Signal Tranducers and Activators Transcription (STAT) 5b Protein as A Candidate of Growth Promoter in Broiler Chicken

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Abstract: The purpose of this study is to determine the molecular weight of the protein STAT-5b that exists in liver tissue as a basis to determine the amino acid composition of broiler STAT protein phosphatase that experiences growth due to increased growth hormone (GH). Broiler liver tissue samples isolated from broilers were maintained for 21 days, then the examination was followed by SDS Page and Western Blott. The results of Western Blott revealed that STAT 5b of 90 kDa had amino acid composition of datnilvspvylypdip or aspartate, alanine, threonine, asparagine, isoleosine, leucine, valine, serine, proline, valine, tyrosine, leucine, tyrosine, proline, aspartate, isoleucine, proline.

1 INTRODUCTION

Growth hormone has an important role in regulating body growth and metabolism. GH metabolic effects occur when GH receptors associate with and activate tyrosine kinases. The bonding of GH to its receptor can activate Janus Kinase 2 (JAK 2) and further phosphorylate tyrosine in the JAK-2 GH-receptor complex. This tyrosine then forms the bonding site for a number of signalling proteins, such as signal transducers and activators of transcription (STAT) to induce the growth effect. STAT proteins that play a role in providing healing signals are STAT 1, STAT 3, STAT 5a and STAT B. STAT proteins play a significant role in regulating metabolic and growth effects.

Increased growth in animal husbandry has great implications and appeal to the world of poultry. However, signalling of STAT protein and its expression patterns in broilers during growth period have not been identified. So, the identification of the molecular weight and the composition of amino acids protein signalling STAT in broilers during the growing period due to increased GH can be used to make synthetic STAT protein to spur the growth of the broilers.

Until recently, it has only been known that the weight of STAT1 protein molecules in broilers was 91 kD, while the weight of STAT 3 protein was 83 kD. Therefore, further research is needed to determine the molecular weight of STAT 5b proteins, as well as the amino acid composition of STAT 5b proteins to be used as the basis for making synthetic STAT proteins to stimulate broiler growth.

2 MATERIALS AND METHODS

The chickens were placed in a battery cage with a capacity of one chicken per cage receiving feed twice a day at 06.00 pm and 18.00 pm in an amount of 10% less than standard. At the age of 21 days, the chickens were cut to be sampled for their hepatic tissue for the following tests: (1) Isolation of signalling proteins STAT 5a and STAT 5b from broiler liver tissue, (2) Analysis of STAT 5a and STAT 5b protein signalling from broiler liver tissue by using Dot Blot method and then continued with SDS-PAGE (sodium dudecyl sulphate polyacrylamide gel electrophoreses), (3) Identification of molecular weight of signalling protein STAT 5a and STAT 5b using Western Blot technique by means of electrophoresis elucidated protein from polyacrylamide gel, and (4) Identification of amino acids by MALDI-TOF method.

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3 RESULT AND DISCUSION

3.1 SDS Page for STAT protein of the broilers' liver

The result of SDS-PAGE of STAT protein of broiler chicken liver tissue showed the presence of STAT protein as in Figure 1.

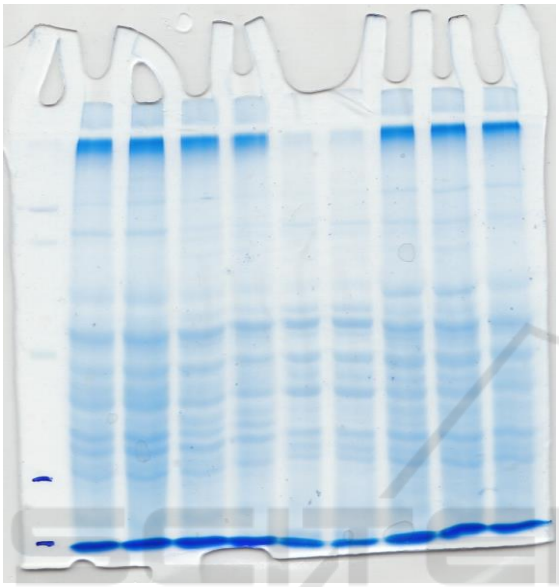

Figure 1. SDS-PAGE of STAT protein from broilers'

The results of SDS-PAGE on broilers' liver tissue revealed the presence of STAT protein. The SDS-PAGE results showed that there were several bands visible. The markers 260 with 140 kDa, 140 with 100 kDa, and 100 kDa marker with 70 kDa each portrayed one protein band. The protein band formed between 100 kDa and 70 kDa markers was suspected of STAT protein. The protein bands formed in the liver were very clear, indicating that the liver tissue illustrationed strongest antibody antigen reaction.

Results of SDS-PAGE on the liver tissue proteins showed the presence of protein bands between 100 kDa and 70 kDa markers. These were proteins with molecular weights of 90 kDa and 91 kDa. However, there has not been able to determine whether these results were STAT 5a and STAT 5b proteins or not because several other protein bands were also formed between these markers. To prove that the formation of protein band with molecular weight of 91 kDa and 90 kDa was indeed STAT 5a and STAT 5b, it was necessary to make further test using Western blot test.

3.2 Western Blot for STAT 5b protein

The result of Western blot for STAT protein on hepatic tissue showed the presence of STAT 5b protein with a molecular weight of 90 kDa, as in Figure 3.

