced cAMP signaling in poultry regulates the ubiquitin pathway. Besides, it would be interesting to investigate if PACAP can regulate ubiquitin signaling during stress response via CRH altered by HPA axis stimulation. Therefore, this review highlights a summary of research studies that indicate the potential interaction of the PACAP and ubiquitin signaling pathways on different molecular and physiological parameters in poultry species through the cAMP and stress signaling pathways.

## 1. Introduction

Developing fundamental sciences and their application to better animal husbandry emphasize the physiological processes of regulation through active biological substances like proteins, peptides, and hormones (Kotodziejcki et al., 2021).

The pituitary adenylate-cyclase-activating polypeptide (PACAP), a 27- or 38-amino acid neuropeptide, is primarily expressed in nervous tissues and plays a role in energy metabolism, including thermogenesis, appetite, mobilization of energy storage, and activity (Rudecki and Gray, 2016; Wang et al., 2022). The family of vasoactive intestinal peptides (VIP), a hormone family of peptides regulating class B G-protein coupled receptor (GPCR) activity, also known as the secretin receptor family, bears high similarity to PACAP. In addition, PACAP is

identified as a member of the glucagon/gastric inhibitory polypeptide (GIP)/GHRH family (Segre and Goldring, 1993; Nowak and Zawilska, 2003; Hirabayashi et al., 2018). The peptides of the glucagon superfamily have two types of receptors to bind with: type I receptors (PAC1-R) show affinity much higher and are specific only for PACAP; however, type II receptors (VPAC1-R and VPAC2-R) show resemblance affinity for both polypeptides PACAP and VIP (Vaudry et al., 2009). A large amount of PAC1 receptors have been found in the brains of chickens besides VPAC membrane receptors (Zawilska et al., 2003).

When PACAP is present, rat pituitary cells release more growth hormone (GH), adrenocorticotrophic hormone (ACTH), luteinizing hormone (LH), and prolactin (PRL) (Miyata et al., 1989). Avian species also express prolactin through VIP from the anterior pituitary gland (Macnamee et al., 1986; El Halawani et al., 1990). Researchers found that

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<https://doi.org/10.1016/j.ygcen.2024.114577>

Received 10 April 2024; Received in revised form 13 June 2024; Accepted 20 June 2024

Available online 22 June 2024

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PACAP increased the levels of LH, follicle-stimulating hormone (FSH), and TSH in the gonadotrophin pituitary glands of mammals. It also increased the expression of the PACAP receptor (PAC1R) and the gonadotropin-releasing hormone (GnRH) receptors in the hypothalamus (Oride et al., 2018). Moreover, enterochromaffin-like (ECL) cells can stimulate the PAC1 receptor, leading to PACAP's stimulation of histamine secretion in the stomach, which is responsible for secreting acids needed for digestion (Barnes et al., 2001; Reglodi et al., 2018).

Miyata and his colleagues (1989) discovered and isolated PACAP from the hypothalamus of ovine, functioning as an activator for the production of cyclic adenosine monophosphate (cAMP) in the anterior pituitary cells (Oride et al., 2018). McRory and his colleagues (1997) were the first to isolate PACAP in chicken, and they proved that growth hormone-releasing factor (GRF) and PACAP belong to the glucagon superfamily as in mammals. They also reported that the chickens only expressed PACAP and GRF peptides in the brain and gonads. Later studies proved that PACAP plays a non-neuronal role after they found PACAP was present in the gonads, the intestine, and many other organs (Jozsa et al., 2001; Pirone et al., 2011). After cAMP production is stimulated, PACAP initiates several biological processes, including neurotropic effects (Nowak et al., 1999b; Somogyvari-Vigh and Reglodi, 2004). This stimulation initiated by PACAP has an effect on the levels of corticosterone during stress, as PACAP is recognized as a master regulator in stress response by exerting an effect on CRH secretion from the hypothalamus (Tachibana et al., 2004; Agarwal et al., 2005; Jozsa, 2006). As a result, elevated levels of stress hormones like corticosterone and cAMP stimulation affect the ubiquitin–proteasome system (UPS) that stimulates the expression of its related molecules, including ligases and genes that are involved in protein degradation and are proven to be involved in stress regulation in poultry (Nakashima et al., 2004; Li et al., 2011; Lu et al., 2017; Sun et al., 2022).

