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Effectivity of Immunoglobulin Y Anti Lipase as a Pancreatic Lipase Inhibitor for Prevention of Obesity*

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Abstract: Obesity has been a world-wide health problem and associated with many degenerative diseases. Two approved anti-obesity compounds, sibutramine and orlistat, have various serious side effects which limit their uses. IgY anti lipase will inhibit the activity of pancreatic lipase in the gastrointestinal tract. The objective of this study was to evaluate the effectivity of IgY anti lipase obtained from yolk as pancreatic lipase inhibitor for prevention of obesity. IgY anti lipase was obtained from the yolk of hens immunized with porcine pancreatic lipase and was purified with sodium sulphate precipitation. The protein was analyzed by SDS-PAGE and was identified by Immunoblotting. The ability of IgY anti lipase to inhibit pancreatic lipase was tested by ELISA colorimetric microplate assay at five grades of concentration. Yolk containing IgY anti lipase was then tested in rabbit to evaluate its ability to inhibit fat absorption *in vivo*. The result showed that IgY contained two proteins with molecular weights of 61.2 and 26.9 kDa and was positively reacted with the rabbit anti chicken coupled with HRP. IgY anti lipase and orlistat did not differ in inhibition capacity at various concentrations (p>0.05). The inhibition capacity of IgY obtained from unimmunized hen was significantly lower than IgY anti lipase and orlistat (p<0.05). Yolk containing IgY anti lipase also had the ability to inhibit dietary fat absorption *in vivo*, but its inhibition capacity was lower than orlistat (p<0.05). It is concluded that IgY anti lipase could be used to inhibit fat absorption to prevent obesity.

Key words: IgY anti lipase, orlistat, anti-obesity, inhibition, pancreatic lipase, fat absorption

INTRODUCTION

Obesity is a pathological condition characterized by the excessive accumulation of fat in the body with body mass index (BMI) is greater than or equal to 30 (WHO, 2013). Obesity has been a serious concern in all countries because it becomes a major risk factor for cardiovascular diseases, diabetes mellitus, musculoskeletal disorder and cancer with an increasing prevalence. It was estimated that obesity treatment accounted for 2 to 7% of total health-care costs. There are also other aspects to be considered such as reduced quality of life and the loss of productivity due to medical leave (WHO, 2003). According to a global estimate by World Health Organization (WHO), in 2008 there were about 1.4 billion overweight adults and among them at least 500 million adults were obese. The WHO further projects that by 2015, approximately 2-3 billion adults will be overweight and more than 700 million will be obese (WHO, 2006, 2013).

Currently, there are only two types of anti-obesity: appetite suppressant (amfepramone) and lipase inhibitor (orlistat). Previously approved anti-obesity such as sibutamine and rimonabant were withdrawn from the market in EU because of its side effects such as increased blood pressure, dry mouth, constipation, headache and insomnia (de Simone and D'Addeo, 2008; Dujovne *et al.*, 2001;

Tonstad *et al.*, 2016). Lipase inhibitor is more preferable because it works locally in gastrointestinal tract by its binding to pancreatic lipase so that it can be used in a longer period and its side effects are supposed to be minimum (USDHHS, 2004). In 2009, the US Food and Drug Administration issued a warning about a possible link between orlistat and serious liver injury and approximately 20% of patients develop unacceptable side effects, such as diarrhea, flatulence, bloating, abdominal pain and dyspepsia (FDA, 2012; Padwal *et al.*, 2003).

Pancreatic lipase is responsible for the hydrolysis and absorption of 50-70% dietary fat and its inhibition is the most widely studied mechanisms for determination of the potential efficacy of many bio actives as anti-obesity agents (Birari and Bhutani, 2007). Orlistat (tetradi-hydrolipstatin) is the only lipase inhibitor that is used for obesity treatment. Orlistat covalently binds to the binding site of pancreatic lipase (serine-152) with strong, stable and irreversible binding (Hadvary *et al.*, 1991). Its binding will reduce triglyceride absorption and reduction in gastrointestinal tract lipid digestion is related to the decrease in the intra-abdominal fat content (Rubio *et al.*, 2007). Beside its side effect, the major limitation of using orlistat as an anti-obesity agent is the high price that makes it unaffordable by all social classes.

