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Increasing of Plasma Cholecystokinin Level and Jejunum Histological Changes After Treatment with Soybean Extracts Protein

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It is well known that soybean has beneficial health effects. There are lot of active compounds in soybean, like protein and anti nutrition factors (ANF). Trypsin inhibitor and lectin, two kinds of ANF have an adverse effect on the morphology and function of digestive tract in animals. β-conglycinin in soybean protein, has been proven has reducing body weight effect through increasing cholecystokinin (CCK) level. The aim of this study was to measure plasma CCK level and the histological changes of jejunum in Wistar rats after treatment with protein extract of *Willis* raw soybean (PEWS), protein extract of *Detam 1* raw soybean (PEDS) and protein extract of *Detam 1* tempeh (PEDT) for 14 days. This study was also to ascertain whether β-conglycinin and ANF contribute to reducing body weight by giving PEWS, PEDS, and PEDT to 4 groups of 6 rats for 14 days. We observed food intake, body weight, CCK level, and histological profile of jejunum. As a conclusion, PEWS, PEDS, and PEDT treatment to Wistar Rats for 14 days caused increasing CCK plasma level and jejunum villi atrophy. The reducing body weight is caused not only by β-conglycinin but probably by ANF as well.

Key words: protein extract of soybean, β-conglycinin, anti nutrition factors, histological changes of jejunum

INTRODUCTION

Soybean is called as 'a food for the future' because it has many benefits. The content and the quality of proteins in soybean make it a perfect source of dietary supplementation for both human and animals. Nutritional intervention studies performed in animals and human show that dietary soybean has beneficial health effects. For example, several studies have shown that the isoflavones and soybean protein, the major components of a soy diet can decrease the profile of lipid plasma, like cholesterol and triglycerides, and reducing body weight (Anderson 1999; Aoyama *et al*. 2000; Anosike *et al*. 2008).

There are several mechanisms of soybean to reduce body weight, depending on the active compounds. Isoflavones and soybean protein, have been prove in reducing body weight although the mechanisms were unclear (Jang *et al*. 2008). β-conglycinin in soybean protein, induced the secretion of Cholecystokinin (CCK), a neuropeptide hormone in gastro intestinal tract (GIT) regulating food intake and eventually reducing body weight (Nishi *et al*. 2001; Nishi *et al*. 2003). Besides active compounds, called anti nutrition factors (ANF), like trypsin inhibitor, lectin, poliphenol, phitic acid, saponin, and antivitamin have negative affects to the absorbtion of food in GIT (Palacios *et al*. 2004; Godlewski 2006).

Based on the previous studies, the most potential ANF in soybean were *Trypsin Inhibitor* and *Lectin*. Several studies reported that both ANF can cause the changes in histological mucosae of small intestine, like decreasing the height of villi and the depth of crypt. However, the mechanisms are still unclear. This histological changes can interfere the small intestine function to digest, secrete and absorb food. Since the food was absorbed in small intestine especially jejunum, the changes of the histological jejunum profile can manifest in many symptoms like diarrhea, malnutrition and reducing body weight (Yen *et al*. 1977; Feng *et al*. 2007).

Glycine max L.merr *Detam 1* variety is a high quality soybean, and was approved by Minister of Agricultural decree no 240/Kpts/SR.120/3/2008 date March 6th 2008. This soybean has a yellow seed covered with hard black seed skin. It contained much higher protein level (45.36% from the dry seed weight) than protein in other soybean variety (Hidayat *et al*. 2010). This soybean may contain a high level of bioactive compound in its seed. The soybean *Detam 1* variety was planted in Research Institute for Nuts and Tubers or Balai Penelitian Kacang dan Umbi-umbian (Balitkabi) plantation in Malang. This variety developed from a cross of the 9837 introduction variety and the *Kawi* variety. *Detam 1* variety produced 2.51 ton per acre, after 84 days of cultivation. Another Indonesian local soybean is *Wilis* variety commonly planted by farmers in Indramayu. This soybean has a yellow seed with yellow skin. It contained about 39% protein (percentage from dry weight) (Gizi Departemen Kesehatan RI 1981; Hidayat *et al*. 2010).

