

POTENTIAL OF PLANT-DERIVED GHRELIN PROTEIN AS AN ENERGY BALANCE REGULATING MATERIAL FOR FEED EFFICIENCY

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DOI: [10.5281/zenodo.13622758](https://doi.org/10.5281/zenodo.13622758)

Abstract

The aim of this research is to identify plant-derived ghrelin proteins that could help regulate energy balance and improve the efficiency of broiler chicken feed. The study demonstrated that ghrelin protein is present in natural sources such as kale leaves (*Ipomoea aquaticum*), water lettuce leaves (*Nasturtium officinale*), and cassava leaves (*Manihot esculenta*). Following this, the amino acid sequence of the ghrelin protein was synthesized at Science Genetics. The synthetic ghrelin protein was then tested on various experimental animals. This development of synthetic ghrelin protein offers a means to enhance energy balance regulation, thereby increasing feed efficiency.

Keywords: Ghrelin, Synthetic Ghrelin, Energy Balance, Feed Efficiency.

INTRODUCTION

Ghrelin is a neuroendocrine signal that plays a crucial role in regulating various biological processes, highlighting the significant interaction between the stomach and the brain (Inui et al., 2004). This peptide hormone, produced primarily in the stomach, is essential for controlling food intake. Plasma levels of ghrelin increase gradually before meals and rapidly decrease afterward. Intravenous infusion of ghrelin has been shown to enhance food consumption and stimulate appetite, suggesting that ghrelin is integral in inducing hunger and initiating eating behavior. Additionally, ghrelin is involved in weight regulation, as fasting plasma ghrelin concentrations have an inverse relationship with body mass index (Bloom, 2005). Disruptions in stomach-originating signals can significantly affect energy balance, growth, and both gastrointestinal and neuroendocrine functions (Inui et al., 2004).

Ghrelin and leptin, while complementary, operate in opposition; their signaling reflects both acute and chronic changes in energy balance. These hormonal effects are mediated through neuropeptides such as neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the hypothalamus (Inui et al., 2004). However, gastric distension and chemical sensitization alone are insufficient to trigger the ghrelin response. It is possible that post-gastric processes, including insulin secretion—either directly or via incretin stimulation, glucagon-like peptide-1, and gastric inhibitory peptide—are involved. Most studies indicate that insulin can reduce ghrelin levels independently of glucose, though the exact mechanism by which insulin inhibits ghrelin remains unclear. This inhibition may be mediated through direct effects on ghrelin-secreting cells or through humoral or central pathways (Bloom, 2005).

Previous research in broiler chickens has identified the molecular weight of ghrelin protein as 44 kDa and NPY as 11 kDa (Nove et al., 2012). The amino acid composition of ghrelin includes methionine, phenylalanine, leucine, arginine, valine, and isoleucine, whereas neuropeptide Y is composed of threonine, methionine, arginine, leucine, tryptophan, valine, serine, leucine, alanine, glutamate, alanine, tyrosine, proline, and

serine (Nove et al., 2013). The ghrelin receptor protein has a molecular weight of 44 kDa, with an amino acid sequence that includes mregssenrt ggesplrlfp apvltgitva cvllfvvgvl gnltmvlvs rfrdmrttn mflrvillgi llsliltet alagssflsp tykniqqkd trkptarlhr rgesfwdtd etegeddnns vdikfnvpfe igvkiterey qeygqalekm lqdilaenae etrts (Nove et al., 2014).

Understanding the interplay between ghrelin, its receptors, and neuropeptides like NPY, as well as ghrelin's role in regulating gastrointestinal function, energy balance, and growth, remains incomplete. Therefore, further research is warranted to develop synthetic ghrelin as a potential tool for managing energy balance and promoting livestock growth.

METHODS

This study utilized samples of kale leaves (*Ipomoea aquaticum*), watercress leaves (*Nasturtium officinale*), and cassava leaves (*Manihot esculenta*). The research process involved several key steps: (1) Isolation of ghrelin protein from the selected plant samples—kale, watercress, and cassava leaves, (2) Identification of ghrelin protein in these plants using the Immunohistochemistry method, and (3) Synthesis of ghrelin protein within the framework of Genetic Science.

RESULT & DISCUSSION

The immunohistochemical analysis of kale leaves (*Ipomoea aquaticum*) revealed the presence of ghrelin, indicated by the brown staining visible in Figure 1.

