



The composition of the amino acids protein ghrelin and neuropeptide y as the basis for regulating energy balance in broilers

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Abstract

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

Keywords: ghrelin, energy, feed efficiency, broiler

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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 hyposensitization are insufficient to stimulate ghrelin response. This possibility is a postgastric process involving insulin secretion, either directly or indirectly, through the incretin stimulation of glucagon-like peptide 1 and gastric inhibitory peptide hormones. Most studies suggest that insulin will lower ghrelin concentrations 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 had not been entirely clear. Therefore, a study is required to find ghrelin amino acid from broiler chickens so that we can create synthetic ghrelin protein that can be utilized to regulate the energy balance and growth of the livestock.

MATERIALS AND METHODS

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

The day old chickens were placed in a letter cage for 21 days with feed and drink *ad libitum*. After reaching the age of 21 days, the chickens were sacrificed to extract their gastrointestinal and brain tissue as the samples for the following tests; (1) the isolation of ghrelin and neuropeptide Y (NPY) proteins from the gastrointestinal tract and brains, (2) The 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) the analysis of molecular weight of ghrelin protein and Neuropeptide Y by using Western Blot test technique using proteins described electrophoresis of polyacrylamide gel, and last (4) the examination of amino acid structure of ghrelin and neuropeptide Y by using MALDI-TOP method.

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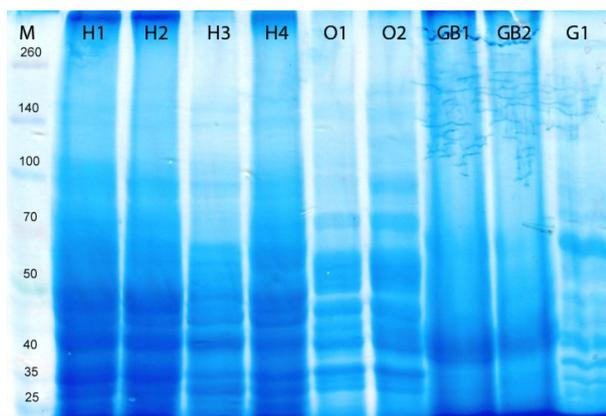


Fig. 1. SDS Page of ghrelin and NPY proteins from the digestive tract and brains

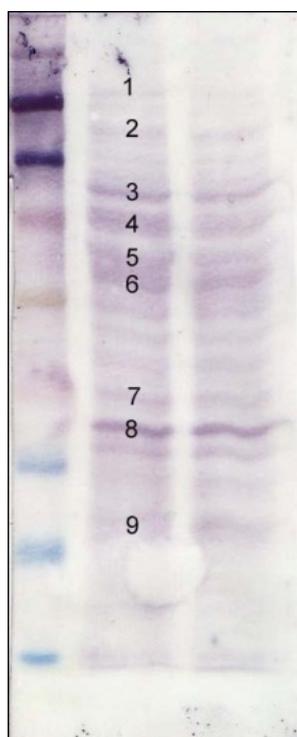


Fig. 2. Western blot test for ghrelin protein from broilers' digestive tract

RESULTS AND DISCUSSION

SDS Page for Ghrelin and Neuropeptide Y Proteins

The results of SDS-PAGE ghrelin and neuropeptide Y (NPY) proteins in the broiler's gastrointestinal tract and brains revealed the presence of ghrelin and neuropeptide Y proteins as seen in **Fig. 1**.

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.

The 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 brains were very clear, indicating that the tissue appears to induce the strongest antibody-antigen reaction.

The SDS-PAGE protein of the gastrointestinal tract and brains of the broiler chickens indicated a protein band between 50 kDa and 40 kDa marker, which was the protein with molecular weights of 44 kDa and 11 kDa. However, it was not confirmed 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 a molecular weight of 44 kDa and 11 kDa were ghrelin and neuropeptide Y proteins, it is necessary to perform further examination.

Western Blot Test for Protein Ghrelin from Broilers' Digestive Tract

The Western blot test of ghrelin protein in gastrointestinal tissue suggested the presence of ghrelin protein with the molecular weight of 44 kDa, as seen in **Fig. 2**.

