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Article

Effects of reducing postprandial hyperglycemia and metabolism of acetate wheat starch on healthy mice

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Recently, the acetate wheat starch (AWS) has been prepared by acetylation with an acetyl content of 2.42%, containing of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) with 25.0%; 22.9% and 34.5%, respectively. In this study, this kind of starch was continuously evaluated with the postprandial blood glucose response and determined short-chain fatty acids (SCFAs) metabolized from AWS in the gastrointestinal tract of healthy mice by HPLC. The result showed that the mice fed with AWS exhibited a very limited increase in blood glucose level and remained stable for 2 hours after meals efficiently comparing with the control group fed with natural wheat starch (NWS). Simultaneously, the content of SCFAs produced in the caecum of the mice fed with AWS was significantly higher than mice fed with NWS, especially with acetic and propionic acids by 28% and 26%, respectively. Thus, AWS has shown to limit the postprandial hyperglycemia in mice effectively through the resistance to amylase hydrolysis in the small intestine. When going into the caecum, it is fermented to form SCFAs providing a part of energy for the body's activities, avoiding rotten fermentation causing digestive disorders which are inherent restrictions of normal high cellulose and fiber food.

Keywords: Acetate wheat starch. Glucose. SCFA, Caecum.

INTRODUCTION

Diabetes is one of the considerable concerns of society because of the rapid increase in the world. This is a potential threat to endocrine diseases and cardiovascular diseases. In the treatment regimens for diabetes, not only drug therapy, diet and lifestyle changes also play very important roles. Beside recommending to limit the amount of starch consumed in people with diabetes, nutritionists are looking for foods that provide energy for daily activities without increasing postprandial blood glucose levels. So resistant starch (RS), a type of starch that is resistant to hydrolysis of amylase in the gastrointestinal tract, is one of the practical options in many recent researches (Birt *et al*., 2013).

All over the world, there have been many studies on RS. Those studies have confirmed the role of this starch due to its effect on the resistance to amylase. This type of starch is not digested in the small intestine, which helps to significantly reduce the sudden increase in blood glucose levels *in-vivo* as well as in clinical. Besides, after passing out of the small intestine and going into the large intestine, RS will be fermented into short-chain fatty acids (SCFAs), which are fatty acids structured from 1 - 6 carbon and existing in the form of straight or branched chains. After being absorbed into the circulation, SCFAs have supplied the body with energy as well as affected the biochemical parameters of diabetic patients. Thus, FDA approved and authorized some RSs as functional foods to support the treatment of diabetes in 2016 (FDA Announces Health Claim For Resistant Starch, 2016).

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In 2018, Uyen *et al.* began to study on the semi-synthesis of acetate wheat starch (AWS) oriented for supporting the treatment of diabetes mellitus. This is a type 4 resistant starch (RS4) formed by chemical modification and possessing a strong resistance to the enzyme amylase *in-vitro*. For further knowledge about the improvement in postprandial glycemic response on living body of AWS and the metabolism of this starch, this study has continued with objectives to evaluate the postprandial hyperglycemic effects of ACWS in healthy mice compared with natural wheat starch and to determine SCFAs metabolized from this starch in the gastrointestinal tract of experimented mice by HPLC.

MATERIAL AND METHODS

Material

Natural wheat starch (NWS) and acetate wheat starch (AWS) were prepared by acetylation with an acetyl content of 2.42%, containing of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) with 25.03%; 22.85% and 34.54%, respectively.

Male and female white Swiss mice which weighed 16 - 20g, were 4 weeks old, mature and healthy were obtained from the Institute of Vaccines and Biological Medical of Nha Trang, Vietnam. Mice were fed for two weeks with a normal diet to adapt to the experimental environment. All procedures were subject to regulations of animal experimentation from the Ethics Committee of College of Medicine and Pharmacy, Hue University and the Guide for the care and use of laboratory animals, Copyright 2011 by the National Academy of Sciences, USA.

Evaluation of AWS's ability to limit postprandial hyperglycemia in mice

Thirty healthy mice which were chosen in random with body weight 22 – 25g were divided into 3 groups in different cages (N = 10). Mice have fasted 16 hours overnight before the test. Three of each suspension containing 5g of powder of different starch in 10ml distilled water were prepared before the experiment by mixing in 5 minutes. Each of mice groups was fed with only a volume of each suspension equivalent to 5g/kg body weight by injection with an oral feeding needle. The first group (NWS) was fed with suspension containing NWS, the second group (NWS/AWS) was fed with suspension containing the mixture NWS/AWS following ratio 50/50 (w/w). The third group was fed with suspension containing AWS.

