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ORIGINAL PAPER

Inhibition on JAK-STAT3 Signaling Transduction Cascade Is Taken by Bioactive Peptide Alpha-S2 Casein Protein from Goat Ethawah Breed Milk

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ABSTRACT

Background: RA is a systemic inflammatory disease that causes developing comorbidity conditions. This condition can cause by overproduction of pro-inflammatory cytokine. In a previous study, we have found bioactive peptide CSN1S2 from Ethawah goat milk for anti-inflammatory for repair the ileum destruction. However, the signaling transduction cascade of bioactive peptides inhibits inflammation still not clear yet. Therefore, we analyzed the signaling transduction cascade via JAK-STAT3 pathway by in vivo and in silico. Methods: The ileum was isolated DNA and amplification with specific primer. The sequence was analyzed using the Sanger sequencing method. Modeling 3D-structure was predicted by SWISS-MODEL and virtual interaction was analyzed by docking system using Pymol and Discovery Studio 4.0 software. Results: This study showed that STAT3 has target gene 480bp. The normal group and normal treating- CSN1S2 of goat milk have similarity from gene bank. Whereas, RA group had transversion mutation that the purine change into pyrimidine even cause frameshift mutation. Interestingly, after treating with the CSN1S2 protein of goat milk shows reverse to the normal acid sequence group. Based on in silico study, from eight peptides, only three peptides of CSN1S2 protein, which carried by PePT1 to enter the small intestine. The fragments are PepT1-41-NMAIHPR-47; PepT1-182-KISQYYQK-189 and PepT1-214-TNAIPYVR-221. We have found just one bioactive peptide of f182-KISQYYQK-189 is able bind to STAT3. The energy binding of f182-KISQYYQK-189 and RA-STAT3 amino acid, it was Σ = -402.43 kJ/mol and the energy binding of f182-KISQYYQK-189 and RAS-STAT3 amino acid is decreasing into Σ = -407.09 kJ/mol. Conclusion: This study suggested that the fragment 182-KISQYYQK-189 peptides from Ethawah goat milk may act as an anti-inflammatory agent via JAK-STAT3 signal transduction cascade at the cellular level.

Key words: Goat milk Casein, Ileum, Inflammation, Rheumatoid Arthritis, STAT3.

1. INTRODUCTION

Rheumatoid arthritis (RA) is systemic inflammatory diseases characterized by inflammation at joint synovial (1). Several cases show that this disease can increase developing comorbidity conditions caused by high level inflammatory substance (2). In addition, the increasing self-reactive antibodies and pro-inflammatory T-lymphocytes was contributed the gut inflammation comorbidity condition on RA (3). According to Rohmah et al., 2015 (4), the RA condition was pro-inflammatory cytokine level increasing that influence of villi ileum destruction. However, the mechanisms of intestinal inflammation in RA are still unknown and need further research.

Signal transducer and activator of transcription 3 (STAT3) proteins is a transcription factor which plays a key regulatory in various cytokine-controlled cellular processes such as immune responses, differentiation, proliferation, and cell survival (5). The activation and expression of STAT3 on autoimmune disease has been well studied. The activated STAT3 was founded in inflamed tissues histology also appeared the total STAT3 protein increasing compared with non-inflammatory control cell (6,7).

Functional food may provide nutritional benefits that have several active components(8). Goat milk is one of functional food has play important role for healthy, nutrition also therapeutic effects of neonates and adults. Goat milk was contributed for reduction gastrointestinal disorder and chronic disease risks (9). Recent study, we found the eight peptides of goat Ethawah breed milk CSN1S2 protein that suggest have multifunction(10). *In vivo* study was explained that the CSN1S2 protein act as an anti-inflammatory agent that could repair the villi ileum destruction (4) and reduce the pro-inflammatory cytokine in synovial rheumatoid arthritis rat (11). In *vitro* study shows that the CSN1S2 protein of goat milk can increase proliferation MC3T3E1 pre-osteoblast cells due to methylglyoxal exposure (12). Beside that, the modeling biological study was explained that the CSN1S2 protein act as an inhibitor of AGEs-RAGE interaction at cellular level(13). We predict that bioactive peptides may act as reducing agent of inflammation on RA via STAT3 signaling transduction. Therefore, we analyzed the role of STAT3 mechanism and to design modeling structure the bioactive peptide against STAT3 that cause inflammation on ileum RA.

