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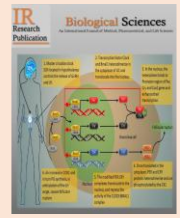

Wheat gluten protein and the physiology of the ghrelin hormone in chickens: a novel therapeutic approach

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ABSTRACT

Ghrelin, a 26-amino acid peptide, is known to stimulate the release of growth hormone and motilin in chickens. Wheat is a staple food in many countries, and the whole grain contains 13% protein, 75% of which is gluten protein. Wheat gluten is hydrolyzed by digestive enzymes, producing gliadin and gluten-exorphin. Gluten-exorphin acts on the gastrointestinal mu receptor and can slow down motility, providing an anti-diarrheal effect. Wheat gluten has also been found to have hepatoprotective effects in rats. Recent studies suggest that gluten may also have ghrelin-like effects in rats, which could promote growth and provide other health benefits. This review article focuses on the potential pharmaceutical applications of wheat seed compounds, including the use of whole wheat in poultry feed and the development of pharmaceuticals using in-vitro hydrolyzed gluten protein. This study provides a comprehensive review of the pharmacological and nutritional properties of wheat gluten protein in poultry and other animals, with an emphasis on its potential as a source of anti-diarrheal, hepatoprotective, and growth-promoting agents.

Introduction

The use of whole grains in poultry diets has become increasingly popular in many countries due to lower feed costs and potential improvements in feed conversion efficiency. Substituting whole wheat (Triticum aestivum) for ground wheat has been shown to enhance broiler productivity in some studies [1,2]. Whole wheat consists of three main components: the bran layer, embryo, and endosperm, with the latter accounting for 85% of the grain and containing the gluten protein mixture. Wheat is a rich source of metabolizable energy and protein for broilers, with the potential to satisfy up to 70% of their energy and 35% of their protein requirements. However, the quality of wheat can vary and impact chicken productivity [3]. Gluten is a type of seed storage protein found in various cereal grains. Wheat gluten mainly comprises glutenin and gliadins, which can be further divided into high and low molecular weight glutenin and α/β, γ and Ω gliadins [4]. In some individuals, gluten can cause inflammatory, immunological, and autoimmune responses, resulting in gluten-related disorders such as celiac disease [5-8], affecting 1-2% of the population, and non-celiac gluten sensitivity, affecting 0.5-13% of the population, as well as dermatitis herpetiformis, gluten ataxia, and other neurological problems [9]. Physiologically active peptides resembling endogenous molecules like

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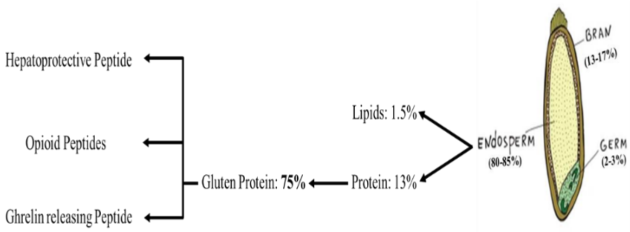

Figure 1. Wheat gluten protein undergoes enzymatic breakdown to produce hepatoprotective, opioid, and ghrelin-releasing peptides.

hormones and neuropeptides have been identified from wheat gluten enzymatic digests. For instance, the pepsin-thermolysin digest of gluten produced opioid peptides called gluten exorphins [10], while a hepatoprotective peptide called pyro glutamyl leucine was discovered in gluten hydrolysate [11]. Recently, a gluten-ghrelin peptide has been identified, with the gluten hydrolysate containing wheat-ghretropin A peptide that enhances the in vitro and in vivo synthesis and secretion of ghrelin hormone in mice [12].

The gastrointestinal tract of birds contains opioid receptors, including mu receptors that are present in both central and peripheral nervous systems as well as the gastrointestinal tract [13]. Opioid receptors interact with Gi/Go proteins and, to a lesser extent, with Gs proteins [14]. Endogenous opioid peptides act through neuromodulatory, hormonal, and paracrine mechanisms, producing analgesia and other physiological effects. These peptides include met-enkephalin, leu-enkephalin, β-endorphin, and dynorphin, as well as possibly endomorphin 1 and 2, which are ligands of opioid receptors [15]. The interaction between opioid receptors and Gi/Go proteins leads to three functional outcomes, including decreased intracellular cAMP levels, increased potassium leakage, and decreased calcium conductance, resulting in decreased smooth muscle contraction [14]. In this review, we focus on the potential pharmaceutical benefits of wheat gluten protein, which has been shown to have hepatoprotective effects and to trigger the release of growth hormones and motilin in chickens. In addition, gluten protein can be hydrolyzed by digestive enzymes to generate bioactive peptides with anti-diarrheal and laxative effects. This review aims to summarize the current state of knowledge on these pharmaceutical benefits of wheat gluten protein and to discuss their potential applications in both poultry feed and pharmaceutical formulations.