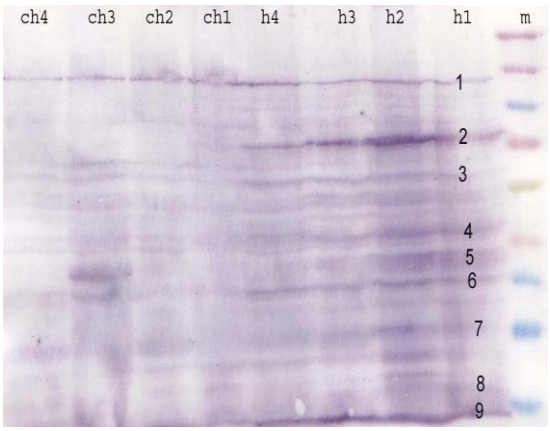

Figure 2. Western Blot for STAT 5b protein from the broilers'

The result of calculating the weight of the STAT 5b protein molecule revealed that its weight was 90 kDa. The formation of protein bands between 100 kDa and 70 kDa markers after calculation apparently had molecular weight of 90 kDa. This suggested that the SDS-PAGE protein tested with Western blot was a STAT 5b protein from growing-phase broiler with a molecular weight of 90 kDa. The formation of a protein band with definite 90 kDa molecular weight was due to a bonding between STAT 5b protein resulting from SDS- PAGE and anti-phospho-STAT 5b(Ser731).

STAT 5b with molecular weight of 90 kDa in hepatic tissue showed that in growing phase broilers STAT 5a and STAT 5b proteins had almost the same molecular weight. It is expected that by identifying the weight of the protein molecules STAT 1, STAT 3, STAT 5a and STAT 5b in broilers, researcher will be able to determine clearly the mechanism of growth hormone in regulating growth and metabolism.

3.3 Amino acid of the STAT 5b proteins

The results of the MALDI-TOP examination revealed that the STAT 5b of 90 kDa had amino acid composition of datnilvspvylypdip or aspartate, alanine, threonine, asparagine, isoleosine, leucine, valine, serine, proline, valine, tyrosine, leucine, tyrosine, proline, aspartate, isoleucine, and proline.

Growth hormone plays a role in regulating body growth and composition (Foster, 1998). Growth hormone has a significant biologic effect that is influenced by insulin-like growth factor I (IGF-I) in improving skeletal muscle growth (Younken, 2000). Provision of in vivo growth factor in broilers led to an increase in growth rate and muscle mass by 15% and required 6.5% less feed than normal. This increase in growth has great implications and appeal to the world of poultry. However, the expression pattern of growth factor gene during growth mass to date has not been known clearly (Killefer, 2000).

STAT protein plays an important role in the regulation of gene transcription by GH and other cytokines that activate Janus Kinase (JAK). STAT proteins originally identified in the signalling interferon pathway (IFN) (Darnell et al., 1994) are cytoplasmic factors that contain the SH-2 domain. In the frequent tyrosyl-phosphorylation through–the-JAK-kinase-initiated cocktail, the cytoplasmic STAT protein forms a complex with another STAT protein through the phosphorylated tyrosine interaction of the SH-2 domain, trans-locates to the nucleus, binds to DNA and then activates transcription of the target gene (Ihle, 1996).

Growth hormone is known to activate STATs 1, 3, 5a and 5b. Tyrosyl phosphorylation of GH-dependent STATs 1, 3, 5a and 5b are found in 3T3-F442A fibroblasts, in the liver of mice with hypophysectomy, in liver cell cultures and in various over-expression systems. Tyrosyl phosphorylation of STATs 5a and 5b are also found in human IM-9 cells and hepatic muscle as well as skeletal muscle of normal mice (Smit et al., 1999)

.STAT1, also called P91, is identified as a member of the factor 3 gene complex that is stimulated by IFNα (FU, 1992). GH signalling analysis of JAK2 deficiency cells and mutated cells in expressing GH receptors showed that activation of GH-dependent STATs 1, 3, 5, and 5b requires activation of JAK2 (Smit et al., 1997). This is consistent with the finding that JAKs activation is required for STAT activation (Muller et al., 1993). JAK1 or JAK2 actively overexpressed in COS cells will stimulate the binding of STAT1 to DNA (Silvennoinen, 1993).

An indirect study has shown that GH stimulates the phosphorylation of STATs 1, 3 and 5 in serine or threonine in the liver. This phosphorylation will increase DNA binding of STAT1, and STAT3 and substantially alter DNA binding of STAT5 (Ram et.al., 1996). STAT 1, 3, and 5a contain conserved consensus sequences for phosphorylation of MAP kinases and preliminary studies show that MAP kinase is responsible for serum phosphorylation of STAT1, STAT3 and STAT 5a. While STAT 5b does not contain conserved consensus sequence, phosphorylation is performed by other kinases other than MAP kinase. Proteins STAT 1, 3, 5a and 5b also contain protein kinase C and casein kinase for phosphorylation process. This suggests that double signalling pathways may converge on STAT proteins for transcriptional activation by GH.

4 CONCLUSIONS

The weight of the STAT 5b of 90 kDa had amino acid composition of datnilvspvylypdip or aspartate, alanine, threonine, asparagine, isoleosine, leucine, valine, serine, proline, valine, tyrosine, leucine, tyrosine, proline, aspartate, isoleucine, and proline. Identified amino acid sequence can be used as a basis for making STAT synthetic proteins which are expected to be used to extend the action or effects of growth hormone. Increased effects of growth hormone will spur livestock growth.

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