The UPS regulates a substantial portion of protein breakdown in eukaryotes, playing an important role in a variety of activities, both physiological and pathological. In the protein degradation pathway, ubiquitin, a 76 amino acid polypeptide that functions as a regulatory protein, was first isolated from the thymus, and subsequent research identified the ubiquitin molecule as an ATP-dependent proteolysis factor (APF-1), emphasizing its role in protein degradation within the cell (Hegde, 2010). It is connected to the substrates via an enzymatic cascade that is highly regulated in a process called ubiquitination. This process starts when an ubiquitin-activating enzyme (E1) binds with ubiquitin and activates it, along with ATP, and passes ubiquitin to another enzyme called a conjugating enzyme (E2), which can directly act or form a complex with a ubiquitin-protein ligase (E3) and ubiquitinate substrate protein. The proteasome is the major target for proteins in the ubiquitin pathway, as it may break down by ubiquitin attachment and reuse substrates. There are two chains of ubiquitin modifications: mono-ubiquitination, where the attachment to a protein is with one ubiquitin molecule, and polyubiquitin chains, which form when multiple ubiquitin molecules attach to either one of seven lysine residues or another ubiquitin molecule N-terminus, depending on the type of protein that binds (Kaiser and Huang, 2005; Swatek and Komander, 2016; Abbas, 2021). Additionally, polyubiquitinated proteins may take a non-degradative pathway where they can regulate the target protein's activity or intracellular transport (Bonifacino and Weissman, 1998). The ubiquitin system has an essential role in maintaining protein homeostasis through protein turnover regulation. These proteins play a significant role in a variety of regulatory pathways, such as those that include DNA damage and its repair, apoptosis, cell cycle progression, protein processing, immune response, signal transduction, and receptor-mediated endocytosis (Kona et al., 2013; Chitra et al., 2012).

The involvement of these polypeptides in such a wide range of molecular regulation makes them an interesting topic of focus. In addition, the molecular studies on mammals and rodents suggested that PACAP may potentially regulate ubiquitin signaling pathways and stimulate further biological interactions that are ubiquitin-related via cAMP

signaling, stress response and regulation, and other possible pathways (Huang et al., 2013; Rinaldi et al., 2015). In this review, we will highlight the summary from research studies that outline the potential interaction of these polypeptides with different molecular and physiological parameters in poultry species via the corticosterone and cAMP signaling pathways.

## 2. The possible routes where PACAP can affect the ubiquitin signaling pathway in poultry

### 2.1. The regulation of the cAMP signaling pathway

The extracellular ligand (e.g., PACAP) binds to the G protein-coupled receptors (GPCR) link (e.g., PAC1-R) at the cell membrane surface and interacts with the stimulatory G-protein  $\alpha$  subunit ( $G_{\alpha s}$ ) to activate the adenylate cyclases (AC) that produces cAMP (Cooper and Tabbasum, 2014). The equilibrium between the two classes of enzymes, ACs and cyclic nucleotide phosphodiesterases (PDEs), regulates the cAMP levels, and the cAMP cascade regulates the signaling scaffolds and regulatory protein networks (Walsh and Van Patten, 1994; Taskén and Aandahl, 2004; Taylor et al., 2005). The AC are responsible for catalyzing the production of cAMP using ATP, and to regulate the cyclic nucleotide signaling, cAMP levels are decreased by the cAMP-PDEs enzymes, which hydrolyze cAMP into 5-AMP (Maurice et al., 2014).