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Research on effects of fermented soybean products like tempeh to reducing body weight has rarely been done. Fermented process can decrease the protein content in soybean but the amount of absorbable protein is increased because ANF in soybean become inactive by heating in fermentation process. Fermentation is increasing the active compound (Aglycon) in isoflavon (Hermana *et al*. 1999).

Protein extracts of *Detam 1* soybean contain rich of β-conglycinin. The extracts may contains ANF, especially in the raw soybean extracts and this ANF can affect the body weight (Yen *et al*. 1977). Therefore, we observe CCK plasma level and the histological changes of Jejunum in Wistar rats after treatment with protein extract of *Willis* raw soybean (PEWS), protein extract of *Detam 1* raw soybean (PEDS), and protein extract of *Detam 1* tempeh (PEDT) for 14 days. The aim of this study were to ascertain whether β-conglycinin and ANF contribute to reducing body weight, by reviewing the increasing CCK level and the histological changes of jejunum.

MATERIALS AND METHODS

Animal. 24 male wistar rats (5-6 weeks), weighing 200-230 g, from Biology Department ITB Bandung, each were put in a cage separately.

Method. This is an experimental study to normal male wistar rats, which has approved for the ethical clearance from Ethical Committee of Maranatha Christian University (No.148/KEP FK UKM-RSI/V/2009). Data were analyzed by ANOVA continued with Duncan test, ANOVA continued with Tukey HSD test, and paired t-test.

Fermentation Soybean Procedures to Make Tempeh (Hermana 1999; Santoso 2003). We made tempeh ourselves using this following procedures. The procedures include 8 steps process, boiling, skin seed peeling, soaking, washing, steaming, giving the yeast, packaging, and stewing. *Detam 1* and *Wilis* soybean seed were weighed 500 g each, and then boiled. The next process was peeling the skin seed to enable the mycellium breaking through the epidermis which contains horny materials. Soaking was the next step that made an acid condition. The soybean seed (without skin) were then washed until the seed is not sleek. Then the materials were steamed until the seed were soft and wellcooked.

After giving the yeast or tempeh inoculum to the soybean seed, then the materials were cooled. The dosage was 1 g of inoculum for 1 kg soybean seed. The soybean seed which were already mixed with inoculum were packed in plastic bags. Then the packages of this materials were incubated for 2 days in 18-24 °C with good airflow (Hermana *et al*. 1999; Santoso 2003). From 500 g raw soybean seed we produced 650 g tempeh.

Soybean and Tempeh Protein Extraction (Panthee Method Modification). Ten grams soybean or tempeh material we ground in cool water (20 °C, Knifetec 1,095 Sample mill for 20 second). This process produced soybean flour with relatively uniform particle size. Soluble protein was extracted for 1 h at 18-24 °C by stirring 1 g of full fat soybean flour in a 1:15 (w/v) ratio with 0.2 M Tris HCl buffer, pH 8.0 that contained 0.1 M β-*mercaptoethanol*. The mixture was centrifuged (10,000 X g) 10 min at 4 °C. Upon removal of the fat layer, an aliquot (1 ml) of crude protein extract or supernatant was taken from each sample. Storage proteins and their polypeptides were dissociated in the crude extract by adding an equal volume of both 5% SDS and 0.1 M β-*mercaptoethanol* solution and warmed in a waterbath for 10 min (Panthee *et al*. 2004; Delwiche *et al*. 2007).

Diets and Animal Experimental Protocol. The rats were given soyfree pellet feed standard for rats and water *ad libitum*. After 7 days adaptation, 24 male wistar rats were divided into 4 experimental groups of 6 rats each group, refers to Table 1. The extracts were given for 14 days once a day orally via intragastric sonde in dosage 20 mg/kg BW based on Nishi's study and modification from our previous study (Nishi *et al*. 2003; Hidayat *et al*. 2010).

Every day the food intake and body weight of the rats were measured. The body weight were measured at the 10th and the 14th day. The CCK level were measured on the first day before treatment and the 14th day using enzym linked immuno sorbent assay (ELISA) method.