Subsequently, blood was collected from the tail vein before meal and 30; 60; 90; 120 minutes after feeding to determine blood glucose levels with the Accu Check Performa blood glucose meter (Roche, Germany). The experiment was repeated three times to determine the mean values (Shimotoyodome *et al*., 2009; Kimura *et al*., 2013).

The differences between groups were compared with the use of ANOVA test, and differences in the same group at two times were compared with the use of paired t-test, at 95% confidence intervals for both tests.

Determination of SCFA content in gut sections of mice

Twenty healthy mice which were chosen in random with body weight 22 – 25g were divided into 2 groups in different cages (N = 10). The suspensions containing 5g of powder of NWS or AWS starch dispersed in 10ml distilled water were continuously used for this experiment. During 21 days, each mice group was fed with each suspension containing different starch by injection with the oral feeding needle. The first group was fed with a volume of suspension equivalent to 5g of NWS/kg body weight in the morning and the same one in the afternoon. The second group was fed with a volume of suspension equivalent to 5g of AWS/kg body weight in the morning and the same one in the afternoon. During the trial period, mice were permitted to drink unlimited amount of carbohydrate-free milk and distilled water on a daily basis.

On the 21st day, mice were anesthetized and had surgery to obtain metabolic fractions in the gastrointestinal tract including the small intestine, caeca, colon and rectum. The samples were collected to determine SCFAs by hydrazide derivative reaction with 1-EDC-HCl (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride), 2-NPH-HCl (2-nitrophenyl hydrazine hydrochloride)) in pyridine and analyzed on HPLC with ZORBAX Eclipse XDB-C8 column (150 x 4.6 mm, 5 μm) at 40°C, mobile phase consisting of MeOH - ACN - 0.057 mM TFA buffer (pH 4.5) Time: 0:13:87 (0 - 9 minutes) - 10:20:70 (10 - 35 minutes) - 0:13:87 (36 - 40 minutes). The flow rate was 1.4 ml / min, the injection volume was 20 μl, detected at 396 nm. The internal-standard substance is 2-ethylbutyric acid (Khanh, 2017).

RESULTS AND DISCUSSION

Evaluation of AWS's ability to limit postprandial hyperglycemia in mice

The results of the study on the effects of diets containing NWS, NWS/AWS and AWS on postprandial blood glucose levels in mice were presented in Table I. Mean blood glucose values were obtained from three groups (n = 10) right before the trial and 30, 60, 90, 120 minutes after feeding.

TABLE I - Blood glucose levels in three mice groups over the time

	Time (min)	Group NWS	Group NWS/AWS	Group AWS	ANOVA test
Conc. of blood glucose (mmol/l)	0	8.0	9.1	7.6	p > 0.05
	30	14.3	15.5	11.5	p > 0.05
	60	16.1	12.9	10.3*	p < 0.05
	90	12.9	11.3	7.7*	p < 0.05
	120	9.9	9.8	7.6	p > 0.05

* *Statistical significance*

The results showed that blood glucose concentration before feeding was not significantly different between groups (p>0.05). After diet, although there was an increase of postprandial blood glucose concentration in among all groups at 30 minutes. However, postprandial blood glucose concentrations at 60 minutes and 90 minutes in the AWS group (10.30 and 7.7 mmol/l) were significantly lower than the NWS group (16.1 and 12.9 mmol/l) (p < 0.05).

To better illustrate the pre- and postprandial glucose response, the data in Table 1 was converted to the percentage of increased blood glucose at 30; 60; 90 and 120 minutes compared with fasting blood glucose and shown in Figure 1. The Figure 1 showed that postprandial blood glucose levels of the AWS and NWS/AWS groups varied almost negligible compared to the NWS group. This is showed very clearly at the slopes of the graphics. 30 minutes after the diet, the glucose levels in both of the AWS and NWS/AWS groups were also highest (55.8% and 70.5%, respectively), because of the hydrolysis almost completely of the RDS and SDS in these diets by amylase enzymes, but still lower than the NWS group (80.9%) although this difference was not statistically significant (p> 0.05). However, after 60 minutes, blood glucose levels of the AWS and NWS/AWS groups reduced gradually (36.7% and 42.3%, respectively) and after 90 minutes, they were almost equal to fasting glucose levels. At the same time, blood glucose levels in the NWS group continued to rise and peaked at 60 minutes (106%) (p <0.05).