2. MATERIAL AND METHODS

2.1. Isolation of CSN1S2

Milk and yogurt was taken from Ethawah breed goat milk, at UPTD Indonesian local goat, and Singosari, Malang. Isola-

tion of milk and yogurt goat CSN1S2 protein was performed according to the previous study (12) with some modifications.

2.2. Experimental Animals

The animal condition and handling was performed according to the previous study(11) with some modifications. All rats were grouped into normal rats group (N), normal rats group RA treated with milk CSN1S2 protein (NM), normal rats group RA treated with yogurt CSN1S2 protein (NY), CFA-induced rheumatoid arthritis rats group (RA), RA rats group treated with milk CSN1S2 protein (RAM) and RA rats group treated with yogurt CSN1S2 protein (RAY).

2.3. DNA Isolation

Ileum samples from rat were isolated of DNA based on Starke et al., 2014(14) with some modifications. Quality and quantity of DNA were measured by using NanoDrop spectrophotometer and 1% agarose gel electrophoresis then visualized with BioRad Gel Documentation.

2.4. DNA amplification

Blood DNA was amplified by primer STAT3-F-1873 & STAT3-R-2330. Our primer was designed specifically of STAT3 gene. PCR program: hot start 94°C for 1min, denaturation 94°C for 30s, annealing 54°C for 30 s, and extension 72°C for 45s (35 cycles), and then post extension 72°C for 7 min. PCR products were measured qualitatively by using 2% agarose gel electrophoresis. PCR products were sequenced by same primer to identified STAT3 gene.

2.5. DNA sequencing

Amplification product was purified based on Greco et al., 2014(15) with some modifications. The sequencing was performed by The ABI 3730xl DNA Sequencer (Koeln, Germany) using Sanger sequencing method. The sequences were alignment by ClustalX software.

2.6. STAT3 protein peptide sequence retrieval

The protein sequences of NSTAT3, NSSTAT3, NYSTAT3, RASTAT3, RASSTAT3, and RAYSTAT3 was taken from DNA sequencing. The DNA sequence was translated into protein using Bioedit v.7.2.5. The peptide sequence fragments of caprine milk CSN1S2 protein was isolated and identified by MALDI-TOF(10).

2.7. Protein modeling 3D-structure Preparation

Modeling 3D-structure of PepT1; NSTAT3, NSSTAT3, NYSTAT3, RASTAT3, RASSTAT3, and RAYSTAT3 and peptide sequence fragments of caprine milk CSN1S2 protein were predicted by SWISS-MODEL web server by homology modeling method (16,17,18,19).

2.8. Docking of Bioactive peptide–Protein interaction and their Visualization

To analyze the virtual interaction among PePT1 and peptide sequence fragment of caprine milk CSN1S2 protein; NSTAT3, NSSTAT3, NYSTAT3, RASTAT3, RASSTAT3, RAYSTAT3 and peptide sequence fragments using Cluspro 2.0 (20, 21, 22) and Patchdock (23, 24). Interaction visualization among them was showed off by Pymol and Discovery Studio 4.0 as proper.