On the 14th day, all rats were killed and the small intestine was taken, the proximal jejunum were processed into histological slide with haematoxilin eosin staining. There are two reasons why we chose jejunum segment. First because jejunum is the most suitable segment in small intestine to digest, balance electrolytes and absorb nutrition. Second, result from previous study showed that significant changes occured mostly in jejunum.

Prosedure to Measure Cholesistokinin Plasma Level in Rats using ELISA Method. On the first day before treatment, and on the14th day after fasted for 12 hours, plasma cholesistokinin level each rats in all groups were measured by taking blood sample from tail vein 1 cc, then put in a lavender vacutainer (# VT-6450) tube containing 2 mg EDTA. The tube were then shaken and made like number eight movement to make a well mix plasma. Then centrifuge 1600 x g for 15 minutes at 4 °C. First, we prepared 5 tube to make a standard solution. Add 50 µl/well of standard, sample, or positive control, 25 µl primary antibody and 25 µl biotinylated peptide. Incubate at room temperature (20-23 °C) for 2 hours. The immunoplate were washed 4 times with 350 µl/well of 1 x assay buffer then add with 100 µl/well of SA-HRP solution. Incubate at room temperature (20-23 °C) for 1 hour. The immunoplate were washed 4 times with 350 µl/well of 1x assay buffer then add with 100 µl/well of TMB substrate solution. Incubate at room temperature (20-23 °C) for 1 hour. The reaction were terminate with 100 µl/well of 2N HCl then read absorbance O.D at 450 nm and calculate results.

Haematoxilin-Eosin Staining of Jejunum. The jejunum block specimen were prepared and cut in horizontal position of proximal jejunum 1 x 0.5 x 0.5 cm^3 then fixed with 10% formalin for 3 days to preserved the cell morphology and molecule composition. The specimen were dehidrated with alcohol 70, 80, 90, 95% and absolute alcohol each for 3 hours to change the cell solution with organic solvent and after that flooded with 3 kind of absolute alcohol each for 3 hours. The specimen were put into paraffin blocking 1, 2, 3 each for 1 hour in incubator

at 60 °C and put it in room temperature. Paraffin block were sectioned with microtome 5 µm thick 5 slices each block and were floating in cold and warm water separately then were attached on the object glass. The sections were dried in incubator and stained with Hematoxillin Eosin by putting the object glass into Hematoxillin solution for 5 minutes. It was washed with aquadest and running water for 30 minutes after that put into Eosin solution for 1 minute and was dehydrated with alcohol 70, 80, 90, and absolute alcohol. Washed it two times with xylol.

The data of histological small intestine profile were taken by observing the histological slide of jejunum with Haematoxilin Eosin staining through light microscopic visual (10 x 10 magnification) for qualitative and quantitative measurements. Qualitative measurement was to describe the quality of structure mucosae jejunum from each group, small intestine absorptive epithel (enterocyte), lamina propria, musculaire mucosae and serosae. While quantitatively we measured the height of villi and the depth of Lieberkühn crypt from 10 units villi-cryptae in 5 view field from each sample of rats small intestine through light microscopic visual with 10 x 10 magnification, by using a micrometer in the ocular lense.

RESULTS

After being given extracts soybean treatment for 14 days, every groups of rats showed decreasing food intake. However, only group PEDS and PEDT showed significant results (P = 0.044), group PEDS gave a significant result after it was analyzed by paired t-test (P = 0.022) (Table 1 & 2).

In body weight, after 10 days of treatment, group PEWS and PEDS showed significant reduction (P = 0.002) and (P = 0.011) (Table 1 & 3). After 14 days treatment, group PEDS showed a significant reduction (P = 0.020) (Table 1 & 4).

After 14 days treatment, there was tendency increasing CCK plasma level in group PEWS, PEDS, and PEDT although the results are non significant. The PEDS group reached the highest level of CCK [pre 16.23 ng/ml (SD 5.69), post 36.03 ng/ml (SD 15.43) increased 19.80 ng/ml] (Table 1 & 5).

