

The Potential of Protein Ghrelin as Material for Energy Balance Setting for Feed Efficiency in Broiler Chicken

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Keywords: Ghrelin, energy, feed efficiency, broiler.

Abstract: The purpose of this study was to determine the molecular weight of the protein ghrelin as a basis to determine amino acid composition of protein ghrelin and subsequently to make synthetic ghrelin protein whose function is to control energy balance in broilers. Samples were isolated from the digestive tract and brain tissue of the broilers and then examined by SDS Page and Western blot. Based on the results, it can be concluded that the protein ghrelin had the molecular weight of 44 kDa and neuropeptide Y of 11 kDa.

1 INTRODUCTION

Ghrelin and leptin are complementary but work antagonistically. Their signals reflect acute or chronic energy balance changes and their effects are mediated by hypothalamic neuropeptides such as neuropeptide Y (NPY) and agouti related peptide (AgRP) (Inui *et al.*, 2004).

Gastric distension and gastric hyposensitisation are insufficient to stimulate ghrelin response. This possibility is a postgastric process involving insulin secretion, either directly or indirectly, through the incretin stimulation of the hormone glucagon such as peptide 1 and gastric inhibitory peptide. Most studies suggest that insulin will lower ghrelin concentrations that are independent of glucose. The insulin mechanism inhibiting the effect of ghrelin concentration is not fully known. These insulin effects may be mediated by the direct effects of ghrelin secreting cells or the effects of humoral mechanisms or central mechanisms (Bloom, 2005).

Association between ghrelin, stomach, hypothalamus and the implications of ghrelin on gastrointestinal function control, energy balance, and current growth has not been entirely clear. Therefore, a study is needed to find ghrelin amino acid from broiler chickens so that we can create synthetic ghrelin protein that can be used to regulate the energy balance and growth of the livestock.

2 MATERIALS AND METHODS

This study used samples of male Lohman (MB 202 P) broiler chickens which were maintained from the age of 1 day up to 21 days in letter cages, as many as 25 chicken.

Day old chicken were placed in a letter cage for 21 days with food and drink ad libitum. After reaching the age of 21 days, the chickens were sacrificed to be sampled in the form of gastrointestinal and brain tissue for the following tests (1) Isolation of ghrelin and neuropeptide Y (NPY) proteins from the gastrointestinal tract and brains, (2) Identification of ghrelin and neuropeptide proteins (NPY) of the gastrointestinal tract and brains of broilers using SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoreses) method, (3) Analysis of molecular weight of ghrelin protein and Neuropeptide Y by blotting method *i.e.* Western Blot technique using proteins described electrophoresis of polyacrylamide gel, (4) Examination of amino acid structure of ghrelin and neuropeptide Y by MALDI-TOP method.

3 RESULTS AND DISCUSSION

3.1 SDS Page for Ghrelin and Neuropeptide Y Proteins

Results SDS-PAGE ghrelin and neuropeptide Y (NPY) proteins in the broiler's gastrointestinal tract and brain showed the presence of ghrelin and neuropeptide Y proteins, as shown in Figure 1.

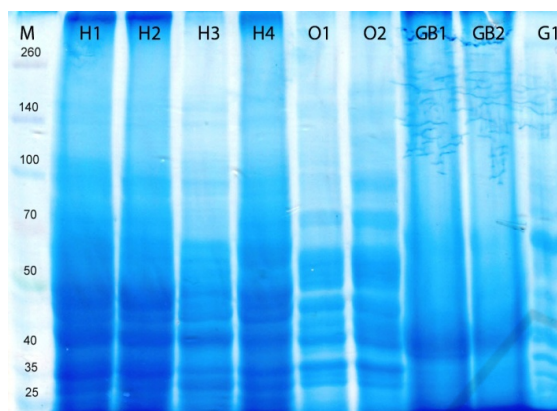


Figure 1 : SDS page of ghrelin and NPY proteins from the digestive tract and brain.

The results of SDS-PAGE on gastrointestinal tract and brains of broilers revealed ghrelin protein and neuropeptide Y. SDS-PAGE results showed that there were several visible bands. One protein band was found each in the markers between 260 and 140 kDa, 140 and 100 kDa, 100 kDa and 50 kDa, 50 kDa and 40 kDa, and between 25 kDa and 10 kDa.

