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POTENTIAL OF GHRELIN PROTEIN ORIGINATED FROM PLANT AS ENERGY BALANCE SETTINGS FOR FEED EFFICIENCY

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ABSTRACT

The purpose of this study was to determine the molecular weight of ghrelin protein as a basis to determine its amino acid sequence and then to make the protein's synthetics with a function to regulate energy balance in broilers. Samples were isolated from gastrointestinal tract and brain tissue from the broilers and examined by SDS Page, Western blot and MALDI-TOP. Based on the results, it can be concluded that the molecular weight of the protein ghrelin is a 44kDa with the amino acid sequence mflrvil. By knowing the molecular weight and amino acid composition of the ghrelin protein, we can produce synthetic ghrelin protein to regulate energy balance of the broilers. Exploration of natural ingredients derived from salad leaves, spinach leave, and cassava leaves showed the presence of ghrelin protein.

Keywords: *ghrelin, broilers, energy*

INTRODUCTION

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

Gastric distention and gastric hyposensitization are insufficient to stimulate ghrelin response. Possibly, the postgastric process is involved in insulin secretion, either directly or indirectly, through incretin stimulation of the glucagon-like peptide 1 hormone and gastric inhibitory peptide. Most studies suggest that insulin will lower ghrelin concentrations independent of glucose. The mechanism of insulin in 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 or central mechanisms (Bloom, 2005).

The knowledge that shows the relationship between ghrelin, stomach, hypothalamus and the implications of ghrelin on the control of gastrointestinal function, energy balance and growth is still not entirely clear. Therefore, research needs to be done to find the amino acid of ghrelin protein and ghrelin's natural origin so that synthetic ghrelin protein can be made that can be used to regulate the energy balance and growth of livestock.

MATERIALS AND METHODS

This study used a sample of male Lohman strain (MB 202 P) broiler reared from the age of 1 day to 21 days in letter cages. A total of 25 day-old chickens were placed in letter cages until the age of 21 days with feed and water ad libitum. Upon reaching the age of 21 days, the chickens were sacrificed to take a sample of tissue from gastrointestinal tract and brain for the following examinations: (1) isolation of the protein ghrelin in tissues of gastrointestinal tract and brain of the broilers; (2) identification of the protein ghrelin in tissues of gastrointestinal tract and brain of the broilers using SDS-PAGE (sodium dudecyl sulphate polyacrylamide gel electrophoreses); (3) analysis of the molecular weight of ghrelin protein by blotting method, the Western blot technique, by using the protein elaborated electrophoretically from polyacrylamide gels; (4) examination of ghrelin's amino acid sequence by MALDI-TOP method and (5) examination of natural materials - the lettuce, spinach leaves and cassava leaves.

RESULTS AND DISCUSSION

SDS page of ghrelin protein

The result of ghrelin protein SDS-PAGE on broiler gastrointestinal tract and brain showed the presence of ghrelin protein. SDS-PAGE in the gastrointestinal tract and brains of broilers shows the presence of ghrelin protein. The SDS-PAGE results show several bands. Marker 260 with 140kDa shows one protein band, marker 140 with 100kDa one protein band, 100kDa marker with 50kDa one protein band, 50kDa marker with 40kDa one protein band, and 25kDa marker with 10kDa also one protein band.

The protein bands formed between the 50kDa marker and 40kDa are thought to be ghrelin proteins. The protein bands formed in broilers' gastrointestinal tract and brain are very distinctive. This suggests that, in the tissue, the antigen antibody reaction occurred most powerfully.

The result of SDS-PAGE protein of gastrointestinal tract and brain of broiler showing the existence of protein band between 50kDa marker with 40kDa is a protein with molecular weight 44kDa. Proteins with a molecular weight of 44kDa of SDS-PAGE results have not been able to show for certain whether it was ghrelin protein or not. This was because, among these markers, several other protein bands were also formed. To prove that the formation of protein band with molecular weight of 44kDa was ghrelin protein, it was necessary to examine with Western blot.

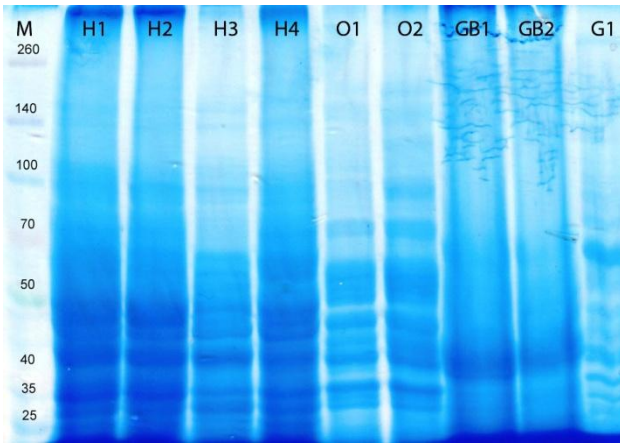

Figure 1. SDS-PAGE protein of the gastrointestinal tract and the brain of broiler chickens

Western blot of ghrelin protein from broilers' digestive tract

Western blot of protein ghrelin in gastrointestinal tissue showed the presence of ghrelin protein and ghrelin receptors with a molecular weight of 44kDa. Calculation of molecular weight of ghrelin protein showed that the molecular weight of ghrelin protein was 44kDa. After calculation, the protein band formed between the 50kDa marker with 40kDa had a molecular weight of 44kDa. This suggests that protein produced by SDS-PAGE tested with Western blot was a ghrelin protein in a growth-period broiler chicken with a molecular weight of 44kDa. The apparent formation of protein bands with a molecular weight of 44kDa was due to the occurrence of a binding between the ghrelin protein resulting from SDS-PAGE and rabbit pAb ghrelin (data Sheet Rev. 102203F).