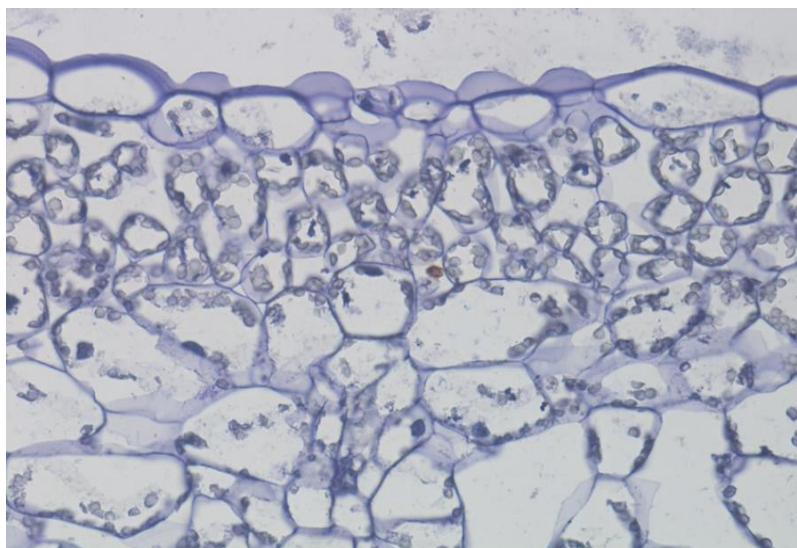


Figure 1: Ghrelin on kale leaves (*Ipomoea aquaticum*) with 400X Magnification

The immunohistochemical assessment of watercress leaves (*Nasturtium officinale*) confirmed the presence of ghrelin, as evidenced by the brown coloration depicted in Figure 2.

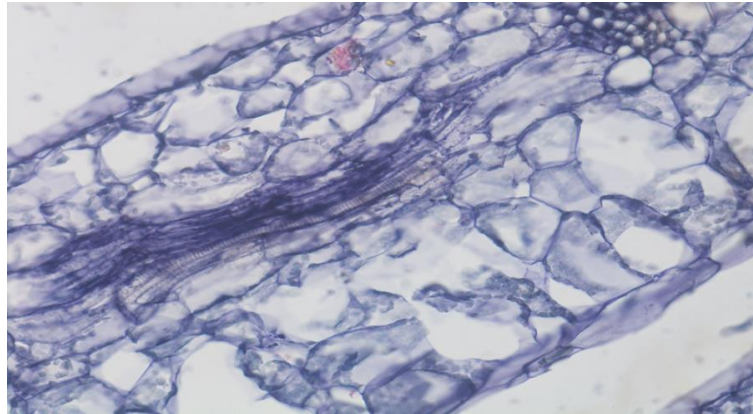


Figure 2: Ghrelin on water slada leaves (*Nasturtium officinale*) with 400X magnification

The immunohistochemical analysis of cassava leaves (*Manihot esculenta*) revealed the presence of ghrelin, as indicated by the brown staining observed in Figure 3.

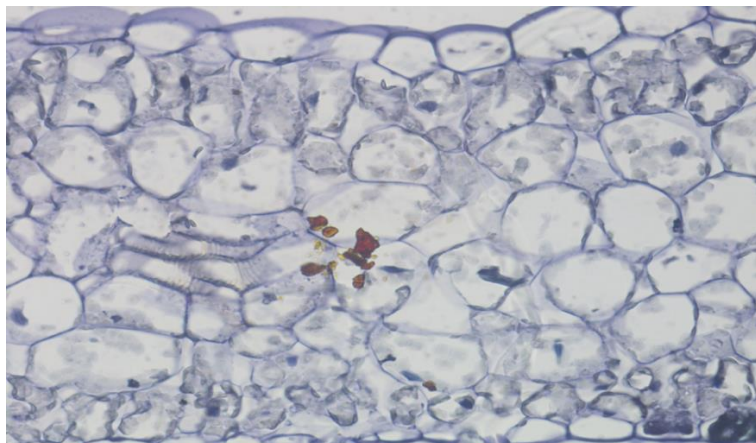


Figure 3: Ghrelin on cassava leaves (*Manihot esculenta*) with 400X magnification