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Since obesity is so prevalent, the demand for anti-obesity treatment is very high. Many researches have been conducted to find new lipase inhibitors and various phytochemicals like saponin, polyphenol, flavonoid and caffeine from various plants like *Panax janonicus*, *Playticodi ratix*, *Salacia reticulata*, *Nelumbo nucifera*, *Cnidium officinale*, *Coleus forskohlii*, *Salacia reticulate* and *Sesamum indicum* were tested and confirmed as lipase inhibitors, but none of them has been produced commercially (Badmaev *et al.*, 2015; Kazemipoor *et al.*, 2012; Mo *et al.*, 2016; Yun, 2010; Zheng *et al.*, 2010).

Based on those facts, there should be a new methodological approach for the invention of new lipase inhibitor. Using IgY anti lipase from laying hens immunized with pancreatic lipase is one of the new and prospective approaches. It was reported that IgY anti lipase obtained from the eggs of immunized laying hen could inhibit pancreatic lipase activity and reduce body fat deposition (Pimentel, 2008). This experiment was conducted to study the feasibility of using IgY anti lipase in yolk to inhibit the activity of pancreatic lipase in the gastrointestinal tract. IgY anti lipase will be an interesting alternative for anti-obesity because it is safe and natural.

The molecular weights of IgY and porcine pancreatic lipase are 180 and 50 kDa, respectively, with ratio of 3.6: 1. IgY has two binding sites on its heavy chain, it means the ratio between the amount of IgY and porcine pancreatic lipase for total inhibition is 1.8: 1. The amount of IgY in one egg is 100-150 mg (Akita and Nakai, 1992; Carlender, 2002; Schade *et al.*, 1991), while the total secretion of human pancreatic lipase after eating a high fat meal is 200-250 mg (Carriere *et al.*, 2005). It is assumed that total amount of IgY anti lipase in yolk of one egg is enough to inhibit 30-55% of pancreatic lipase secretion in one person.

Immunization of laying hen to produce antibody against pancreatic lipase is not a problem because chicken has a developed immune system that is sensitive to foreign protein. Immunization of laying hens with mammalian pancreatic lipase will produce a high and specific antibody in the serum and yolk because of the phylogenetic distance between chicken and mammals (Larsson *et al.*, 1988). The stability of IgY in oral gastrointestinal tract has been well-documented and IgY has been used in the treatment or prevention of dental caries, periodontitis, gingivitis, gastritis and gastric ulcer (Nolan and Mine, 2004; Rahman *et al.*, 2013). With recent trends in consumer preference for natural products, yolk containing IgY anti lipase could be functional for treatment and prevention of obesity. The main problem is whether the IgY anti lipases produced have the abilities to neutralize pancreatic lipase activities and IgY anti lipases administered orally do not lose their activities by the destruction of the molecule by HCl and proteolytic enzymes produced in the gastrointestinal tract. This experiment was designed to produce IgY anti lipase that could inhibit lipase activities in the gastrointestinal tract and used for obesity treatment.

MATERIALS AND METHODS

Purity analysis of porcine pancreatic lipase: Porcine pancreatic lipase was purchased from AppliChem (catalog #A9520). Purity analysis was done because there was no information about purity in the label and porcine pancreatic lipase used was partially soluble in water (PBS). Porcine pancreatic lipase was diluted in phosphate buffer saline (2.5 mg/mL) and centrifuged at 3000 x g for 20 min. The supernatant and pellet were collected separately. The purity of porcine pancreatic lipase solution at various fractions was monitored by sodium dodecyl sulphate-polyacrilamide gel (SDS-PAGE). Protein concentration was determined by Bradford method using bovine serum albumin as a standard protein. The injected lipases were calibrated based on their protein concentrations.