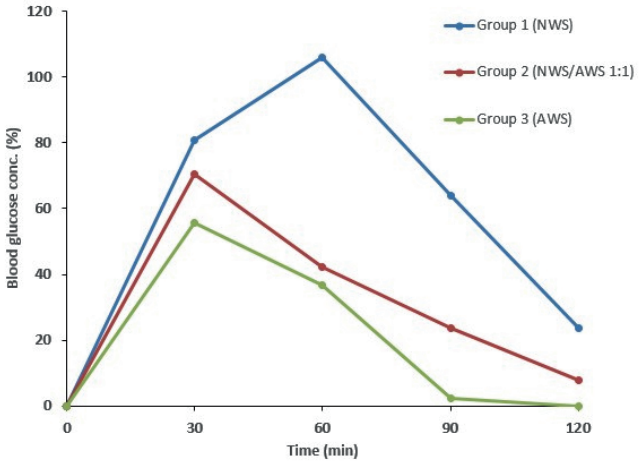

FIGURE 1 - Postprandial glycemic response of three experimental mice groups.

The result found that diets containing AWS had limited the sudden increase in blood glucose level compared to NWS. This explained that AWS contains a higher RS content (34.5%) than NWS, which would inhibit the hydrolysis of carbohydrate portion by amylase enzymes to release glucose into the small intestine effectively. Therefore, it indicated a better control of resistant starch in the change of postprandial blood glucose response compared with the nature starch. This has proven that AWS is capable of regulating blood glucose levels very well. This finding matched with the study of Shimotoyodome *et al.* (2009) who conducted in a model of high-fat diet rats. In their study, the blood glucose level monitored through 120 minutes in the group fed with modified corn starch was significantly lower than the group fed with natural corn starch. In addition, the study also demonstrated that the amount

of insulin secreted to regulate blood glucose levels in mice fed with RS4 was significantly lower in the control group (Shimotoyodome *et al.*, 2009).

Determination of SCFA content in gut sections of mice

After forming hydrazide derivatives and analyzing on HPLC, the content of SCFAs included succinic, acetic, propionic, butyric and valeric acids in the gut sections in each group (NWS and AWS) is shown in Table II.

TABLE II - SCFA content in the gut sections of experimental mice

SCFA (µmol/g)		Suc.	Ace.	Pro.	But.	Val.	Total SCFA
Group NWS	Small intestine	0.7	1.7	0.23	0.1	0.0	2.7
	Caeca	0.9	29.6	5.2	2.3	0.1	38.1
	Colon	0.7	33.5	4.0	1.5	0.1	39.8
	Rectum	0.02	7.2	1.9	0.3	0.1	9.52
Group AWS	Small intestine	0.3	3.0	0.2	0.1	0.0	3.6
	Caeca	2.7	37.8	6.5	2.4	0.1	49.5
	Colon	1.5	42.0	6.2	3.1	0.1	52.9
	Rectum	0.2	14.4	3.6	0.9	0.1	19.2

To make it clearer, the content of SCFA in each digestion segment of the mice is shown in graph of Figure 2. The result showed that there was a variation in SCFA content in both groups in the digestive tracts. Initially, the SCFA content identified in the small intestine was negligible, but in the segment of the caeca and colon, the SCFA content increased dramatically. But in the rectum, the SCFA content was significantly lower than in the previous sections ($p < 0.05$).

The result in Figure 2 demonstrates that most SCFAs are produced by fermentation of anaerobic microorganisms in the large intestine. These acids are produced by the anaerobic fermentation in the caeca and the near colon then reabsorbed in the distal colon, demonstrated by a decrease in the level of SCFAs in the rectum, to provide significant energy for the activities of the body as well as intestinal bacteria. This finding is consistent with the study of Govers *et al.* (1999) in pigs with diets containing RS2. Level of SCFAs in the caeca and the near colon was significantly higher than that in the distal colon (Govers *et al.*, 1999). In addition, the study by Le Leu *et al.* (2006) also performed on rats fed with high-amylose starch diets with different ratios. The results showed that SCFAs were produced in the caecum and gradually decreased in the next segments of the gastrointestinal tract (Le Leu *et al.*, 2007).