2.9. Analysis for Binding interaction and Binding Energy

The type of binding among receptor, protein peptide and other ligand was identified using Cluspro 2.0 (20, 21, 22) and Patchdock such as amino acids residue; atoms belong to

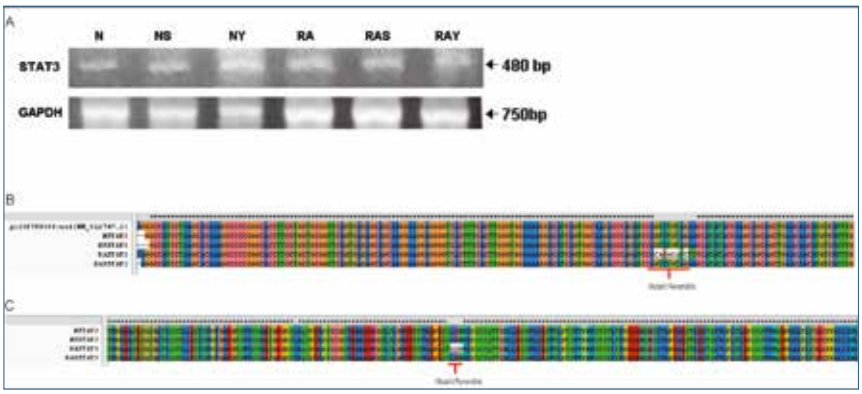

Figure 1. *The Level of STAT3 gene of Rat ileum was comparing with the GAPDH gene as a control. (A) A part of the STAT3 gene of Rat ileum is 480bp and GAPDH gene is 750bp. N: Normal rat group; NS: normal rat induced by Ethawah breed goat milk CSN1S2 protein; RA: Rheumatoid Arthritis rat; RAS: RA rat induced by Caprine milk CSN1S2 protein. (B) The STAT3 nucleic acid sequences Rat ileum in all groups is aligned with STAT3 gene NM_012747.21. (C) The STAT3 protein sequence of Rat ileum all groups is displayed the most of amino acid sequences almost conserve, but only amino acid residue Leu ® Asp, Asp ® Ile, Asn ®Phe as mutant that reversible after treating Caprine milk CSN1S2 protein.*

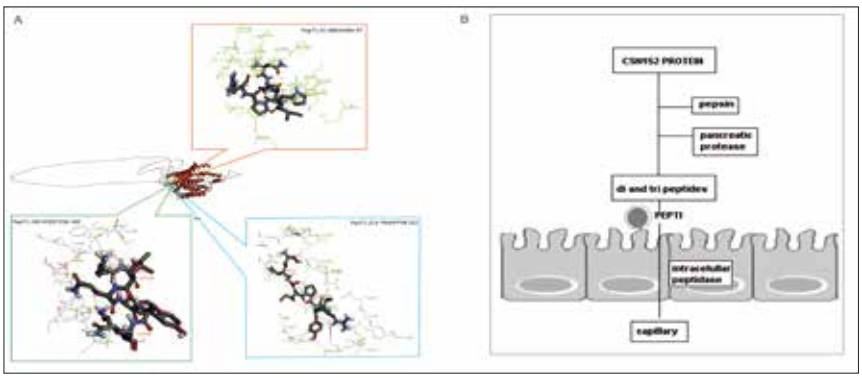

Figure 2. *Insertion modeling of peptide fragments of Caprine alpha-S2 casein protein into small intestine through PePTI protein. A) Possibility interaction modeling between PePTI bind to peptide fragments of Caprine alpha-S2 casein protein. The red rectangular callout is an interaction between PepT1-41-NMAIHPR-47 peptide fragment of Caprine alpha-S2 casein protein. The green rectangular callout is an interaction between PepT1-182-KISQYYQK-189 peptide fragment of Caprine alpha-S2 casein protein. The blue rectangular callout is an interaction between PepT1-214-TNAIPYVR-221 peptide fragment of Caprine alpha-S2 casein protein. B) Modelling of insertion caprine CSN1S2 peptide into the small intestine through PepTI protein.*

the protein and ligand and also type of hydrogen bonds, van der Waals contacts and covalent bonds. The binding energy of their interaction was calculated by Cluspro 2.0.

2.10. Ethics

This study has been evaluated and approved by the research ethics committee of Faculty of Sciences, Universitas Brawijaya, Malang, East Java, Indonesia (Registration number, KEP-90-UB).