The study findings revealed that immunohistochemical analysis of kale leaves (*Ipomoea aquaticum*), water lettuce leaves (*Nasturtium officinale*), and cassava leaves (*Manihot esculenta*) confirmed the presence of ghrelin. Further examination of the ghrelin protein composition using MALDI-TOF mass spectrometry identified amino acids including methionine, phenylalanine, leucine, arginine, valine, and isoleucine. Based on this amino acid profile, synthetic ghrelin protein was produced at PT. Genetics Science Indonesia in Jakarta. The synthetic ghrelin protein achieved a crude purity of Peptide 1 according to HPLC analysis, with a sequence of mflrvil.

CONCLUSION

The study concludes that synthetic ghrelin protein can be developed using the amino acid sequences found in natural sources like kale leaves (*Ipomoea aquaticum*), water lettuce leaves (*Nasturtium officinale*), and cassava leaves (*Manihot esculenta*). This synthetic protein can then be utilized in feed formulations to help regulate energy balance. Improved energy balance from this feed can enhance feed efficiency, ultimately reducing the overall feed requirements.

References

- 1) Ahmed S and Harvey S. Ghrelin : a hypothalamic GH releasing factor in domestic fowl (*Gallus domesticus*). 2002. *Journal of Endocrinology*, 172: 117-125.
- 2) Baudet ML and Harvey S. 2003. Ghrelin-induced GH secretion in domestic fowl in vivo and in vitro, *J. Endocrinol.* 179 97–105.
- 3) Baynes JW and Dominickzak MH, 2005. *Medical Biochemistry*. 2nd. Edition. Mosby International Limited.
- 4) Blom WAM, Stafleu A, Graf CD, Kok FJ, Schaafsma G, and Hendriks FJ, 2005. Ghrelin response to carbohydrate-enriched breakfast is related to insulin. *Am J Clin Nutr* 81:367-375
- 5) Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M.S., Suganuma, T., Matsukura, S., Kangawa, K. and Nakazato, M., 2000. Ghrelin, a novel growth hormone-releasing acylated peptide is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology*, 141, 4255–4261
- 6) Ganong WF, 2006. *Review of medical physiology*. 20Th.Ed, USA: Appleton & Lange, pp 365-375
- 7) Gualillo O, Lago F, Reino JG, Casanueva FF, Dieguez C, 2003. Ghrelin, a widespread hormone : insights into molecular and cellular regulation of its expression and mechanism of action. *FEBS letters* 552:105-109
- 8) Inui A, Asakawa A, Bowers CY, Mantovani G, Laviano A, Meguid MM and Fujimiya M, 2004. Ghrelin, appetite, and gastric motility : the emerging role of the stomach as an endocrine organ. *The FASEB Journal* 18:439-456
- 9) Kaiya Hiroyuki, Saito Ei-Suke, Tachibana Tetsuya, Furuse Mitsuhiro, Kangawa Kenji. 2007. Changes in ghrelin levels of plasma and proventriculus and ghrelin mRNA of proventriculus in fasted and refed layer chicks. *Domestic Animal Endocrinology*. Elsevier. 32 (2007) 247-259.
- 10) Lu, S., Guan, J.L., Wang, Q.P., Uchara, R., Yamada, S., Goto, N., Date, Y., Nakazato, M., Kojima, M., Kangawa, K. and Shioda, S., 2002. Immunocytochemical observation of ghrelin-containing neurons in the rat arcuate nucleus. *Neuroscience Letters*, 321, 157–160
- 11) Murray RK, Granner DK, Mayes PA and Rodwell VW, 2003. *Harper's Biochemistry*. 27th. Edition. Prentice-Hall International, Inc
- 12) Nove H, Sarmanu dan Anwar M. 2003. Peran fisiologis sekresi leptin sebagai dasar pencegahan obesitas. *Majalah Ilmu Faal Indonesia* 5(1): 18-23, Surabaya.
- 13) Rosicka M, Krsek M, Jarkovska Z, Marek J, Screiber V, 2002. Ghrelin a new endogenous growth hormone secretagogue. *Physiol Res* 52:61-66
- 14) Zhou X, De Schepper J, Hooghe-Peters EI, 1998. Pituitary growth hormone release and gene expression in cafeteria-diet induced obese rats. *J Endocrine* 159:165-172.