PACAP stimulates cAMP formation in the avian brain (Nowak et al., 1999a, 1999b; Nowak and Kuba, 2001). Nowak and Kuba (2001) found that the expression of PACAP is highest in the hypothalamus part of the brain, in addition to PACAP's strong ability to stimulate cAMP synthesis in the 2–3 week-old male chicks. Moreover, the level of cAMP increased with the increasing doses of chicken PACAP but was significantly lower when using mammalian PACAP, which shows the importance of using avian PACAP drug when testing their effect on cAMP synthesis for more reliable results. Nowak and Zawilska (2003) found that PACAP stimulated the cAMP accumulation in the 3 to 4-weeks-old brains of avian species more than VIP in an in vitro study. Moreover, PACAP27 stimulated cAMP accumulation mainly in 5 to 6 month ducks and hens' hypothalamus and cerebral cortex.

Cyclic AMP plays a crucial role in poultry, particularly in host defense peptides (HDP) expression regulation, which is important for antimicrobial therapy and immunomodulatory regulation (Sunkara et al., 2014). cAMP has a role in cartilage development in embryonic chicks besides regulating the melatonin levels in chick pineal cells (Burch and Lebovitz, 1981; Nikaido and Takahashi, 1989). It also has a significant role in poultry physiology, particularly in the regulation of sugar accumulation in chicken intestinal cells (Moretó et al., 1984). Furthermore, cAMP inhibits lipid synthesis in the liver of chickens (Boyd et al., 1975; Bricker and Marraccini, 1975).

In eukaryotes, Protein Kinase A (PKA) is the main mediator of cAMP-induced effects through initializing its activation, as PKA has an important role in transmitting the signal downstream to the intended substrates or effectors, thereby participating in the propagation of the signal cascade (Taylor et al., 2005). Additionally, A-kinase anchoring proteins (AKAPs), functioning as scaffolding proteins, regulate critical biological processes like hormone release, differentiation, cell vitality, synaptic transmission, memory, learning, and metabolic activity, which mediate the localization of PKA in the cell through controlling the cAMP signal distribution inside the cell (Alto et al., 2002; Taskén and Aandahl, 2004). As scaffold proteins, AKAPs act as regulators for negative and positive feedback signaling through activating or inactivating components, depending on the required cellular response. AKAPs also associate with PKA and other signaling components to assemble multi-protein complexes that are essential for preserving the specificity and effectiveness of cAMP signaling (Raslan et al., 2015; Zaccolo et al., 2020; Ostrom et al., 2022). The activation of cAMP signaling pathways by ligands (e.g., PACAP) that leads to the activation of downstream effectors such as PKA and Epac plays a crucial role in guiding PKA to particular

substrates and subcellular compartments. It also has a regulatory effect on gene expression, protein synthesis, and other cellular processes involved in muscle growth which is mainly regulated through the ubiquitin system (Reynolds et al., 2007). Besides, several experiments collectively emphasize the regulatory influence of cAMP in the differentiation of chick muscle cells (Zalin, 1973; Zalin and Montague, 1974; 1975).

The UPS can control the AKAPs at the post-translational level, which gives it the ability to strictly control these biological responses by adjusting the protein levels. Based on the specific combinations of cyclic nucleotide generators (AC), effectors (PKA, the exchange protein activated directly by cAMP (EPAC), and cAMP-gated ion channels), degrading PDEs, and scaffold proteins (AKAPs), the so-called “signalosome” is established, which is based on this protein–protein interaction (Rinaldi et al., 2015). This protein-to-protein interaction is also proven to be mediated via the UPS (Schnell and Hicke, 2003), suggesting that the ubiquitin ligase signals can exert or mimic the same effects of cAMP signaling (Huang et al., 2013). It was also found that the UPS could control PDEs by phosphorylating PDE4D at the same time by casein kinase 1 (CK1) and glycogen synthase kinase 3 $\beta$  in mammals (Zhu et al., 2010). It is important to determine whether these regulations are similar in poultry to mammals or if a different regulatory signaling pathway is involved. Post-stimulus receptor desensitization is a result of the UPS's ubiquitination and subsequent receptor proteolysis at the cell membrane (Bonifacino and Weissman, 1998). PKA simultaneously inhibits the deubiquitinase USP20 (ubiquitin-specific processing protease 20), which encourages ubiquitination and NEDD4-mediated receptor degradation (Nabhan et al., 2010; Kommaddi et al., 2015). Agonists cause ubiquitylation of receptors and  $\beta$ -arrestins, which are  $\beta$ -receptor regulatory proteins, affecting the endocytosis of receptors. Recycling allows internalized GPCRs to degrade or return to the cell surface (Shenoy et al., 2001).