Chicken immunization: Four ISA Brown hens (*Gallus gallus domesticus*) with 10 weeks of age were immunized intramuscularly with 1 mL of porcine pancreatic containing lipase (2.5 mg in 1 mL PBS) using an equal volume of Quil-A adjuvant (2 mg in 1 mL PBS, Brenntag) and one hen was used as a control. Each hen was injected at two different sites (500 µL per site) in the breast muscle. Two booster injections, with Quil-A adjuvant, were given at 4 weeks interval following the first immunization. After the third immunization, the sera were collected and their antibody titers were determined by Enzyme Linked Immunosorbent Assay (ELISA). When the titer of antibody remained stable or reached plateau, the immunization was stopped and the eggs laid were collected daily for one week and stored at 4°C. The egg yolk was separated and pooled and then frozen prior to IgY isolation and purification.

IgY purification from yolk: Purification of IgY was carried out by the method described by Jenesius and Koch (1997). Egg yolk was separated from the albumen and the yolk was diluted with 4 volumes of TBS (50 mM Tris, 150 mM NaCl pH 7.4). The solution was then centrifuged at 3000 x g for 20 min at 25°C and the supernatant was collected. Dextran sulphate (10% solution, AppliChem) and 1 M $CaCl_2$ were added at 120 and 50 µL, respectively per mL supernatant and incubated for one hour at 25°C. This solution was then centrifuged again and the supernatant was collected. Successful delipidation was indicated by a clear supernatant. Addition of Dextran Sulphate and $CaCl_2$ was indicated by a clear supernatant. Delipidated yolk was stirred and 20 g sodium sulphate powder was added slowly. After all the sodium sulphate dissolved, stirring was allowed for 30 min. The solution was centrifuged at 3000 x g for 20 min at 25°C. The pellet was collected and dissolved in 10 mL TBS and centrifuged again. The supernatant was collected and then stirred with

addition of 36% sodium sulphate. The solution was centrifuged again and the pellet was collected, dissolved in PBS and dialyzed against PBS to produce high purity IgY. Protein concentration of IgY was determined using Bradford method.

SDS-PAGE and immunoblotting: The purity of IgY protein was monitored by SDS-PAGE. SDS PAGE was carried out by the method described by Laemlli (1970) using Mini-PROTEAN III Cells (Bio-Rad Laboratories), 10% separating gel and 4% stacking gel. Electrophoresis was done under reducing condition, samples were diluted with sample buffer containing 5% (vol/vol) b-mercaptoethanol and heated for 5 min at 100°C. Fifteen microliters of sample was loaded into each well (3 µg of protein per well). Prestained SDS-PAGE standard (Bio-Rad Laboratories) was used as a molecular weight marker. The purity confirmation of IgY was done with Immunoblotting using the rabbit anti chicken IgG coupled with HRP (Sigma-Aldrich #A9046) and pure nitrocellulose membrane 0.45 µm.

ELISA: ELISA was performed to measure antibody titer against porcine pancreatic lipase; the method was adapted from Akita and Nakai (1992) with a modification. A micro plate (Maxisorp, Nunc) was coated with porcine pancreatic lipase (8 µg in 1 mL 0.1 carbonate buffer pH 9.6) and incubated at 4°C overnight. The micro plate was then blocked with nonfat skim milk (50 mg/mL). After blocking, the purified IgY was added using two folds serial dilution. The plate was then incubated 2 hours at room temperature and was washed with washing buffer (0.2 g KCl, 0.2 g KH_2PO_4, 500 µl Tween, 1.15 g Na_2HPO_4 and 37.5 g NaCl in 1 liter aquabidest). The rabbit anti chicken IgG coupled with HRP at dilution 1:2500 (Sigma #A9046) was added and the plate was then washed with washing buffer five times. ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) was added as a substrate. After incubation for 15 min, the absorbance was measured at 420 nm using micro plate reader (Multiskan EX, Thermo).