Wheat as Exorphin

Wheat is a rich source of protein, with gluten protein making up 80-85% of the total protein content. During gluten digestion, opioid peptides called gluten exorphins (GEs) are produced. High molecular weight glutenins release gluten exorphins A4, A5, B4, B5, and C5, while wheat-gliadin releases gliadorphin-7 [16]. Stuknyte et al. have demonstrated that GEs A5 and C5 can cross the human intestinal mucosa via the paracellular pathway [17], suggesting the potential for opioid-like action associated with the consumption of wheat-derived foods. This work also highlights the possibility of complete peptide penetration by food derived GEs [18]. An in-silico method has been proposed for predicting opioid peptides derived from gluten [19].

Although the digestion of gliadin, a polymer of wheat proteins, breaks down gluten epitopes into amino acids, it does not completely break down gluten, resulting in a higher concentration of gluten exorphins. Gluten exorphins have been found to make cross linkages with tissue transglutaminase and form tTG antigen, leading to antibody formation and the development of celiac disease [20]. In birds, gluten exorphins act on the mu receptor in the gastrointestinal tract [21], slowing down motility, which can be used as an anti-diarrheal agent. However, this also creates an opportunity for opportunistic pathogens to proliferate in the cecum [22]. Celiac disease patients release gluten exorphins as part of their allergic immunological reaction. Because celiac disease weakens the intestinal walls, certain gluten exorphins can cross the gut lining and enter the circulation, potentially affecting the central nervous system [23].

Overall, gluten exorphins have potential effects on gastrointestinal motility and hormone secretion and may play a role in the development of celiac disease. The study of opioid peptides derived from gluten is a growing field with the potential for clinical applications.

Wheat as Hepatoprotective

Wheat gluten hydrolysate contains a hepatoprotective peptide, pyroGlu-Leu, which has been shown to reduce serum ALT and AST levels in rats with d-galactosamine-induced acute hepatitis [11]. PyroGlu-Leu is found in certain food protein hydrolysates and traditional Japanese fermented foods and has been shown to reduce dysbiosis in mice with experimental colitis [24]. Wheat has also been shown to protect the liver against ethanol-induced harm [25], and wheat extracts have been proposed as potential nutraceuticals for treating and preventing liver damage and oxidative stress [26].

Studies have suggested that wheat could be an effective treatment option for reducing liver damage caused by oxidative stress, inflammation, apoptosis, and liver cells [27]. PyroGlu-Leu has been shown to block the activation of iNOS gene expression at transcriptional and post-transcriptional phases, preventing NO generation and potentially offering therapeutic potential for liver damage [28]. iNOS-generated NO is known to contribute to liver damage, and the suppression of iNOS expression and NO generation are seen as signs of liver protection in hepatocyte models [29].

Although no data has been published which enshrines the hepatoprotective role of wheat hydrolysate in poultry, the potential therapeutic uses of pyroGlu-Leu in various hepatic diseases in chickens is promising. Further research is needed to explore this potential, and to assess the effectiveness of wheat hydrolysate in poultry health.

Wheat as ghrelin-producing peptide in Chicken

Ghrelin-immunopositive cells have been found in the proventriculus mucosa (oxyntic mucosal cells) in birds, which corresponds to the first glandular area of the stomach where digestive enzymes combine with food before the gizzard [30]. Expression of the preproghrelin gene has also been observed in various organs including the brain, lungs, heart, gizzard, gallbladder, extramural glands, kidney, and small and large intestine in chickens [31,32]. Recently, a study was conducted on ghrelin-producing cell lines derived from mice to evaluate the effects of novel ghrelin-releasing peptides derived from wheat protein [12]. The in vivo study supported the wheat-ghretropin effect in mice. Consumption of whole grains, as opposed to processed wheat, has been shown to decrease hunger but did not appear to have a significant impact on acute energy balance. Interestingly, postprandial responses of PYY and ghrelin hormones were altered by wheat fiber ingestion [33]. Changes in PYY hormone concentrations were correlated with changes in appetite evaluations [34]. Furthermore, ghrelin and leptin, as well as brain-derived neurotrophic factor (BDNF), may improve the quality of life of coeliac disease (CD) patients on a gluten-free diet (GFD) [35]. Digestive hydrolysates of different plants, including wheat, have been shown to regulate appetite [36].