These signaling pathways that link the relationship between cAMP and ubiquitin signaling pathways are mostly studied in mammals and rodents. However, many of these molecules related to the ubiquitin signaling pathway and their expressions, such as the two E3 ubiquitin-ligases, muscle atrophy F-box (mafbx, also called atrogen-1) and muscle ring finger-1 (MuRF-1), in addition to the role of the UPS effect on protein degradation via ubiquitination, have been studied in avian species and proven to contribute to many physiological activities and responses, such as affecting meat quality, growth performance, immunity response, and skeletal muscle development (Bodine et al., 2001).

Besides the proteasome, cAMP signaling controls the UPS. As a result, increasing cAMP in rodent skeletal muscle both in vitro and in vivo reduced levels of atrogen-1, which is involved in muscle atrophy, as well as proteasome activity and ubiquitin-protein conjugates (Gonçalves et al., 2009). Furthermore, after fasting, which significantly influences the production of PACAP, which has an undeniable effect on cAMP production, the E3 ubiquitin-ligases atrogen-1 and MuRF-1 were induced in skeletal muscle. These regulations were suppressed when PDE4 was inhibited when using a selective PDE4 inhibitor (Nakashima et al., 2005; Jozsa, 2006; Lira et al., 2011; Saneyasu et al., 2015).

Studies indicated that cAMP signaling promotes muscle growth and protein synthesis by inhibiting proteolysis by both calpains and the proteasome, both of which play an essential role in muscle atrophy, via regulating the expression of genes involved in muscle development, such as MuRF1 and Atrogen1, preventing protein degradation, and reducing muscle protein loss (Bonaldo and Sandri, 2013; Vainshtein and Sandri, 2019; Pang et al., 2023). Modulating cAMP signaling can affect the action of this system by potentially reducing the breakdown of muscle protein and averting atrophy. For disorders marked by muscle wasting, such as age-related atrophy and muscular dystrophy, such regulation holds potential for therapy. The interaction between cAMP signaling and the UPS is critical for maintaining muscle mass and function by controlling muscle protein turnover. Adjusting these pathways helps to establish a delicate equilibrium between protein synthesis and

degradation in muscle cells, promoting muscle development (Navegantes et al., 2003; Berdeaux and Stewart, 2012).

The cAMP stimulates the protein kinase B (PKB) pathway and inhibits the FOXO signaling pathway in muscle cells (Gonçalves et al., 2009). The Forkhead O (FOXO) signaling pathway is a subset of transcription factors that are involved in various physiological processes, including autophagy, repairing damaged DNA, cell cycle termination, and oxidative stress (Xing et al., 2018). Reduced cAMP stimulation triggers FOXO transcription factors, which in turn enhance the expression of genes linked to protein breakdown, including MuRF1 and Atrogen1. This ultimately leads to an increase in proteolysis and muscle atrophy in specific circumstances (Sandri et al., 2004; Stitt et al., 2004; Berdeaux and Stewart, 2012). It is important to carefully control PKB/Akt signaling and FOXO-mediated E3 ubiquitin ligase expression to control the protein turnover and homeostasis. The complex interaction between Akt, FOXO transcription factors, and muscle-specific E3 ubiquitin ligases determines the control of muscle protein degradation and turnover, which affects overall muscle health and function. The feeding status regulates the ubiquitin–proteasome proteolytic pathway via PKB in mammals, whereas PKB can be regulated by cAMP (Filippa et al., 1999). It has been demonstrated that growth factors (insulin or IGF-I) and nutrients (such as amino acids) strongly influence the expression of the E3 ubiquitin ligase atrogen-1 in avian fibroblasts through signaling that involves the PKB/Akt-FOXO transcription factors and the signaling axis between PKB and the drug rapamycin (Tesseraud et al., 2007).