The result of ghrelin protein molecular weight calculation suggested that the molecular weight of ghrelin protein amounted to 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 finding suggested that the protein produced by SDS-PAGE tested with the Western blot test was a ghrelin protein of growing-phase broilers with the molecular weight of 44 kDa. The formation of the protein band with 44 kDa molecular weight was definite because there was binding between the ghrelin protein resulted from SDS-PAGE and rabbit pAb ghrelin (data Sheet Rev. 102203F).

Western Blot Test for NPY Protein from Broilers' Brains

The result of the Western blot test of neuropeptide Y protein on the broilers' brain tissue revealed the existence of Y neuropeptide protein with 11 kDa molecular weight, as seen in **Fig. 3**.

The results of the molecular weight calculation of neuropeptide Y protein suggested 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 finding suggested that the SDS-PAGE protein tested with the Western blot test was a neuropeptide Y protein of the growing phase of the broiler chickens with a molecular weight of 11 kDa. The formation of a protein band of 11 kDa molecular weight was definite because there was binding between the ghrelin protein resulting from SDS-PAGE with neuropeptide Y antibody (data Sheet ab30914).

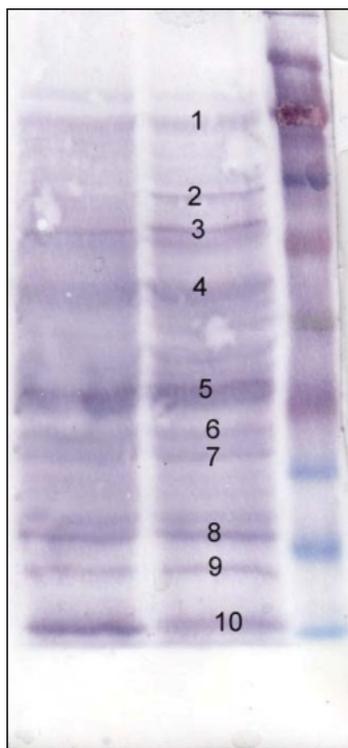


Fig. 3. Western blot test from Neuropeptide Y protein from broilers brains

The Amino Acid of the Ghrelin and Neuropeptide Y Protein

The examination of ghrelin amino acid protein was performed in Proteomic International Australia using MALDI-TOF method. The results showed that the sequence of amino acid ghrelin protein composed of mflrvil or methionine, phenylalanine, leucine, arginine, valine, isoleucine, and leucine.

The sequence of neuropeptide Y protein was composed of tmlwsvltlaeayps or threonine, methionine, arginine, leucine, tryptophan, valine, serine, valine, leucine, threonine, leucine, alanine, glutamate, alanine, tyrosine, proline, and serine.

Ghrelin is a gastric peptide that plays a crucial 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 are 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 neuropeptides such as neuropeptide Y (NPY) and agouti related peptide (AgRP).

Eating is the basis of behavior that is highly essential for life. The lack of eating for a long time can cause death. Ghrelin is a gastrointestinal hormone that can improve eating behavior. This is based on the increased circulation of ghrelin before eating, which is then continued to initiate the desire to fulfill food intake (Cummings et al. 2001). Although the circulating levels of ghrelin are strongly regulated by nutritional status or obesity. However, the mutations in ghrelin and ghrelin receptors are not common in obese individuals (Wang et al. 2004).

Shosha et al. (2005) reported that adult quails injected with low-dose intra-peripheral ghrelin would increase their appetite but, if given at high doses, would inhibit the appetite. On the other hand, chicks administered with ghrelin directly to the brain (intra-cerebrovascular vascular) will experience appetite inhibition (Saito et al. 2002). Similarly, if ghrelin is injected intra-vascularly in chicks, it will not cause appetite (Kaiya et al. 2007).

CONCLUSIONS

The molecular weight of ghrelin protein was 44 kDa with amino acid structure consisted of methionine, phenylalanine, leucine, arginine, valine, isoleucine, leucine and neuropeptide 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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