Ghrelin hormone and its physiological functions

In mammals, ghrelin is a 28 amino acid protein while in chickens, it consists of 26 amino acids [37, 38]. Ghrelin was first reported in rats in 1999 and then in humans in 2002 [39, 40]. Avian ghrelin was discovered in 2002 following the isolation, purification, cloning of a cDNA, and primary evaluation of biological activity from the proventriculus, which is the glandular part of the avian stomach [31]. Subsequently, preproghrelin genes and predicted amino acid sequences for six different avian species have been identified [41-43]. The acylation (n-octanoyl) of the Ser3 takes place by an enzyme ghrelin-O-acyltransferase (GOAT) [38, 44-46], and the 116 amino acid sequence of preproghrelin is cut by prohormone convertase (PC1/3 and PC 2) [37].

No sequence variations have been observed in the preproghrelin of the red junglefowl, which is the ancestral species for current commercial chicken breeds [32]. The physiological actions of ghrelin, a gut-brain peptide, include increased gastrointestinal motility, growth hormone secretion, and increased appetite [39, 47, 48, 49, 50, 51]. We can predict that feeding on a wheat diet can increase the expression of the preproghrelin hormone, GOAT, PC1/3, plasma growth hormone level, feed intake, and increased motilin and obestatin levels in chicken [52]. Therefore, wheat-ghretropin can be used to increase growth, feed intake, and weight gain in chickens. Recent evidence points to increased cortisol secretion brought on by the ghrelin hormone as the cause of improved body weight and feed conversion ratio (FCR) [41, 53].

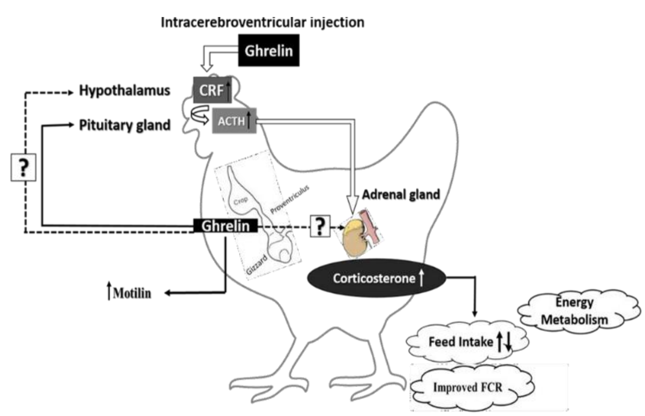

Figure 2. Through the hypothalamus-pituitary-renal axis, ghrelin raises cortisol levels, and motilin levels rise because of their co-secretion. It is debatable whether ghrelin has an immediate impact on the adrenal gland and brain. Modified from "Ghrelin in birds: its structure, distribution, and function," by Kaiya H, Darras VM, Kangawa K., 2007, The Journal of Poultry Science; [44:1-18].

Intestinal enteroendocrine cells play a critical role in the regulation of appetite and satiety in poultry by producing and secreting hormones such as GLP-1, PYY, CCK, leptin, and ghrelin in response to metabolite sensing via specific receptors [54-57]. In poultry, ghrelin-producing cells can be classified into two types: closed-type cells and open-type cells, with distinct morphologies in the stomach and intestine [30]. The gluten-derived ghretropin peptide is thought to act on open-type cells in the intestine, which can sense the contents of the lumen12. Activation of the GHS-R receptor by acyl-ghrelin stimulates cell growth hormone secretion in poultry [32,45,50,58,59]. Ghrelin also stimulates neuronal activity in the hypothalamus via binding to glutathione reductase 1 (GSHR1) [60,61], as it can cross the blood brain barrier [62]. Some studies suggest that wheat may have therapeutic potential as a growth promoter in poultry [49,50]. Wheat can also be used as a nutraceutical agent to increase gastrointestinal motility, as motilin and ghrelin are co-localized on secretory granules, indicating their potential co-secretion [63]. The effect of ghrelin on feed and water intake in poultry is still unclear, as studies have shown both orexigenic and anorexigenic effects [64-68]. Some studies suggest that an increased ghrelin level in poultry may have a negative impact on feed and water intake [69-71]. However, capromorelin, a ghrelin receptor agonist, has been shown to increase feed and water intake, as well as growth hormone and insulin, in broiler chickens [72,73]. Overall, the regulation of appetite and satiety in poultry is complex and involves a variety of hormones and signaling pathways. Understanding the role of ghrelin and other hormones in this process is crucial for improving poultry health and productivity.