Lipase inhibition assay: Lipase inhibition assay was adapted from ELISA colorimetric micro plate assay reported by Choi *et al.* (2003). The assay was based on the spectrophotometric quantification of free thiols with chromogenic 5,5'-dithiobis (2-nitro benzoic acid) (DTNB, Ellman's reagent) released by porcine pancreatic lipase from the 2,3-dimercapto-1-propanol tributyrate (DMPTB, 97%).
The porcine pancreatic lipase was prepared as a stock at 10 mg/mL in buffer L (10 mM KCl, 10 mM Tris-Cl, pH 7.5) and stored at -80°C. The working samples of enzyme were diluted to 2 mg/mL in buffer L. The standard reaction mixtures containing 20 µL of 10 mM DMPTB, 20 µL of 40 mM DTNB, 2 µL of 0.5 M EDTA 0.05%, 5 µL 10% Triton

X-100 and 50 µL Tris-Cl 1 M were mixed in a micro-centrifuge tube and 803 µL deionized water was added. Micro plate wells were filled with 10 µL of working sample enzyme and 10 µL of lipase inhibitors (orlistat/Xenical®, IgY anti lipase and IgY from unimmunized hen) at five grades of concentrations. The concentrations of inhibitors were counted based on their molecular weight ratio between pancreatic lipase and inhibitor. Micro plates were immediately transferred to a 37°C incubator to stimulate the binding of lipase with the inhibitor. Each micro plate well was then filled with 180 µL of reaction mixture and was incubated at 37°C for 30 min. The absorbance of each well at 405 nm was measured with micro plate reader. Inhibition of lipase activity was expressed as percentage of decrease in the OD between lipase incubated without inhibitor and with inhibitor.
Inhibition of dietary fat absorption in gastrointestinal tract Twelve six-month old New Zealand White male rabbits were used in this experiment. The experimental rabbits were housed in individual cages. The experimental rabbits were acclimated for 1 week and during acclimation the rabbits were fed ad libitum with Indo Feed® K-03 Super with ingredients (corn, pollard, rice bran, molasses, anti-mold, antioxidant, vitamin and mineral) and nutrient level (Crude Protein 16%, Fat 5%, Crude Fiber 13%, Ash 9%, TDN 70%, Ca 0.8%, P 0.6%) and had free access to water. The experimental rabbit were assigned into four groups of treatment with 3 replications. Group 1 (Negative control) was fed with 50 g standard ration/rabbit twice a day. Group 2 (Positive control) was fed with 50 g standard ration (5% fat)/rabbit twice a day and 5 g yolk (34% fat)/rabbit twice a day (high fat ration). Group 3 was fed with high fat ration twice a day and orlistat 5.6 g/kg twice a day. Orlistat was given one hour after meal in one week. Group 4 was fed with high fat ration and 3 g yolk containing IgY anti lipase twice a day. The feces from each rabbit were collected one day after treatment and the fat content was measured. The apparent fat digestibility was determined as the difference between the dietary fat intake and the fat excreted in feces.

Statistical analysis: The data on inhibition activity of porcine pancreatic lipase, total fat feces content and fat percentage in feces were analyzed on group linear model of SPSS. All data were presented as mean±SD. When necessary, mean separation was accomplished by using Duncan's multiple-range test (Duncan, 1955), a probability p-value of less than 0.01 and 0.05 was considered significant.

RESULTS
Porcine pancreatic lipase solution and its supernatant had proteins having similar molecular weight (50.1 kDa and 49.1 kDa, respectively) (Fig. 1). On the other hand, the pellet contained protein having molecular weight of 20.6 kDa lipase solution (2.5 mg/mL in PBS) contained 2.0

mg/mL protein and its supernatant contained 1.9 mg/mL with Bradford method. It meant that the protein concentrations were 80.7 and 77.9%, respectively.