3. RESULT

3.1. STAT3 gene analysis

The SH-2 and transactivation domain of STAT-3 protein is an activation region to phosporylation when interact with another protein. To identify any mutation in this region, we were observing the nucleic acid sequences of STAT3 gene (480bp) on N, NS, RA, and RAS (Figure 1a). The alignment of sequencing product was shown that normal (N) group, normal treating-CSN1S2 milk (NS) have similarity compared with STAT3 gene NM_012747.21 from Genbank (Figure 1b). Otherwise, RA group had transversion mutation that the purine change into pyrimidine ($_{2111}$G into $_{2111}$C, $_{2112}$T into $_{2112}$A, $_{2113}$C into $_{2113}$G, $_{2114}$T into $_{2114}$A, $_{2115}$G into $_{2115}$C, $_{2116}$T into $_{2116}$A, $_{2117}$A into $_{2117}$T, $_{2118}$G into $_{2118}$C, $_{2119}$A into $_{2119}$T, $_{2120}$A into $_{2120}$T). This transversion mutation induced the amino acid residues in normal (Leu-Asp-Asn, red line in Figure 1C) also changed into Asp-Ile-Phe. Interestingly, we found that the STAT3 gene was appearance normally of the Rat group after inducing with caprine milk CSN1S2 protein, the nucleic acid sequence mutation (^{2111}**C-A-G-A-C-A-T-C-T-T**^{2120}) reverses into tthe normal nucleic acid sequence (^{2111}G-T-C-T-G-T-A-G-A-A^{2120}). The STAT3 protein also displayed the reversibility of amino acid sequence residue (Leu into Asp, Asp into Ile, Asn into Phe) (Figure 1B).

3.2. Virtual docking PepT1 and peptide sequence fragment of caprine milk CSN1S2 protein interaction

The possibility interactions of PepT1 and peptide sequence fragments of caprine milk CSN1S2 protein were shown in Figure 2. The sequence fragment can interact with PepT1 just only three fragments. These fragments are PepT1-41-NMAIHPR-47; PepT1-182-KISQYYQK-189 and PepT1-214-TNAIPYVR-221. Total energy binding was shown in Table 1. The energy binding between peptide fragments 41- 47 of CSN1S2 and PepT1 was -440,79kJ/mol. More ever, the CSN1S2 peptides are required as acceptor for PepT1 (ASN582- HIS45) and as donor for PepT1 (ASN41-ASP431; ASN41-VAL448; ASN41-PRO446; ASN41-GLY447; ILE44- THR580; ILE44- VAL581) by hydrogen bonds. Whereas, energy binding between PepT1-182-KISQYYQK-189 was -540,59kJ/mol and CSN1S2 peptide play role as donor for PepT1 (LYS182- ASP645, LYS182- ASP 645, LYS182- ASP645, LYS182- ASP 645, ILE183- ASP645, ILE183- ASP645, SER184- ASP645, GLN185- SER114, TYR186- LYS182, TYR186- ILE183, TYR187- ILE183, TYR187- SER184, GLN188- GLN185, LYS189- TYR186, LYS189- ASP48, LYS189- ASP48). The profile also appeared in the interaction between PepT1-214-TNAIPYVR-221 was -630,20kJ/mol with the result that the caprine milk CSN1S2 protein as donor for PepT1 (THR214- ASP50, ILE217- ASP645). This study indicated that the caprine milk CSN1S2 protein interacted into PepT1 properly.