Protein bands formed between 50 kDa and 40 kDa markers and between 25 kDa and 10 kDa markers were suspected as ghrelin and neuropeptide Y proteins. The protein bands formed on the gastrointestinal tract and the broiler's brain were very clear, indicating that the tissue appears to induce the strongest antibody antigen reaction.

SDS-PAGE protein of gastrointestinal tract and brain of broiler chicken showed protein band between 50 kDa and 40 kDa markers, which were protein with molecular weights of 44 kDa and 11 kDa, but it has not been certain whether it was ghrelin protein and neuropeptide Y as several other protein bands were also formed between these markers. To prove that the formation of protein bands with molecular weight of 44 kDa and 11 kDa was ghrelin and neuropeptide Y protein, it was necessary to perform further examination.

3.2 Western Blot for Protein Ghrelin from Broiler's Digestive Tract

The Western blot of ghrelin protein in gastrointestinal tissue showed the presence of a 44 kDa molecular weight of ghrelin protein, as displayed in Fig. 2.

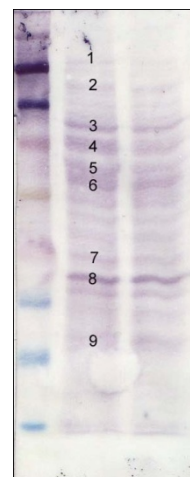


Figure 2 : Western blot for ghrelin protein from broilers' digestive tract

The result of ghrelin protein molecular weight calculation showed that the molecular weight of ghrelin protein was 44 kDa. The formation of protein bands between 50 kDa and 40 kDa markers, after being calculated, apparently showed a molecule with molecular weight of 44 kDa. This suggested that the protein produced by SDS-PAGE tested with Western blot was a ghrelin protein of growing-phase broiler with a molecular weight of 44 kDa. The formation of the protein band with 44 kDa molecular weight was definite because there was a binding between protein ghrelin resulted from SDS-PAGE and rabbit pAb ghrelin (data Sheet Rev. 102203F).

3.3 Western Blot for NPY Protein from Broiler's Brain

The result of Western blot protein of neuropeptide Y on brain tissue showed the existence of Y neuropeptide protein with 11 kDa molecular weight, as shown in Figure 3.

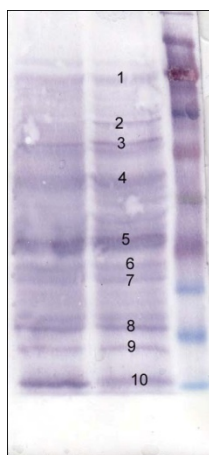


Figure 3 : Western blot for neuro peptide Y protein from broiler's brain.

The results of the molecular weight calculation of neuro peptide Y protein showed that it had a molecular weight of 11 kDa. The formation of protein bands between 25 kDa and 10 kDa markers, after being calculated, was found to be 11 kDa. This suggested that the SDS-PAGE protein tested with Western blot was a neuro peptide Y protein of growing phase broiler chicken with a molecular weight of 11 kDa. The formation of a protein band of 11 kDa molecular weight was definite because there was a binding between the protein ghrelin resulting from SDS-PAGE with neuro peptide Y antibody (data Sheet ab30914).

Ghrelin is a gastric peptide that plays an important role in the regulation of food into the body (food intake). Before eating the plasma, ghrelin concentration rises gradually and immediately goes down after eating. The addition of ghrelin intravenously increases food intake and appetite, which proves that ghrelin plays a role in hunger and the beginning of a meal initiation. Ghrelin is also involved in weight control because the body mass index is negatively controlled by plasma ghrelin concentrations at the time of fasting. Abnormalities of the signal from the stomach signal is related to energy balance disorders and growth, and this is related to gastrointestinal and neuroendocrine function.

Ghrelin and leptin are complementary but work antagonistically, their signals reflect acute or chronic energy balance changes and their effects are mediated by hypothalamic neuro peptides such as neuro peptide Y (NPY) and agouti related peptide (AgRP).

4 CONCLUSIONS

The molecular weight of ghrelin protein was 44 kDa with amino acid structure consisted of methionine, phenylalanine, leucine, arginine, valine, isoleucine, leucine and neuro peptide Y molecular weight was 11 kDa with threonine, methionine, arginine, leucine, tryptophan, valine, serine, valine, leucine, threonine, leucine, alanine, glutamate, alanine, tyrosine, proline, and serine. By identifying the molecular weight and the arrangement of amino acids, we can create synthetic ghrelin protein to regulate the energy balance of broiler chickens.

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