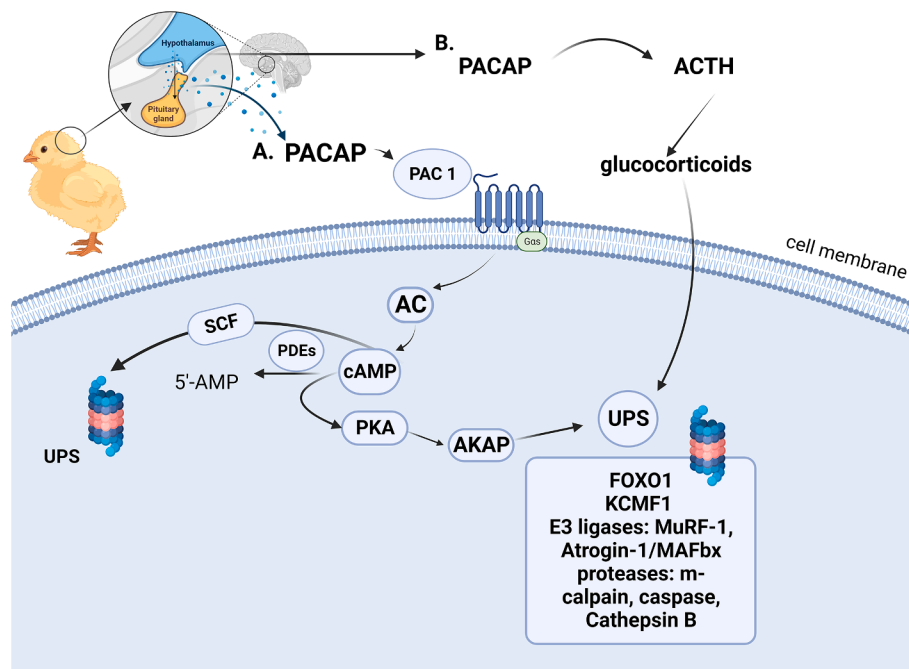
The studies demonstrate that the cAMP signaling pathway may be one of the potential pathways where PACAP can affect the ubiquitin signaling pathway as it controls the cAMP level stimulation. The latter has a part in regulating the expression of genes and proteins related to the UPS, especially the E3 ubiquitin ligases atrogen-1 and MuRF-1. These ubiquitin-related membranes play a vital role in the protein degradation system, which affects meat quality mainly through apoptosis, muscle myopathy, and skeletal muscle differentiation (See Fig. 1 A).

## 2.2. PACAP controlling CRH secretion which affects UPS

In our previous discussion, we explored the various ways in which PACAP influences physiological parameters in poultry. Tachibana et al. (2004) studied whether the intracerebroventricular (ICV) injection of PACAP and VIP affects the corticotropin-releasing factor (CRF). They found a significant increase in the CRF after 30 min from the administration of 188 pmol of PACAP and VIP in 3-day-old male layer chicks, concluding that PACAP and VIP regulate the stress response. In addition, co-injection of astressin, an antagonist for the CRF receptor, completely reduces the corticosterone-releasing effect of PACAP, but this effect was only partially present for VIP. Thus, CRF neurons modulate PACAP and VIP, which are responsible for hypophagia. In another study, after the central administration of CRF, responsible for activating the hypothalamic–pituitary–adrenal (HPA) axis in the chicken hypothalamus, significantly reduced feed intake and plasma glucose levels, suggesting a relationship between CRF and HPA axis activation and glucagon-induced anorexia in chicks but not hyperglycemia (Honda et al., 2012).

Birds subjected to environmental stress factors, like thermal temperature change, have been proven to negatively affect their performance (Surai and Fisinin, 2016; Chen et al., 2015; Farag and Alagawany, 2018). PACAP is one of the polypeptides responsible for stimulating stress hormone secretion. Studies have proven that PACAP controls the physiological balance of the autonomic nervous system, as PACAP is recognized as a master regulator in stress response (Tachibana et al., 2004; Stroth et al., 2011a, 2011b; Szentléleky et al., 2019). The release of corticosterone hormone in response to stress conditions, which stimulates CRH, can negatively impact various physiological parameters in poultry, including immunity and meat quality, which are significantly regulated by the UPS (Li et al., 2011; Tachibana and Tsutsui, 2016; Abo-Al-Ela et al., 2021).