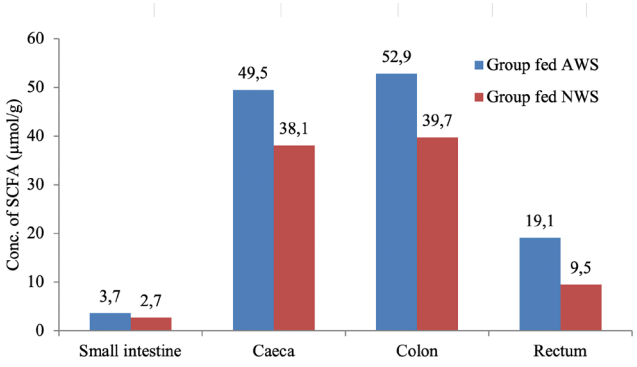

FIGURE 2 - Total SCFA in mice intestinal segments after 21 days of feeding NWS and AWS.

The variation in each SCFA content in mice digestive tract was shown in Figure 3. Acetic acid and propionic acid were still present in high proportions of the total SCFA metabolized in the caeca (77% and 13% for acetic and propionic acid, respectively) in both groups. It showed that acetic acid and propionic acid accounted for a large proportion of the total SCFAs produced in the caecum on both NWS and AWS groups (approximately 90-95%) although the two diets were completely different. Acetic acid had the highest ratio (76-77%), followed by propionic acid (13%). This explained that although microorganisms exist throughout the gastrointestinal tract, the phylum Bacteroidetes, which are responsible for fermentation producing acetic and propionic acids, account for the largest proportion (Macfarlane, Macfarlane, 2003; Ley *et al.*, 2005).

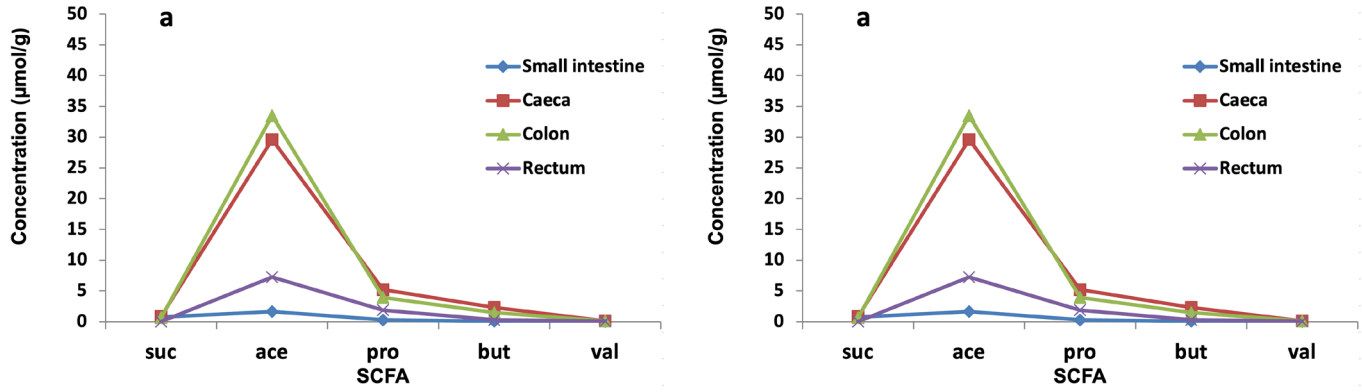

FIGURE 3 - SCFA content in intestinal segments in NWS group (a) and AWS group (b)

To further illustrate the differences in SCFA ratios of mice fed with the AWS versus NWS group, the variation in SCFA content in the caecal segment was shown in Figure 4. The results showed that the mice fed with AWS had a higher SCFA content than the corresponding NWS group, especially for acetic acid and propionic acid (28% and 26%, respectively, $p < 0.05$).