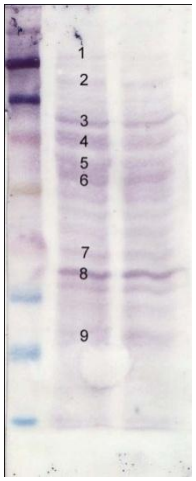

Figure 2. Western blot of ghrelin protein from broiler's digestive tract

Amino acid of ghrelin protein

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

Ghrelin is a gastric peptide that plays an important role in the regulation of food that enters into the body (food intake). Before eating, plasma ghrelin concentration rises gradually and immediately falls after eating. The addition of ghrelin intravenously increases the intake of food (food intake) and triggers appetite. This 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 signals originating from the stomach are related to abnormalities of energy balance and growth. This is related to gastrointestinal and neuroendocrine function.

Ghrelin and leptin are complementary, but act antagonistically. Their signals reflect acute or chronic energy balance changes and their effects are mediated by hypothalamic neuropeptides, such as neuropeptide Y (NPY) and augouti-related peptide (AgRP).

Eating is a fundamental behavior that is essential for life. Lack of eating for a long time may cause death. Ghrelin is a gastrointestinal hormone that can improve eating behavior. It is based on an increase in ghrelin circulation before meals, which is then continued to initiate a desire to meet food intake (Cummings et al., 2001). Although ghrelin circulation rates are strongly regulated by nutritional status or obesity, ghrelin gene and ghrelin receptor mutations are not common in obese individuals (Hinney et al., 2002; Korbonits et al., 2002; Wang et al., 2004).

Shosha et al. (2005) reported that adult quails injected intraperiferally with low dose ghrelin increased their appetite, but, if given at high doses, their appetite was inhibited. However, chicken given with ghrelin directly to the brain (intra-cerebrovascular) experienced appetite inhibition (Furuse et al., 2001; Saito et al., 2002). Similarly, intravascular injected ghrelin in chicken did not result in increased appetite (Kaiya et al., 2007).

Ghrelin from natural ingredients

The results of exploration of natural materials from kale leaves, lettuce leaves and cassava leaves immunohistochemically showed the presence of ghrelin protein. Proteins from these natural ingredients can then be used as ingredients to make synthetic ghrelin. This can be seen in Figure 3, Figure 4 and Figure 5.

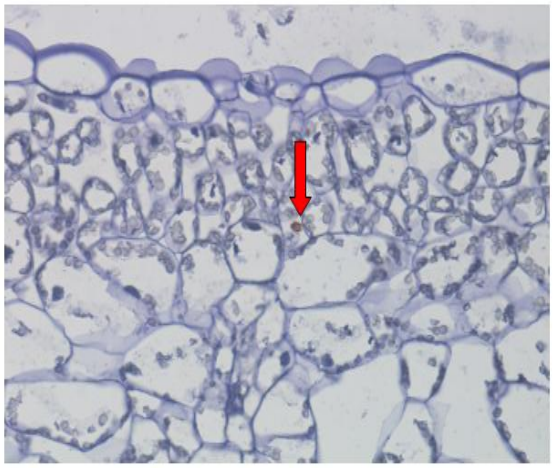

Figure 3. Ghrelin in kale leaves (Ipomoea aquaticum) in 400X magnification

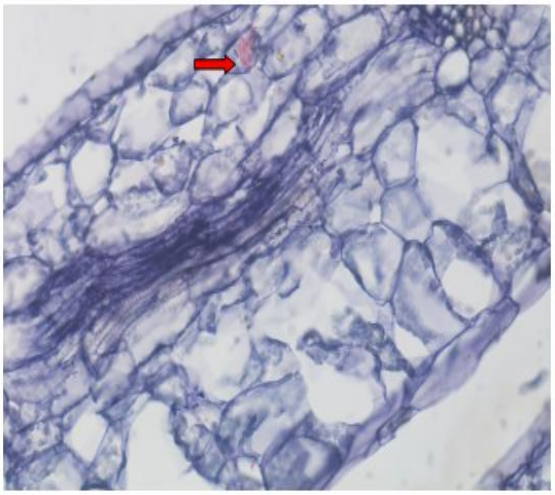

Figure 4. Ghrelin in lettuce leaves (Nasturtium officinale) with 400X magnification

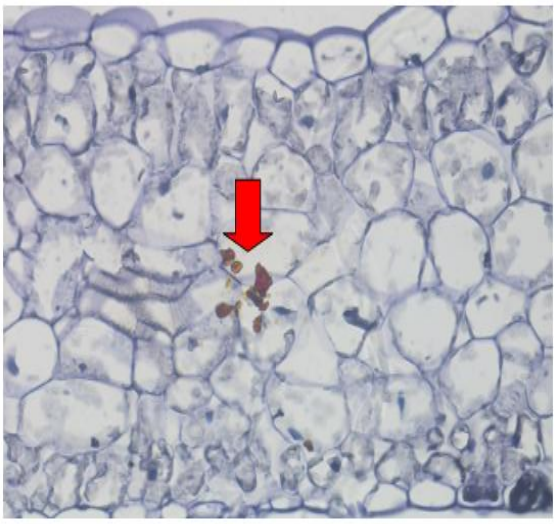

Figure 5. Ghrelin on cassava leaves (Manihot esculena) with 400X magnification

CONCLUSION

The weight of ghrelin protein molecule is 44kDa, with the amino acid structure for ghrelin as mflrvil or methionine, phenylalanine, leucine, arginine, valine, isoleucine and leucine. Exploration of natural materials from kale leaves, lettuce leaves, cassava leaves showed the presence of ghrelin protein.

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