Histological Quantitative Measurement. After being given extracts soybean treatment for 14 days, the height of villi in histological slide which were measured by micrometer measurement (10 x 10 magnification) showed

Table 1. Four experimental groups of Wistar Rats

Code	Content	Sample abbreviation	Sample amount
A	Protein extract of *Wilis* soybean raw seed	PEWS	(n=6)
B	Protein extract of *Detam 1* soybean raw seed	PEDS	(n=6)
C	Protein extract of *Detam 1* soybean tempeh	PEDT	(n=6)
D	Negative control Aquadest	NCA	(n=6)

PEWS: protein extract of *Willis* raw soybean, PEDS: protein extract of *Detam 1* raw soybean, PEDT: protein extract of *Detam 1* tempeh, NCA: negative control aquadest.

Table 2. Comparison average food intake per day (g) pre and post treatment from 14 days measurement

Group treatment	Measurement pre treatment	Measurement post treatment	Duncan	t test paired	p value
PEWS	18.63 ± 1.85	14.56 ± 2.06	a, ab, bc	2.335	0.145
PEDS	21.77 ± 2.82	12.60 ± 0.89	a*	6.585	0.022*
PEDT	16.90 ± 1.85	12.53 ± 0.68	a*	3.022	0.094
NCA	19.30 ± 1.41	17.83 ± 0.76	bc	1.743	0.223
F (ANOVA) 1.029		2.216			
p value 0.453		0.044*			

p value < 0.05 → Significant (*), PEWS: protein extract of *Willis* raw soybean, PEDS: protein extract of *Detam 1* raw soybean, PEDT: protein extract of *Detam 1* tempeh, NCA: negative control aquadest.

Table 3. Comparison body weight after 10 days pre and post soybean extracts treatment

Group treatment	Measurement pre treatment	Measurement post treatment	t test paired	p value
PEWS	228.00 ± 23.89	200.67 ± 24.11	22.743	0.002**
PEDS	215.00 ± 26.90	188.67 ± 29.36	9.651	0.011*
PEDT	210.67 ± 17.92	185.00 ± 0.00	2.480	0.131
NCA	220.67 ± 25.58	225.33 ± 11.68	-0.555	0.635

p value < 0.05 → significant*, p value < 0.01 → highly significant**, PEWS: protein extract of *Willis* raw soybean, PEDS: protein extract of *Detam 1* raw soybean, PEDT: protein extract of *Detam 1* tempeh, NCA: negative control aquadest.

Table 4. Comparison body weight after 14 days pre and post soybean extracts treatment

Group treatment	Measurement pre treatment	Measurement post treatment	t test paired	p value
PEWS	248.00 ± 23.89	208.67 ± 3.78	3.371	0.078
PEDS	238.33 ± 21.38	189.33 ± 27.30	6.976	0.020*
PEDT	230.67 ± 17.92	199.67 ± 9.45	2.103	0.170
NCA	240.67 ± 25.58	236.33 ± 22.50	0.212	0.852

p value < 0.05 → Significant*, PEWS: protein extract of *Willis* raw soybean, PEDS: protein extract of *Detam 1* raw soybean, PEDT: protein extract of *Detam 1* tempeh, NCA: negative control aquadest.

Table 5. Cholecystokinin (CCK) plasma level pre and post soybean extracts treatment

Group treatment	Measurement pre treatment (ng/ml)	(Normal range 0-100 ng/ml) post treatment (ng/ml)	t test paired	p value
PEWS	17.96 ± 9.45	33.50 ± 22.25	-2.029	0.180
PEDS	16.23 ± 5.69	36.03 ± 15.43	-2.390	0.139
PEDT	23.87 ± 13.26	34.80 ± 7.00	-3.212	0.085
NCA	38.60 ± 10.15	29.00 ± 22.30	0.529	0.065

PEWS: protein extract of *Willis* raw soybean, PEDS: protein extract of *Detam 1* raw soybean, PEDT: protein extract of *Detam 1* tempeh, NCA: negative control aquadest.