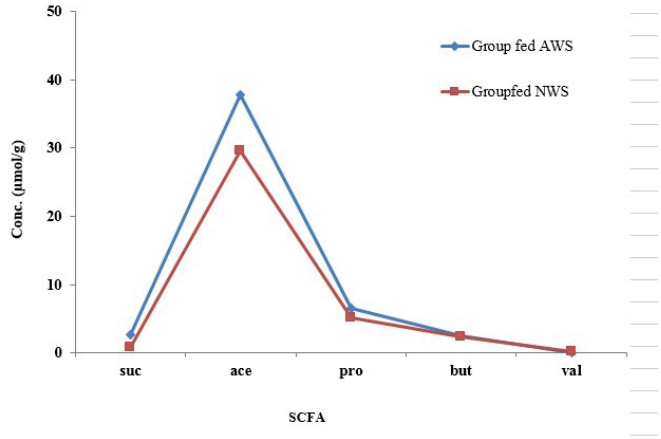

FIGURE 4 - The content of SCFAs in the caeca of mice in two group NWS and AWS.

From the results shown in Table 2 and Figure 4, total SCFA content as well as the acetic and propionic acid content of the mice fed with AWS were higher than the control group in each segment of the digestive tract, especially in the caeca (28% and 26%, respectively, $p < 0.05$). This result demonstrates that AWS resist to the hydrolysis of amylase in the small intestine. These carbohydrates, which were introduced into the caeca, have become the "food" for anaerobic bacteria and metabolized into SCFAs. This finding was consistent with the study of Tatsuya Morita *et al.* (2005) in rats showing that the diets containing acetate starch had a significantly higher SCFA content in the caeca compared with the control group fed with corn starch. In addition, the study also showed that the SCFA ratio increased with the rate of acetate starch in the diet (10-30%) (Morita *et al.*, 2005). The results of Le Thanh *et al.* (2014) who performed on rats also showed similar results of the higher SCFA content of the acetate starch group compared to the natural starch group in the caecal portion (Le Thanh-Blicharz *et al.*, 2014).

Several studies have demonstrated that the role of SCFAs is closely related to some of the pharmacological effects *in-vivo*. Ryoko Shimada *et al.* (2015) demonstrated that large SCFAs stimulate the intestinal nervous system to release PYY (Peptide YY) hormone, which inhibits the brain's appetite center, creates the feeling of fullness and loss of appetite, so limiting the amount of food consumed during the day (Shimada *et al.*, 2015). This has been induced by the fact that rats with RS diet had a significantly reduced body weight index compared to the control group. Moreover, the study of Xu Si et al. (2016), who performed in obesity rats for 6 weeks, resulted in a significant reduction in triglycerides, LDL-cholesterol, total cholesterol in the blood relative with role of SCFAs (Si *et al.*, 2017).

In addition, SCFAs also increase the ability to balance glucose uptake in cells of muscle and adipose tissue through increasing insulin sensitivity.

This result was demonstrated in Robertson's *et al.* study (2005) that a rich-RS diet (Hi Maize 260) produced SCFA content (mainly propionic and acetic acid) which was significantly higher than placebo, leading to an increase of SCFA content absorbed and distributed to the muscle tissue and adipose tissue. At these tissues, the SCFAs have increased their binding density to the G-protein coupled receptor (GPR 41 & 43). The activation of these receptors increased insulin sensitivity, resulting in increased intracellular glucose retention (Kimura *et al.*, 2013; Robertson *et al.*, 2005). In addition, it was demonstrated that propionic acid after produced in caeca was quickly absorbed by the portal vein into the liver. Here, propionate was an important precursor for the process of sugar accumulation and it also reduced the accumulation of fats in the liver, thus contributing to improve the insulin sensitivity in obese diabetic rats (Den Besten *et al.*, 2013; Weitkunat *et al.*, 2016).

From the study, it was showed that the acetylated wheat starch was able to control the postprandial hyperglycemia in mice because of its resistance to hydrolysis of amylase in the small intestine. Undigested starch when going into the large intestine will be fermented to form SCFAs, which provide energy for body's activities and avoid rotten fermentation maybe causing digestive disorders, which are inherent restrictions of normal high-cellulose and fiber food.

These SCFAs were easy to solute in body elutes and metabolized in Krebs Cycle to create essential energy for body, which was different from free fatty acids, the long-chain organic acids difficult to solute in body elutes, absorbed in the circulatory system causing the insulin resistance and cardiovascular disease.

Experimental mice were continuously followed with the side effects after three days, no digestive tract dysfunction or abnormal activities of mice groups happened. Based on the results of this study, we propose in-vivo and clinical trials to further develop AWS as a functional food in the treatment of obesity and diabetes.

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