The average ELISA's optical density (absorbance at 420 nm) of serum from immunized hens from pre-immunization until the third immunization were 0.3±0.1, 0.5±0.2, 2.0±0.4 and 2.2±0.6, respectively. The average ELISA's optical density of serum at the third immunization was 7.6 times higher as compared to that at pre-immunization. The increased ELISA's optical density of serum showed the increased specific antibody against porcine pancreatic lipase in serum. The purified IgY contained two protein bands on SDS-PAGE with molecular weights of 61.2 kDa and 26.9 kDa (Fig. 3a) and was confirmed as IgY because it positively reacted against the rabbit anti chicken IgG coupled with HRP at Immunoblotting (Fig. 2b). The average IgY concentration from immunized and unimmunized hens was 30.1±6.3 and 17.7±2.1 mg/mL yolk, respectively. The average ELISA's optical density of purified IgY from immunized and unimmunized hens were 1.842±0.007 and 0.007, respectively. Eggs were collected and its IgY was purified after the third immunization because the antibody titer was high. It meant that the purified IgY from immunized hens had a high antibody titer against porcine pancreatic lipase because its average ELISA's optical density was 21.32 times higher than control.

IgY anti lipase had the ability to inhibit porcine pancreatic lipase activity at various concentrations (p<0.05) and did not have any difference as compared to orlistat at the same equimolar (Table 1). IgY from unimmunized hen did not have the ability to inhibit porcine pancreatic lipase activity. The IC_{50} of orlistat and IgY anti lipase were 0.996 µg/mg lipase and 340.08 µg/mg lipase, respectively.

in vivo experiment showed that rabbit treated with yolk containing IgY anti lipase and orlistat had fewer apparently digested fat and had higher fecal fat content (p<0.05) as compared to untreated rabbit (Table 2). However, the pancreatic lipase inhibition activity of orlistat was still higher than that of yolk containing IgY anti lipase (p<0.05).

DISCUSSION

Porcine pancreatic lipase used was partially soluble in water, purity analysis with SDS-PAGE and protein measurement with Bradford method was done to ensure that pancreatic lipase was at the soluble fraction. As shown in Fig. 1, porcine pancreatic lipase solution and its soluble fraction had protein having similar molecular weight. De Caro *et al.* (1981) reported that molecular weight of porcine pancreatic lipase was 49.859 kDa. On the other hand, the pellet had a different molecular weight against the soluble fraction, it meant that there was no pancreatic lipase in the insoluble fraction and the soluble fraction contained the same protein as the lipase solution. The lipase solution (2.5 mg/mL in PBS) contained 2.017 mg/mL protein and the supernatant contained 1.949 mg/mL. It meant that the porcine pancreatic lipase used was not entirely composed of protein. The decreased protein concentration might be caused by a partial denaturation of lipase during the preparation.

Immunogenicity of porcine pancreatic lipase in laying hen was well defined at high titer of antibody against lipase in the sera. High titer of antibody against porcine pancreatic lipase was reflected by a higher optical density. The first immunization only increased the optical density by 1.636 times, but the second and the third immunization increased the optical density by 6.94 and 7.6 times, respectively. The increased antibody titer showed that booster immunization could maintain and increase the titer

Table 1: Inhibition of pancreatic lipase activity*

--------------- Orlistat ----------------		-------------- IgY antilipase ---------------		------------------ IgY control -----------------	
Concentration (µg/mL)	Inhibition activity (%)	Concentration (µg/mL)	Inhibition activity (%)	Concentration (µg/mL)	Inhibition activity (%)
12.5	69.8±0.9[a]	5000	65.6±2.2[a]	5000	13.6±6.6[b]
6.25	65.6±0.6[a]	2500	61.1±3.9[a]	2500	19.6±2.3[b]
3.13	56.6±1.1[a]	1250	54.1±1.5[a]	1250	12.1±3.2[b]
1.56	44.6±0.6[a]	625	50.5±0.6[a]	625	17.9±1.3[b]
0.78	39.7±0.9[a]	312,5	43.1±1.2[a]	312,5	15.2±5.8[b]

[a,b]Values within the same column with no common superscript are different significantly, p<0.05. *Values are means±SD

Table 2: Fat intake, fat content of feces and the apparently digested fat in rabbit*