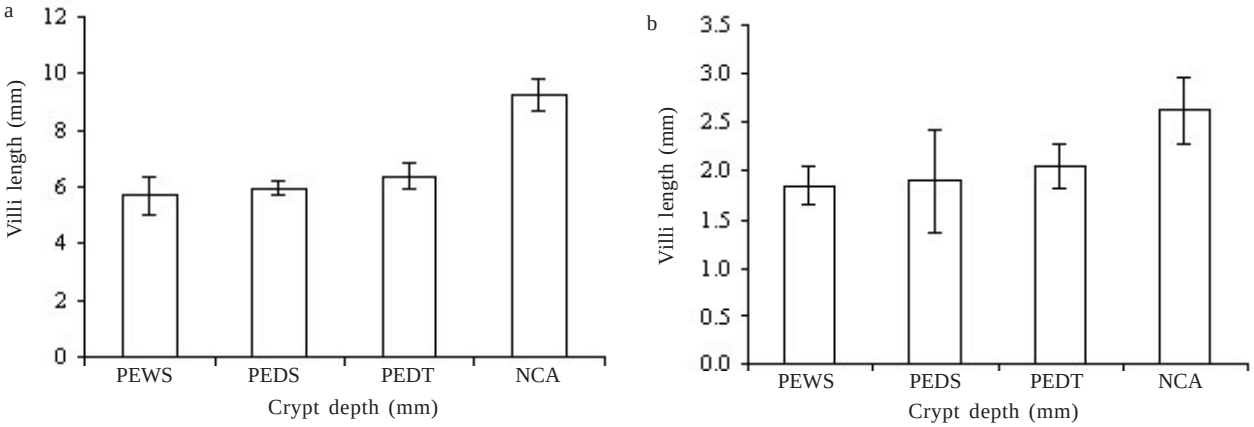

Figure 1. Jejunum histological quantitative measurement after 14 days soybean extracts treatment using parameters. a. Villi length and b. lieberkuhn crypt depth. PEWS: protein extract of *Willis* raw soybean, PEDS: protein extract of *Detam 1* raw soybean, PEDT: protein extract of *Detam 1* tempeh, NCA: negative control aquadest.

significant differences between the height of villi in histological slide treatment groups (PEWS, PEDS, PEDT) and group D (NCA). It means that treatment of 14 days extracts protein of soybean showed significant villi atrophy in the three treatment groups. There was no difference potential among the treatment groups and PEDS group gave the highest effect to make atrophy villi (5.700 square = 2.850 mm) (Table 1 & Figure 1). The average length of PEWS, PEDS, and PEDT villi were significantly reduced. The average height of the PEWS villi was 5.70 ± 0.68 mm, PEDS was 5.94 ± 0.24 mm, PEDT was 6.38 ± 0.44 mm (P = 0.00) (Figure 1) compared with 9.23 ± 0.55 mm in the control group.

After being given extracts soybean treatment for 14 days, the depth of Lieberkuhn crypt in histological slide which were measured by micrometer measurement (10 x 10 magnification) showed significant differences between the crypt depth of treatment groups (PEWS, PEDS, PEDT) and negative control group D (NCA) after they were analyzed by ANOVA continued with Tukey HSD test. It means that treatment of 14 days extracts protein of soybean showed significant diminishing crypt depth in the three treatment groups. There was no difference potential among the treatment groups and group PEDS gave the highest effect to make diminished (1.850 square = 0.925 mm) (Table 1 & Figure 2). The average length of crypt depth PEWS, PEDS, and PEDT was significantly reduced. The average height of the PEWS crypt depth was 1.85 ± 0.20 mm (P = 0.011), PEDS crypt depth was 1.90 ± 0.53 mm (P = 0.006), PEDT crypt depth was 2.05 ± 0.22 mm (P = 0.049) (Figure 6) compared with 2.62 ± 0.35 mm in the control group.

Histological Qualitative Measurement. Among the groups of treatment, group PEDS (B) showed the severest villi atrophy, morphology cells were multiformed, but the goblet cell appearance almost normal. In group PEWS (A) and Group PEDT (C) showed slight atrophy, few foblet cell appearance, but the morphology of cells were slight uniformed. In all groups of treatment there were damaging absorbtive epithel, thinner lamina propria, decreasing the height of villi, diminishing Lieberkuhn crypt and only the the mucosae and serosae were normal. While in control group the villi, absorbtive epithel, lamina propria, height of villi, Goblet cell, Lieberkuhn crypt were normal, morphology cells were uniformed, and the mucosae and serosae normal (Table 1 & Figure 3).