These high levels of hormonal stress may also have other effects, such



**Fig. 1.** The possible way PACAP regulates the ubiquitin–proteasome system in poultry. A. Represent a possible regulatory pathway through cAMP signaling. The PACAP secreted from the hypothalamus stimulates intracellular signaling through the G-protein-coupled receptor (GPCR) at the  $G\alpha$  subunit and stimulates adenylate cyclase (AC) secretion. AC then stimulates the secretion of 3'-5' cyclic adenosine monophosphate (cAMP), which activates the protein kinase A (PKA) and exerts an effect on the ubiquitin–proteasome system (UPS) through the scaffolding protein A-kinase anchoring proteins (AKAP). The cyclic nucleotide phosphodiesterases (PDEs) regulate the level of cAMP by converting it into 5'-AMP, controlling its amplitude and duration (see the review article). These PDEs affect UPS through the scaffold protein complex SCF. B. represents a possible pathway through the stress response by stimulating the secretion of CRH. This effect will occur through the hypothalamic–pituitary–adrenal (HPA) axis pathway, where the stimulation of PACAP from the hypothalamus will cause the pituitary to secrete ACTH. After that, the adrenal gland is stimulated to secrete cortisol or glucocorticoids. The glucocorticoids bind to the intracellular surface and exert an effect on the ubiquitin signaling pathway and the genes expressed. These stimulations will be responsible for several physiological responses like immunity, stress response, feed intake regulation, and others. “This figure was created with [BioRender.com](https://www.biorender.com).”

as regulating protein signaling and immune responses. Lu et al. (2017) found that alpha-lipoic acid treatment restored 24 proteins that are primarily concerned with protein translation, transcription regulation, detoxification, and protein degradation, in addition to immunity and response to stress, contrary to the results in the control group subjected to stress. The expressed proteins during heat stress exposure included high mobility group box 1 (HMGB1), which acts as a major chromatin-associated non-histone protein, acts as a DNA chaperone, and is involved in replication, transcription, DNA repair, chromatin remodeling, and genomic stability (Li et al., 2013), as well as glutathione S-transferase (GST), an essential detoxifying and antioxidant enzyme that catalyzes the conjugation of decreased glutathione to a set of exogenous and endogenous hydrophobic electrophiles and has a critical cell protection role against rapid oxidative stress (Hayes et al., 2005). Both of these proteins have a close connection to the immune system, oxidative stress, and the collagens involved in liver damage. Additionally, the results demonstrate the expression of the de-ubiquitinating enzymes ubiquitin carboxyl-terminal hydrolase isozyme L5 (UCHL5) and ubiquitin carboxyl-terminal hydrolase 24 (USP24), which are involved in ubiquitin-dependent protein catabolic processes. By disassembling polyubiquitin chains, UCHL5 can prevent protein degradation (Chen et al., 2013).

Stroth et al. (2011b) studied the mechanism of PACAP in stress response and found that CRH quickly increased in cultured hypothalamic neurons after PACAP treatment. In the pituitary gland of mice lacking PACAP, Nr4a factors (Nur77, Nurr1) induction was reduced as a response to restraint. Restraint causes the mRNA levels of the adrenocortical for all Nr4a transcription factors to increase in the adrenal glands significantly. Together, these findings demonstrate that PACAP, possibly through transcription factors like Nur77 and Nor1, regulates

the HPA responses to control primarily at the hypothalamic level by increasing CRH. PACAP appears to be required for stimulus-transcription coupling during subsequent adrenocortical steroidogenesis, which suggests a mechanism by which PACAP regulates HPA axis activity during stress. In poultry, another kind of transcription factor-like (Nrf2) is involved during the regulation of oxidative stress, alongside the proteasome of the ubiquitin system that is responsible for cell repair after being damaged by stress conditions (Surai et al., 2019; Chitra et al., 2012). The chick develops the HPA axis during the pre-hatch stage and is regulated at various levels. At the level of the adrenal gland itself, the gland starts secreting glucocorticoids as a sign of its ability to produce these kinds of hormones. The other level occurs at the anterior pituitary gland, where the gland begins to influence the adrenal gland to produce the stress hormone, indicating its regulatory capacity. The last level of regulation is at the hypothalamus, where the secretion of glucocorticoids is controlled, making the hypothalamus the main mediator for stress responses (Jenkins and Porter, 2004).