No	Interaction	Point Interaction	Donor Atom	Acceptor Atom	Type	Chemistry Bond	Energy binding
1	PepT1-41-NMAIHPR-47	**THR310-** GLN307	THR310:H	GLN307:O	Hydrogen Bond	Hydrogen Bond	-440,79 kJ/mol
		THR449- ARG47	THR449:H	ARG47:O	Hydrogen Bond	Hydrogen Bond	
		ASN579- MET42	ASN579:H	MET42:O	Hydrogen Bond	Hydrogen Bond	
		VAL581- THR580	VAL581:H	THR580:O	Hydrogen Bond	Hydrogen Bond	
		ASN582- MET311	ASN582:H	MET311:O	Hydrogen Bond	Hydrogen Bond	
		ASN582- VAL581	ASN582:H	VAL581:O	Hydrogen Bond	Hydrogen Bond	
		ASN582- HIS45	ASN582:H	HIS45:N	Hydrogen Bond	Hydrogen Bond	
		ASN582- HIS45	ASN582:H	HIS45:N	Hydrogen Bond	Hydrogen Bond	
		ASN41- ASP431	ASN41:H	ASP431:O	Hydrogen Bond	Hydrogen Bond	
		ASN41-VAL448	ASN41:H	VAL448:O	Hydrogen Bond	Hydrogen Bond	
		ASN41-PRO446	ASN41:H	PRO446:O	Hydrogen Bond	Hydrogen Bond	
		ASN41-GLY447	ASN41:H	GLY447:O	Hydrogen Bond	Hydrogen Bond	
		ILE44- THR580	ILE44:H	THR580:O	Hydrogen Bond	Hydrogen Bond	
		ILE44- VAL581	ILE44:H	VAL581:O	Hydrogen Bond	Hydrogen Bond	
2	PepT1-182-KISQYYQK-189	**ASP49-** TRP 47	ASP49:H	TRP 47:O	Hydrogen Bond	Hydrogen Bond	-540,59 kJ/mol
		ASP50- ASP48	ASP50:H	ASP48:O	Hydrogen Bond	Hydrogen Bond	
		LYS182- ASP645	LYS182:H	ASP645:O	Hydrogen Bond	Hydrogen Bond	
		LYS182- ASP 645	LYS182:H	ASP645:O	Hydrogen Bond	Hydrogen Bond	
		LYS182- ASP645	LYS182:H	ASP645:O	Hydrogen Bond	Hydrogen Bond	
		LYS182- ASP 645	LYS182:H	ASP645:O	Hydrogen Bond	Hydrogen Bond	
		ILE183- ASP645	ILE183:H	ASP645:O	Hydrogen Bond	Hydrogen Bond	
		ILE183- ASP645	ILE183:H	ASP645:O	Hydrogen Bond	Hydrogen Bond	
		SER184- ASP645	SER184:H	ASP645:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- SER114	GLN185:H	SER114:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- LYS182	TYR186:H	LYS182:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- ILE183	TYR186:H	ILE183:O	Hydrogen Bond	Hydrogen Bond	
		TYR187- ILE183	TYR187:H	ILE183:O	Hydrogen Bond	Hydrogen Bond	
		TYR187- SER184	TYR187:H	SER184:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- GLN185	GLN188:H	GLN185:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- TYR186	LYS189:H	TYR186:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- ASP48	LYS189:H	ASP48:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- ASP48	LYS189:H	ASP48:O	Hydrogen Bond	Hydrogen Bond	
3	PepT1-214-TNAIPYVR-221	**ASP50-** ASP48	ASP50:H	ASP48:O	Hydrogen Bond	Hydrogen Bond	-630,20 kJ/mol
		VAL581- THR580	VAL581:H	THR580:O	Hydrogen Bond	Hydrogen Bond	
		ASN582- VAL581	ASN582:H	VAL581:O	Hydrogen Bond	Hydrogen Bond	
		THR214- ASP50	THR214:H	ASP50:O	Hydrogen Bond	Hydrogen Bond	
		ILE217- ASP645	ILE217:H	ASP645:O	Hydrogen Bond	Hydrogen Bond	

Table 1. *Interaction and total energy binding of PepTI and caprine milk CSN1S2 peptide fragment.*