DISCUSSION

Treatment with PEWS and PEDS can cause significant reduction in body weight after 10 days of treatment. But after 14 days treatment. only PEDS group showed significant reducing effect. The increasing CCK level in PEDS group. although it is not significant statistically. showed a high increasing level (19.80 ng/ml). Actually normal range for CCK is 0-100 ng/ml. In CCK level 36.03 ng/ml it should already given a respon effect. like significant reducing food intake (Average from 21.77 to 12.60 g shows in Table 1) then followed by reducing body weight (Average from 215.00 to 188.67 g on the 10th day shows in Table 2), and 238.33 to 189.33 g on the 14th day shows in Table 3). If we compared the histological measurement of PEDS group treatment with normal cell in NCA group, qualitative results showed severe villi atrophy,

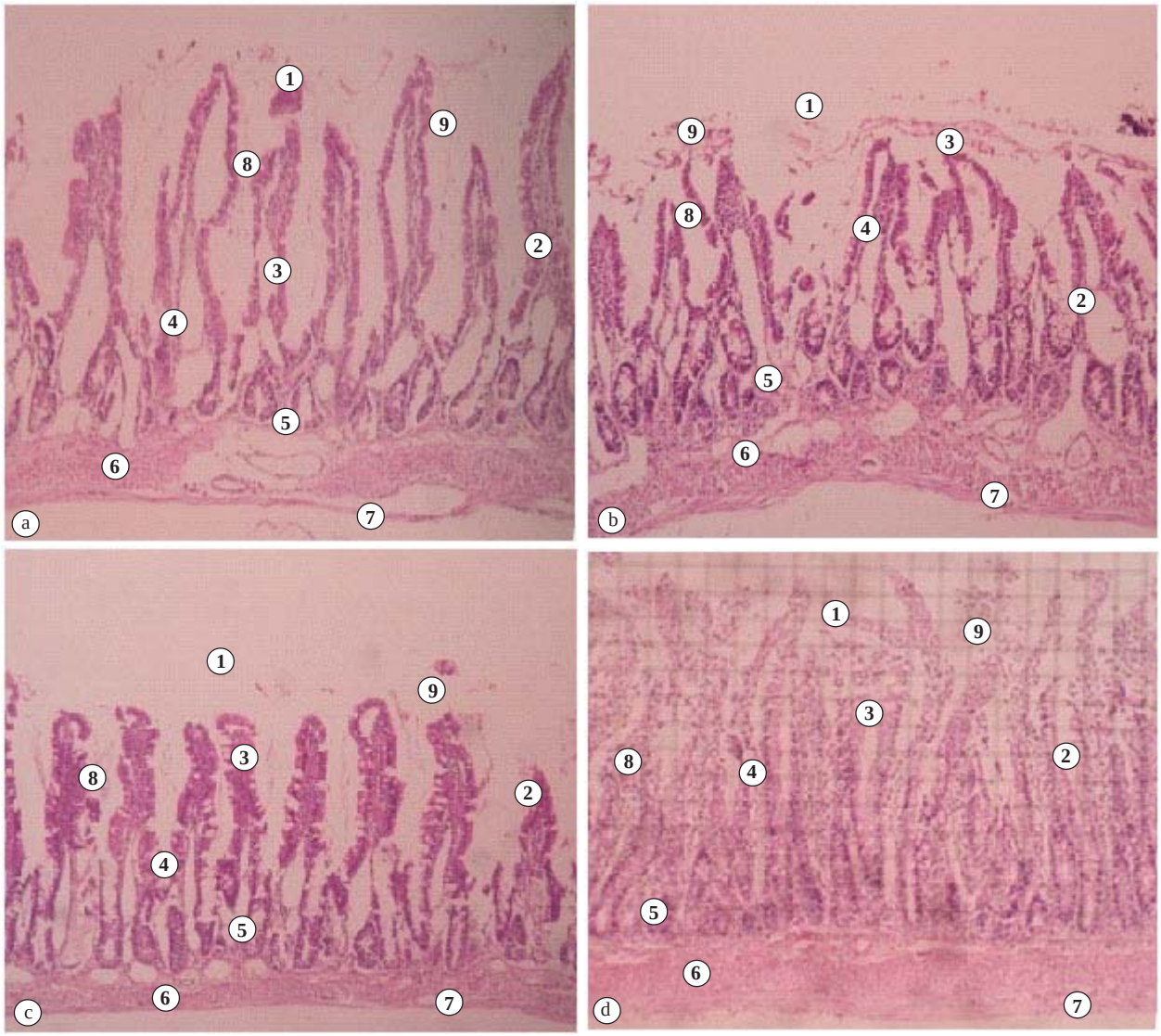

Figure 2. Jejunum histological quantitative measurement after 14 days soybean extracts treatment. a. PEWS: protein extract of *Wilis* soybean raw seed (1. morphology cells were slight uniformed, 2. the height of villi decreased, 3. villi atrophy, 4. lamina propria damaged, 5. lieberkuhn crypt became damaged, 6. tunica muscularis, 7. tunica serosae normal, 8. goblet cell decreased, 9. absorbtive epithel damaged; b. PEDS: protein extract of *Detam 1* soybean raw seed (1. morphology cells were multiformed, 2. the height of villi decreased, 3. severe villi atrophy, 4. lamina propria more damaged, 5. lieberkuhn more damaged, 6. tunica muscularis, 7. tunica serosae normal, 8. goblet cell decreased, 9. absorbtive epithel damaged; c. PEDT: protein extract of *Detam 1* soybean tempeh (1. morphology cells were slight uniformed, 2. the height of villi decreased, 3. villi slight atrophy, 4. lamina propria slight damaged, 5. lieberkuhn crypt slight damaged, 6. tunica muscularis, 7. tunica serosae normal, 8. goblet cell appeared became bigger, 9. absorbtive epithel damaged; d. NCA: negative control aquadest (1. morphology cells were uniformed, 2. the height of villi, 3. villi, 4. lamina propria, 5. lieberkuhn crypt, 6. tunica muscularis, 7. tunica serosae, 8. goblet cell, 9. absorbtive epithel all were normal). Each square micrometer measurement numeric apertura has been counted was 0.5 mm.