Stress activation of HPA axis is believed to cause alterations in the intestinal mucosa and the resulting negative impact on the performance and immune system of broiler chickens under heat stress (Quinteiro-Filho et al., 2010). Moreover, it has been discussed before that PACAP regulates the HPA axis by affecting the stimulation of its main mediator, the CRH, from the hypothalamus. CRH then stimulates ACTH secretion from the pituitary, which then stimulates the secretion of glucocorticoids from adrenals, and showing increased levels of corticosterone in the blood (Agarwal et al., 2005; de Kloet et al., 2005; Costa-Pinto and Palermo-Neto, 2010; Bu et al., 2019). The HPA axis's final effectors, the glucocorticoids (GCs), are involved in the regulation of whole-body homeostasis and the reaction of birds to stress. GCs elevate the expression of the E3 ubiquitin ligases, MuRF1 and atrogin1, activating the

muscle's protein degradation (Menconi et al., 2008; Wang et al., 2016). They play a significant role in affecting the development of muscle by suppressing the expression of insulin-like growth factor 1 (IGF1), which is a main contributor to muscle growth. They can also stimulate the expression of myostatin, which negatively affects muscle growth, and the ubiquitin ligases that degrade muscle protein. These effects are dependent on age and the time of exposure to the stress, as ubiquitin ligase WWP1 was downregulated in 7-day-old chicks after 3 days of exposure but upregulated after prolonged exposure for 7 days. Moreover, for older broiler birds, fasting helped downregulate the expression of the WWP1 gene in the muscle, which explains the fast growth of the broiler because they have a lower protein degradation rate (Hayashi et al., 1985; Song et al., 2011; Saneyasu et al., 2015). Glucocorticoids increased the levels of oxidative stress, leading to an increase in the expression of the FOXO1 gene, activated by the PKB pathway (Tesseraud et al., 2007), which is responsible for activating the E3 ubiquitin ligases that act on protein degradation like Murf1 and Atrogin 1, in addition to increasing the expression of apoptotic genes like Bcl-2, Caspase-3, Bim, and Bax, and decreasing the genes and proteins related to enhancing antioxidant enzymes superoxide dismutase (SOD), catalase activity (CAT), and glutathione peroxidase (GSH-Px) in the reproductive system of laying hens (Bai et al., 2023).

The elevated levels of corticosterone stimulate the secretion of ROS, which can increase the expression of Atrogin-1 through the activation of the UPS, which is responsible for muscle degradation. Other genes related to proteolysis were not expressed, such as the proteasome C2 subunit, cathepsin B, and m-calpain in the pectoralis muscle. Also, FOXO3 expression had the tendency to increase in response to heat stress, but FOXO1 did not show any change, although it is known to be incorporated in the protein degradation in skeletal muscle (Furukawa et al., 2016). In another study, the heat stress also elevated the E3 ligase Atrogin-1 but also affected the expression of m-calpain by decreasing its levels, in addition to decreasing the transcription factor PGC-1 $\alpha$ , which is involved in energy metabolism (Boussaid-Om Ezzine et al., 2010). Nakashima et al. (2004) noted that Calpain, the proteasome, and Cathepsin B, expressed during oxidative stress, act as intracellular proteases that directly influence protein degradation.