No	Interaction	Point Interaction	Donor Atom	Acceptor Atom	Type	Chemistry Bond	Energy binding
1	NSTAT3- f182-KISQYYQK-189	**LEU 757-** SER755	LEU 757:N	SER755:O	Hydrogen Bond	Hydrogen Bond	-184,07 kJ/mol
		SER184- GLU744	SER184:N	GLU744:O	Hydrogen Bond	Hydrogen Bond	
		SER184- GLU744	SER184:O	GLU744:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- PRO727	GLN185:N	PRO727:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- PRO728	GLN185:N	PRO728:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- LYS182	GLN185:N	LYS182:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- LYS182	TYR186:N	LYS182:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- ILE183	TYR186:N	ILE183:O	Hydrogen Bond	Hydrogen Bond	
		TYR187- GLN733	TYR187:N	GLN733:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- GLN185	GLN188:N	GLN185:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- TYR186	GLN188:N	TYR186:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- ALA729	GLN188:N	ALA729:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- TYR186	LYS189:N	TYR186:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- TYR187	LYS189:N	TYR187:O	Hydrogen Bond	Hydrogen Bond	
		LYS189-SER755	LYS189:N	SER755:O	Hydrogen Bond	Hydrogen Bond	
2	NSSTAT3- f182-KISQYYQK-189	**LEU 757-** SER755	LEU 757:N	SER755:O	Hydrogen Bond	Hydrogen Bond	-184,07 kJ/mol
		SER184- GLU744	SER184:N	GLU744:O	Hydrogen Bond	Hydrogen Bond	
		SER184- GLU744	SER184:O	GLU744:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- PRO727	GLN185:N	PRO727:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- PRO728	GLN185:N	PRO728:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- LYS182	GLN185:N	LYS182:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- LYS182	TYR186:N	LYS182:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- ILE183	TYR186:N	ILE183:O	Hydrogen Bond	Hydrogen Bond	
		TYR187- GLN733	TYR187:N	GLN733:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- GLN185	GLN188:N	GLN185:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- TYR186	GLN188:N	TYR186:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- ALA729	GLN188:N	ALA729:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- TYR186	LYS189:N	TYR186:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- TYR187	LYS189:N	TYR187:O	Hydrogen Bond	Hydrogen Bond	
		LYS189-SER755	LYS189:N	SER755:O	Hydrogen Bond	Hydrogen Bond	
3	RASTAT3- f182-KISQYYQK- 189	**LEU730-** PRO728	LEU730:N	PRO728:O	Hydrogen Bond	Hydrogen Bond	-402,43 kJ/mol
		ALA743-GLN185	ALA743:N	GLN185:O	Hydrogen Bond	Hydrogen Bond	
		LEU744-LYS182	LEU744:N	LYS182:O	Hydrogen Bond	Hydrogen Bond	
		GLN747- TYR186	GLN747:N	TYR186:O	Hydrogen Bond	Hydrogen Bond	
		GLN747- TYR187	GLN747:N	TYR187:O	Hydrogen Bond	Hydrogen Bond	
		GLN747- GLN188	GLN747:N	GLN188:O	Hydrogen Bond	Hydrogen Bond	
		GLN747- LYS189	GLN747:N	LYS189:O	Hydrogen Bond	Hydrogen Bond	
		GLN748- GLN185	GLN748:N	GLN185:O	Hydrogen Bond	Hydrogen Bond	
		GLN748- GLN188	GLN748:N	GLN188:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- LYS742	GLN185:N	LYS742:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- ALA743	GLN185:N	ALA743:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- GLN747	TYR186:N	GLN747:O	Hydrogen Bond	Hydrogen Bond	
		TYR187- ILE183	TYR187:N	ILE183:O	Hydrogen Bond	Hydrogen Bond	
		TYR187- SER184	TYR187:N	SER184:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- GLU749	GLN188:N	GLU749:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- GLN185	GLN188:N	GLN185:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- TYR186	GLN188:N	TYR186:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- GLN747	LYS189:N	GLN747:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- LEU744	LYS189:N	LEU744:O	Hydrogen Bond	Hydrogen Bond	
		LYS189-SER745	LYS189:N	SER745:O	Hydrogen Bond	Hydrogen Bond	
4	RASSTAT3- - f182-KISQYYQK-189	**ARG725-** CYS724	ARG725:N	CYS724:O	Hydrogen Bond	Hydrogen Bond	-407,09 kJ/mol
		CYS726- GLN185	CYS726:N	GLN185:O	Hydrogen Bond	Hydrogen Bond	
		ALA729- SER184	ALA729:N	SER184:O	Hydrogen Bond	Hydrogen Bond	
		GLN747- GLN188	GLN747:N	GLN188:O	Hydrogen Bond	Hydrogen Bond	
		SER751- GLN731	SER751:N	GLN731:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- PRO727	GLN185:N	PRO727:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- PRO728	GLN185:N	PRO728:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- LYS182	GLN185:N	LYS182:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- ILE183	TYR186:N	ILE183:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- SER753	TYR186:O	SER753:O	Hydrogen Bond	Hydrogen Bond	
		TYR187- SER184	TYR187:N	SER184:O	Hydrogen Bond	Hydrogen Bond	
		GLN188-GLN185	GLN188:N	GLN185:O	Hydrogen Bond	Hydrogen Bond	
		CYS726- GLN185	CYS726:N	GLN185:O	Hydrogen Bond	Hydrogen Bond	
		ALA729- SER184	ALA729:N	SER184:O	Hydrogen Bond	Hydrogen Bond	
		GLN747- GLN188	GLN747:N	GLN188:O	Hydrogen Bond	Hydrogen Bond	
		SER751- SER753	SER751:N	SER753:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- PRO727	GLN185:N	PRO727:O	Hydrogen Bond	Hydrogen Bond	
		GLN185- PRO728	GLN185:N	PRO728:O	Hydrogen Bond	Hydrogen Bond	
		TYR186- SER753	TYR186:O	SER753:O	Hydrogen Bond	Hydrogen Bond	
		GLN188- ALA729	GLN188:N	ALA729:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- TYR186	LYS189:N	TYR186:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- TYR187	LYS189:N	TYR187:O	Hydrogen Bond	Hydrogen Bond	
		LYS189- SER755	LYS189:N	SER755:O	Hydrogen Bond	Hydrogen Bond	