		--- Fat content in feces (Dry matter) ---		-------- Apparently digested fat --------	
Group	Dry matter fat intake (g)	(g)	(%)	(g)	(%)
1	4.11±0.18	0.78±0.18[a]	2.59±0.17[a]	3.33±0.26[b]	80.8±4.59[a]
2	4.72±0.19	1.10±0.01[b]	2.68±0.23[a]	3.63±0.19[c]	76.6±0.96[b]
3	3.78±0.25	1.22±0.08[b]	5.90±1.80[b]	2.56±0.22[a]	67.7±2.03[c]
4	4.45±0.24	1.14±0.02[b]	3.96±0.57[a]	3.27±0.25[b]	74.1±1.2[b]

Group 1: Normal feeding, Group 2: High fat feeding, Group 3: High fat feeding and orlistat, Group 4: High fat feeding and egg yolk containing IgY anti lipase. [a,b]Values within the same column with no common superscript are different significantly, p<0.05. *Values are Means±SD
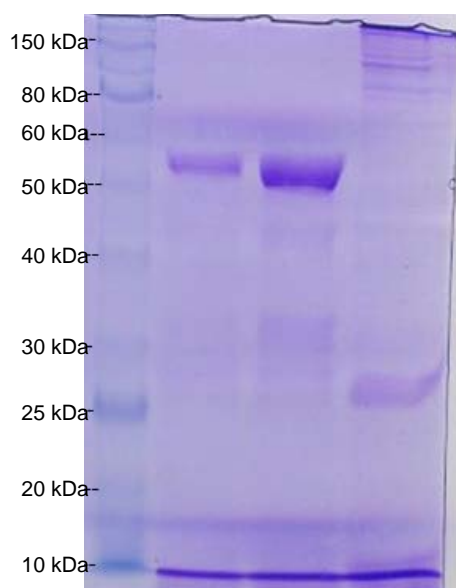
Fig. 1: SDS-PAGE analysis of porcine pancreatic lipase, ST: Protein Marker, L: Lipase solution, S: Supernatant, P: Platelet

reported that chicken would produce a more specific antibody against mammalian protein as compared to mammalian response. Titer of antibody of hens immunized with mammalian protein (bovine serum albumin) increased 14 days after immunization and reached peak 56 days after immunization. Immunization was stopped after the third immunization because the optical density after the second until the third immunization was remained stable. IgY was purified from egg yolk after the third immunization to obtain the high titer of antibody against lipase.

IgY was purified with sodium sulphate precipitation. This method was the first choice for IgY purification because it produced IgY with high purity (Jenesius and Koch, 1997). Hatta *et al.* (1990) produced IgY with high purity (98.7%) of antibody because the immunogen had been recognized by the immune system of the laying hens. Li *et al.* (1998) after three times salting-out with sodium sulphate. The average protein concentration of IgY in immunized hens was 1.7 times higher than the unimmunized hen.

The purified IgY obtained from immunized hen was analyzed using SDS PAGE. As shown in Fig. 2a, the purified IgY contained two protein bands that its molecular weight was dissociated into heavy and light chains of IgY. Hatta *et al.* (1990) found that IgY contained two proteins with molecular weights of 70 kDa for heavy chain and 21 kDa for light chain. In immunoblotting analysis, it was confirmed that the purified molecule was IgY because it positively reacted with the rabbit anti chicken IgG coupled with HRP (Fig. 2b). The purified IgY from immunized hens had a high antibody titer against porcine pancreatic lipase, but purified IgY from unimmunized did not have

antibody titer against porcine pancreatic lipase. This result indicated that IgY produced was specific to lipase. Even though the specific antibody against porcine pancreatic lipase was produced successfully, the ability of IgY anti lipase to neutralize porcine pancreatic lipase must be verified first by *in vitro* lipase assay. It is because the binding site (Ser-152) and active site of pancreatic lipase (His-354) has a low antigenic score, so there is still a probability for IgY anti lipase not to recognize and bind to the binding and active site of pancreatic lipase. The failure of that binding caused pancreatic lipase failed to bind to and hydrolyze their substrate.