Corticosterone treatment increases the protein breakdown through declining myotube diameter and myosin heavy chain (MHC) expression; the latter is proven to be degraded by the E3 Ligase MuRF1 (Clarke et al., 2007), in addition to affecting 3-methyl-histidine (3M-His), which acts as a marker for the myofibrillar protein degradation. Compared to the control, the corticosterone administration elevated the levels of the Atrogin1 protein and upregulated the mRNA expression levels of MuRF1 and Atrogin1, while corticosterone did not significantly affect the levels of MuRF1 and MSTN protein (Sun et al., 2022). These findings show that the ubiquitin–proteasome pathway is involved in breaking down proteins and sending important signal transmissions linked to stress. They also note the importance of studying protein expression as well as gene expression in these kinds of studies to get a clearer picture of the results. In a different experiment, the high expression of ubiquitin-related genes, including KCMF1 (potassium channel modulatory factor 1), UBE2R2 (ubiquitin-associated protein 2-like), and others, was looked at in the muscles of young chickens. This is because of heat stress, which affects their muscle growth and development. These genes play a critical role in apoptosis, lysosomal degradation, and cell repair (Barnes et al., 2019).

The administration of dexamethasone, a glucocorticoid derivative, only increased the expression of Atrogin-1 in the skeletal muscle of chicks without affecting the expression of other proteolytic genes like the 20S proteasome C2 subunit, m-calpain large subunit, and cathepsin B (Nakashima et al., 2016). However, it is important to measure the expression of the E3 ligase MuRF1 gene, which is known for its proteolytic activity in skeletal muscle in chickens, in this kind of stress stimulation to get a clear picture of whether the E3 ubiquitin ligases act differently under different stress factors. In response to oxidative stress, there was a significant increase in the expression of FOXO1 mRNA and

protein. This led to an accumulation of excess FOXO1 protein in the nucleus, increasing downstream apoptotic gene expression (Zhang et al., 2019). This apoptosis is mainly regulated through the ligases and proteases of the UPS. Furthermore, this regulation through FOXO stimulation was discussed in previous parts as being regulated by cAMP, which questions the potential of sync between pathways to regulate stress.

It is now clear that when birds are stressed, PACAP signals from the hypothalamus stimulates the CRH secretion and the adrenal gland will produce GCs. In addition, these GCs affect the UPS through affecting the expression of its main ligases. Studies have shown that under stress, genes that break down proteins, like calpain, proteasome subunit, and cathepsin B, are significantly upregulated. As well as ubiquitin ligases, and other stress-related enzymes like atrogin-1, MuRF1, and UBE2R2. The ubiquitin–proteasome system is in charge of breaking down proteins, and these results show a link between glucocorticoids and this degradation that increases during stress (See Fig. 1 B).

### 3. Conclusion

The objective of this review was to collect data on the function of PACAP in controlling the cAMP pathway and the HPA axis in response to stress conditions. Additionally, the final effectors of HPA, GCs the ubiquitin–proteasome pathway in poultry through ubiquitin ligases, which play a role in physiological responses, addressing a potential of regulation pathway to reduce protein degradation. Furthermore, most studies were carried out on chicks at a young age, which is critical because this period significantly impacts growth performance and later performance, whether as layer or broiler chicks. However, it is essential to understand how these molecules regulate their pathways and expression during the production phase and their impact on performance, including feed intake, reproduction, the immune system, and meat or egg production. Further research is needed to determine the relationship between PACAP and ubiquitin signaling pathways in these areas and to elucidate the pathways involved in PACAP-mediated regulation and their effect on the ubiquitin–proteasome pathway in poultry. Such investigations can be conducted using both in-ovo and ICV injection methods in poultry studies, which gives a clear view of the signaling pathway and determines whether the cAMP and corticosterone hormones secreted and regulate the ubiquitin pathway are the same as those produced by PACAP or whether other pathways contribute to this regulation.

### CRedit authorship contribution statement

**Doha Mohamad Khalifeh:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Levente Czeglédi:** Writing – review & editing, Supervision, Conceptualization. **Gabriella Gulyas:** Writing – review & editing, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

No data was used for the research described in the article.

### Acknowledgments

We would like to thank the Stipendium Hungaricum Scholarship under the Tempus Public Foundation, Hungary for funding the doctoral study of Doha Khalifeh.

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