Table 2. *Interaction and total energy binding of caprine CSN1S2 peptide and STAT3 protein*

3.3. Virtual docking peptide sequence fragment of caprine milk CSN1S2 protein and STAT3 interaction

The interaction of peptide sequence fragments of caprine milk CSN1S2 protein that enters to the small intestine and STAT3 protein was identified on Figure 3 and Table 2. The fragment which interacts with STAT3 is only peptide fragment number 182-KISQYYQK-189. The energy binding of NSTAT3- f182-KISQYYQK-189 and NSSTAT3- f182-KISQYYQK-189 were Σ = -184.07 kJ/mol. Whereas, the energy binding of RASTAT3 was Σ = -402.43 kJ/mol and RASSTAT3 have the lowest energy binding was Σ = -407.09 kJ/mol. The point interactions of NSTAT3 and f182-KISQYYQK-189 are SER184- GLU744; GLN185- PRO727; GLN185- PRO728; TYR187- GLN733; GLN188- ALA729; LYS189-SER755). Whereas the interaction between NS-STAT3 amino acid and f182-KISQYYQK-189 are SER184-GLU744; GLN185- PRO727; GLN185- PRO728; TYR187-GLN733; GLN188- ALA729 and LYS189-SER755. Different point interaction of RASTAT3 amino acid and f182-KISQYYQK-189 are ALA743-GLN185; LEU744-LYS182; GLN747- TYR186; GLN747- GLN188; GLN747- LYS189; GLN748- GLN185; GLN748- GLN188; GLN185- LYS742; GLN185- ALA743; TYR186- GLN747; GLN188- GLU749; LYS189- GLN747; LYS189- LEU744; LYS189-SER745 Conversely, when f182-KISQYYQK-189 interact with RAS-STAT3, the interaction at the CYS726- GLN185; ALA729-SER184; GLN747- GLN188; SER751- GLN731; GLN185-PRO727; GLN185- PRO728; TYR186- SER753; CYS726-GLN185; ALA729- SER184; GLN747- GLN188; GLN185-PRO727; GLN185- PRO728; TYR186- SER753; GLN188-ALA729; LYS189- SER755.