the height of villi decreased, absorbtive epithel (enterocyte) damaged, lamina propria became thinner, but Lieberkuhn crypt diminished and Goblet cell appeared almost normal. And from quantitative measurement, showed significant villi atrophy and crypt depth changes. It means that treatment PEDS for 14 days can caused atrophy villi in jejunum Wistar rats. During the treatment, all rats still looked healthy and no one was died. There is no faeces changes in macroscopically (amount and consistency), although we did not measure the amount of their faeces.

It is well known that Trypsin Inhibitor interfered with the proper function of trypsin and chymotrypsin leading to abnormal intestinal morphology (Liener & Kakade 1993). Previous studies showed that ANF in soybean meal have an adverse effect on the morphology and function of digestive tract in animals (Dunsford *et al.* 1989; Li *et al.* 1991). Antigenic materials in soybean proteins are associated with villi atrophy, increased crypt cell mitosis, and crypt hyperplasia, and thereby causing a malabsorption syndrome (Kenworthy & Allen 1966; Miller *et al.* 1984). So it is suggested that in PEDS contained

ANF and the reducing body weight effect is a synergistic effect by CCK and the ANF although we have not measured the ANF level in this extracts.

Besides PEDS, PEWS group showed significant reducing body weight on the10th day, average from 228 to 200,67 g, but not significant on the 14th day. This group showed a high increasing CCK level (15.54 ng/ml). In CCK level 33.50 ng/ml it should already given reducing body weight effect but the potential was lower if we compare with PEDS. The fact was CCK level in PEWS was lower than in PEDS. If we compared the histological measurement of PEWS group with normal cell in group NCA, qualitative results showed villi atrophy, the height of villi decreased, absorbtive epithel (enterocyte) damaged, lamina propria became thinner, goblet cell disappeared, Lieberkuhn crypt not hyperlasia, but diminished, and from quantitative measurement, showed significant villi atrophy and crypt depth changes. It means that treatment PEWS for 14 days caused atrophy villi in jejunum Wistar rats. It is suggested that in PEWS contained ANF and the reducing body weight effect was a synergistic effect by CCK and the ANF, although we have not measured the ANF level in this extracts either.