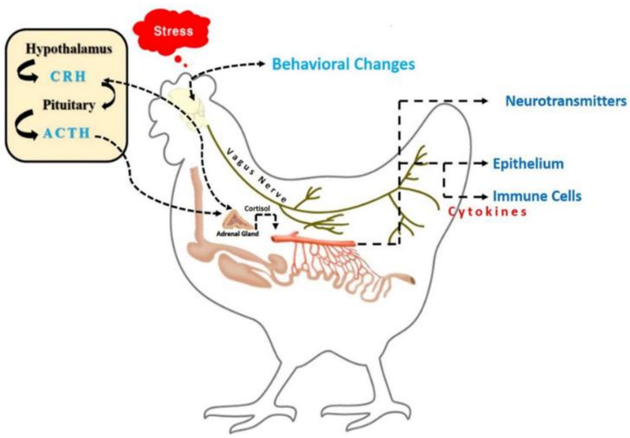

Figure 3. The hypothalamus-pituitary-renal axis in poultry birds.

Metabolic hormones, such as ghrelin hormone, secreted by endocrine glands, strictly regulate glucose [74] and fat metabolism [75-77]. The signaling of ghrelin is essential in several metabolic processes [78] beyond hunger and fat storage, including glucose and energy balance, cardioprotection, muscle atrophy, and bone metabolism. Ghrelin and its receptor, GHS-R, are incredibly desirable therapeutic research targets due to their many functions. Treatment options for obesity and insulin resistance may include ghrelin mimetics and GHS-R antagonists [46,65,79-81]. It is hypothesized that ghrelin-GHSR-1a interactions and ghrelin released from the gastrointestinal tract (GIT) modify eating behavior in response to changes in age, metabolism, and the surrounding environment [82,83]. Some data shows that ghrelin's positive effects on the cardiovascular system [84-86] are mediated by direct actions on cardiovascular cells and control of autonomic nervous system activity; however, the precise mechanisms behind these benefits remain unclear. Ghrelin is a new medicinal drug with great potential for treating heart illness87. Ghrelin is a promising target for the development of treatments for prevention and therapy when autophagy is disrupted, such as in metabolic, cardiac, or neurological disorders [88,89].

To put it another way, IL-27 increases STAT3 and mTOR activity. The expression of the hunger hormone ghrelin is suppressed, and mTOR is inhibited due to IL-27's facilitation of STAT3's interaction with mTOR. Knowing this mechanism of action might pave the way for novel therapeutic methods or result in a more prudent use of already available medications for the treatment of obesity [90,91]. During times of severe nutritional and mental stress, stimulating the ghrelin system may be an effective pharmaceutical strategy for promoting food intake and protecting against hypoglycemia, body weight loss, depression/anxiety, and mortality [92]. Similarly, ghrelin's ability to upregulate miR-21 may lessen the severity of acute kidney injury caused by ischemia/reperfusion [93,94]. Ghrelin itself is now being tested in phase 3 clinical studies [95].

Conclusion

The hydrolysis of the gluten protein found in wheat seeds by various digestive enzymes leads to the production of gluten-exorphin, which affects the mu receptor of the gastrointestinal tract, slowing down its motility and potentially providing a natural anti-diarrheal solution. Additionally, wheat gluten has been shown to have hepatoprotective properties in rats, suggesting its potential use in treating liver damage in different diseases. The discovery of the wheat gluten protein as a ghretropin opens the possibility of developing nutraceutical growth boosters and laxatives. Ghrelin hormone, which is stimulated by the acyl-ghrelin receptor, has been suggested as a potential therapeutic agent for the treatment of metabolic diseases and cardioprotection. These findings highlight the therapeutic potential of wheat beyond the treatment of avian diseases, and in vitro hydrolysis of gluten protein may be employed to produce drugs for treating various medical conditions.

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Conflict of interest

The author declares no conflicts of interest

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