The inhibition activity was tested at five gradient concentrations of inhibitors against porcine pancreatic lipase (2 mg/mL). Different concentrations of inhibitors were used to evaluate correlation between the addition of inhibitor and the increased inhibition activity (Fig. 3). The inhibitor concentration was based on the comparison of molecular weight between inhibitor and pancreatic lipase. It was shown in Table 1 that IgY anti lipase (IC_{50} 340.08 µg/mg lipase) and orlistat (0.996 µg/mg lipase) had the same ability to inhibit the porcine pancreatic lipase activity without a significant difference ($p<0.05$) (Table 1). Ramirez *et al.* (2012) found that the IC_{50} of orlistat was 0.142 µg/mL. The IC_{50} of IgY anti lipase was similar to IC_{50} of several natural products that tested by Ramirez *et al.* (2012), such as *Carmellia sinensis* (IC_{50} 299 µg/mL), *Ludwigia octovalvis* (IC_{50} 202 µg/mL) and *Iosthephane heterophyllia* (IC_{50} 509 µg/mL).

Substrate used in this assay (DMPTB) was analog of triglyceride having two thioester groups which were hydrolyzed by pancreatic lipase (Park *et al.*, 2008). Porcine pancreatic lipase has activity of 30-90 U/mg. It means that this enzyme will release 30-90 µmol DMPTB/minute. With 340.08 µg IgY anti lipase will inhibit the release of 14-45 µmol DMPTB per minute or 5-15 g DMPTB (MW 334.49 g/mol) per minute. Compared with total secretion of human pancreatic lipase after eating a high fat meal (700 mL) which is 200-250 mg, as much as 65-85 mg of IgY anti lipase is needed to inhibit half of the pancreatic lipase activity. This amount could be obtained from 2 g eggs yolks from immunized hen. IgY from unimmunized hen did not have the ability to inhibit the activity of porcine pancreatic lipase (Table 1). It was shown that the inhibition activity of IgY anti lipase originated from antibody produced during immunization and this antibody was specific to the binding and active sites of porcine pancreatic lipase.

Rabbit was used in this research because its pancreatic lipase has 76% similarity in amino acid sequence with human and porcine pancreatic lipases and have the same binding site (Ser-152) (Gomez *et al.*, 1992). As shown in Table 2, rabbits fed with high fat ration (Group 2) had a higher apparently digested fat in gastrointestinal tract as compared to rabbit fed with normal ration. Higher fat ration would increase the secretion of pancreatic lipase
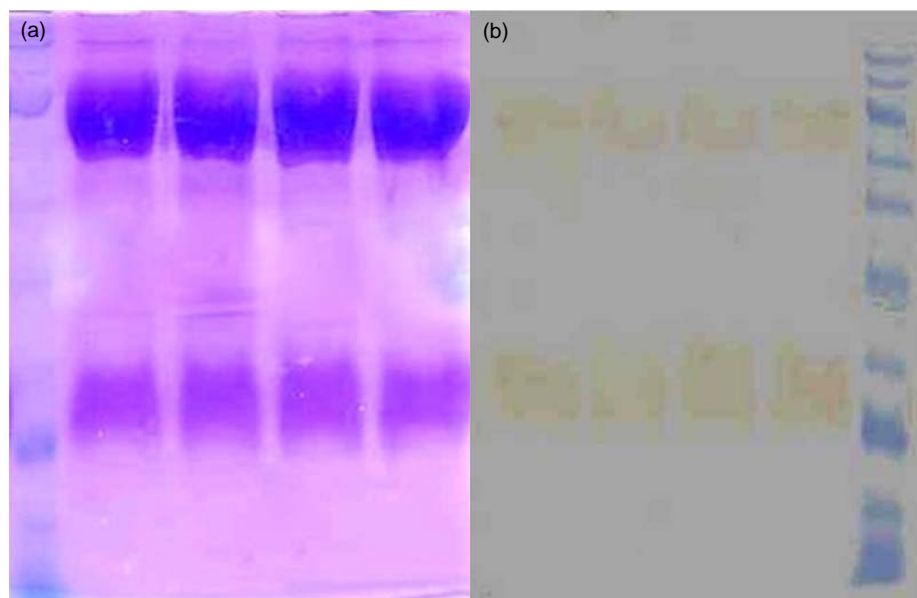
Fig. 2: SDS PAGE analysis of IgY from immunized hens (a), Western Immunoblotting analysis from immunized hens (b)
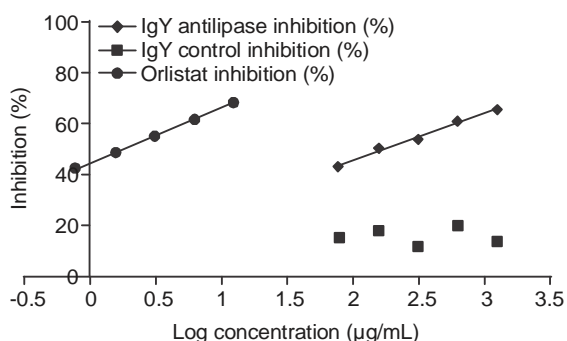