4. DISCUSSION

This study revealed that STAT3 gene transversion mutation (G into C: A into T) on ileum RA model show that inflammatory may can lead to frameshift mutation on STAT protein (Leu into Asp, Asp into Ile, Asn into Phe). Conversely, the treating group- CSN1S2 milk shows the reverse of STAT3 gene sequence became normally as proper. STAT3 protein is a transcription factor that has functions in cytokine signaling in a variety of tissues. However, the STAT3 have important role to regulate of intestinal immune homeostasis (25). STAT3 mutation can cause on develop atopic condition because of the lack of negative regulation of activation (26, 27, 28). Severe atopic also associated with elevated serum IgE due to STAT3 mutation (29, 30). According to Rohmah et al., 2015 (4), RA associated with auto-reactive T cells that promote immune response on ileum RA model resulting in pro-inflammatory overproduction such as interleukin 17 (IL-17) also elevated IgE level both can cause ileum destruction. Interestingly, the CSN1S2 protein can decrease of IL-17 and IgE level.

Previous study reported that Several pathway mechanism of caprine alpha s2-casein fragment (CSN1S2 f41-47, f87-96, f97-107, f131-141, f182-189, f205-213, f214-221 and f214-223 is bind of calmodulin on specific site through Ca^{2+} pump (10). Another study conducted that PePT1 is an oligopeptide transfer family that found in small intestine and kidneys. PePT1 transports di- or tri-peptide into enterocytes are hydrolyzed by intracellular peptidases within cells (31). This study found the CSN1S2 peptide of goat milk enter to small intestine via PepT1 transporter. Interaction possibility of PepT1-214-TNAIPYVR-221 fragment have lowest total energy binding than others (-630.20kJ/mol). That may indicate that the fragment 214-TNAIPYVR-221 CSN1S2 seem binding stronger than others.

Structurally, the STAT3 has five domain, they are an amino-terminal domain, a coiled-coil domain, a DNA-binding domain,a SH2 (Src Homology 2) domain and a carboxyl-terminal transactivation domain (32). Interestingly, only one caprine CSN1S2 peptide fragment 182-KISQYYQK-189 succeed interaction with STAT3 protein. The total energy binding of NSTAT3-f182-KISQYYQK-189 and NSSTAT3-f182-KISQYYQK-189 both has similar total energy binding. According to the sequencing result, the profile normal control (N) and normal treating CSN1S2 (NS) do not find transversion mutation. Meanwhile, the energy binding is between f182-KISQYYQK-189 and RA-STAT3 amino acid residues, it was Σ = -402.43 kJ/mol. The energy binding of f182-KISQYYQK-189 and RAS-STAT3 amino acid residues is elevated into Σ = -407.09 kJ/mol. The lower negative energy shows the strong binding of ligand-receptor. CSN1S2 peptide may indicate have influence different affinity when binding at STAT3 on RA ileum.

According to Szelag et al., 2015 (32), STAT3 can be activated by tyrosine phosporylation of Jak-tyrosine kinase family in response to variety cytokines and growth factors. Here we report that bioactive peptide f182-KISQYYQK-189 can bind at 722-755 at transactivation domain. The increase of STAT3 transactivation activity can increase nuclear localization. This study revealed that the CSN1S2 may be able to influence the transactivation activity to decline the STAT3 nuclear localization(5).

5. CONCLUSION

This study predicted that the fragment 182-KISQYYQK-189 of caprine milk CSN1S2 protein may act as an agent anti- inflammatory via JAK-STAT3 signal transduction cascade at the cellular level.

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CONFLICT OF INTEREST: NONE DECLARED

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