PEDT group showed significant decreasing food intake on the 14th day (average 16.90 to 12.53 g) but no significant effect reducing body weight statistically. Although there was a tendency in reducing body weight (average 230.67 to 199.67 g), its potential was lower compared with PEDS this may due to β-conglycinin in PEDT was lower than in PEDS and CCK level in PEDT is lower than that in PEDS (36.03 and 34.80 ng/ml). Feng *et al*. (2007) stated that fermentation has multiple effects on the nutritional value of soybean and soybean products. If we compared the histological measurement of PEDT group with normal cell in group NCA, qualitatively it showed a slight villi atrophy and it was better if compare with villi atrophy that occur in group PEWS and PEDS. The improvement of intestinal morphology may be associated with the degradation of antigenic materials after fermentation. It was reported that fermentation could degrade large-size protein to small-size peptides (Kiers *et al.* 2000; Hong *et al.* 2004). However, the height of villi still decreased, the absorbtive epithel (enterocyte) damaged, the lamina propria became thinner, a few Goblet cell appeared, Lieberkuhn crypt diminished. Quantitative measurement showed significant villi atrophy and changes of crypt depth. In making tempeh procedures, soybean was boiled at 90-100 °C and it was assumed that ANF in soybean become inactive. But treatment PEDT for 14 days still caused villi atrophy. It is assumed that PEDT still contained residual ANF and the reduction of body weight was a synergistic effect of β-conglycinin and the ANF. The ANF is a thermolabile compound which several component are inactivated by heating. But there was no study stated the duration of heating process to eliminate all ANF completely (Hermana *et al*. 1999).

Morphological changes in cells and organs occured in physiological and pathological condition (Kumar *et al*. 2005). Disability jejunum mucosa to adapt ANF toxicity caused cell injury and will end to apoptosis, the program cell death (Kumar *et al*. 2005; Boudry *et al*. 2007). This will cause atrophy in jejunum villi mucosae which is treated with soybean suplement. The cell recovery of jejunum mucosae by proliferation was not yet seen in this study which only 14 days of treatment. In previous studies, villi atrophy will be followed by hyperregeneration stem cells causing hyperplasia Lieberkuhn crypt to supply new absorbtive epithel (enterocyte) to the villi surface (Yen *et al*. 1977; Dunsford *et al*. 1989; Palacios *et al*. 2004; Feng *et al*. 2007).

The mechanism of soybean trypsin inhibitor and lectin in changing the histology of small intestinal mucosa was still unclear but both trypsin inhibitor and lectin can caused changes of villous height and crypt depth which have already been proved as two indicators small intestine morphometry (Yen *et al*. 1977; Feng *et al*. 2007).

Soybean Trypsin Inhibitor act by binding the pancreas enzyme, Trypsin and Chimotrypsin, and it disturbed the enzyme's function to digest protein in food (Guyton & Hall 2006). This can induce pancreas to produce more digestive enzyme and it caused elevated enzyme level and pancreas hypertrophy. Pancreas disability to compensate will change the microscopic structure of the small intestine (Khayambashi & Lyman 1966). While Soybean Lectin act by binding carbohydrate component in brush border membrane (BBM) in small intestine enterocyte. This interaction is a toxic matter and caused microvilli damage, increasing epithel turnover flow and increasing mucous product, so it caused decreasing enzyme product and ability the small intestine to digest and absorb (George *et al*. 2007). Little is known about the molecular mechanisms that mediate the enterothrophic actions of specific nutrients (Jenkins & Thompson 1994).

As a conclusion PEWS, PEDS, and PEDT Soybean treatment to Wistar Rats for 14 days increasing CCK plasma level and caused atrophy villi and the reducing body weight was caused not only by β-conglycinin effect but probably by Anti Nutrition Factor in soybean as well.

As a suggestion, the ANF level in PEWS, PEDS, and PEDT Soybean need to be measured and study with longer period is needed.

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