Fig. 3: Inhibition activity of orlistat (●), Inhibition activity of IgY anti lipase (♦), IgY from unimmunized hen (■)

would increase the hydrolysis of triglyceride to fatty acid which will be absorbed in small intestine (Dojana *et al.*, 2012). Rabbits treated with orlistat (Group 3) had lower amount and percentage of the apparently digested fat in small intestine than the other groups (Table 2). This result showed that orlistat had ability to inhibit rabbit pancreatic lipase activity. Orlistat decreased the apparently digested fat by 32.3%. Carriere *et al.* (2005) reported that orlistat treatment in human consumed high fat meal inactivated human pancreatic lipase by 51.2±34.6% and inhibited the apparently digested fat by 43.6±16.8%.

Yolk containing IgY anti lipase (Group 4) has the ability to inhibit the apparently digested fat in small intestine. The apparently digested fat of dietary fat in rabbit treated with yolk containing IgY anti lipase was lower (p<0.05) as compared to rabbit fed with high fat ration, but the inhibition is still higher than rabbit treated with orlistat. Based on those data, IgY anti lipase had a potency to be

used as a new anti-obesity compound. The lower inhibition activity of IgY anti lipase might be caused by the partial destruction of IgY anti lipase by proteolytic enzymes in gastrointestinal tract. Jaradat and Marquardt (2000) and Carlender (2002) reported that yolk IgY administered orally could maintain their activity against digestion from pepsin and trypsin because of protective and buffering effects of yolk. Nevertheless, these proteolytic enzymes still had the ability to decrease the IgY activity because IgY will split into F_{ab}, F_{ab2} and F_c fragments. F_{ab} and F_{ab2} still active but their activities were lower than that of the whole IgY molecule.

There are two mode of action of functional food for obesity treatment: reduce energy intake and increase energy expenditure. Foods that have a modified energy density, macronutrient composition and glycemic index can reduce the energy intake. Green tea and oolong tea contain catechin tea and caffeine claimed can increase energy expenditure by inhibition of O-methyl transferase and phosphodiesterase (Choudhary and Grover, 2012). Unfortunately there are little data to support the efficacy of these products and the safety for long term use. Yolk containing IgY anti lipase can reduce energy intake by pancreatic lipase inhibition. Oral administration of yolk containing IgY anti lipase is safe because there was no absorption of antibodies in gastrointestinal tract and no increase in serum immunoglobulin levels after oral administration of immunoglobulin in humans (Blum *et al.*, 1981; Eibl *et al.*, 1988). be taken when administering yolk containing IgY anti lipase to person that has allergic reaction to egg or yolk.

Present result showed that immunization of hens with porcine pancreatic lipase produces high and specific

antibody against porcine pancreatic lipase in the serum and egg yolk, the absorbance of immunized hen's serum was 7.6 times than that of unimmunized hen's serum and the absorbance of purified IgY from immunized hen was 21.3 times than that of unimmunized hen. Purified IgY anti lipase from immunized hen had the same porcine pancreatic lipase activity inhibition with orlistat in *in vitro* pancreatic lipase inhibition assay in various concentrations. Yolk containing IgY anti lipase had the ability to decrease the apparently digested fat in gastrointestinal tract, but its ability was still lower than orlistat.

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