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Hypoglycemic effect of biscuits containing water-soluble polysaccharides from wild yam (*Dioscorea hispida* Dennts) or lesser yam (*Dioscorea esculenta*) tubers and alginate

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Abstract

To determine the hypoglycemic effects on biscuits supplemented with single or mixture of hydrocolloids, crude water soluble polysaccharides (WSP) extracted from tubers of either wild yam (CWY) or lesser yam (CLY) and commercial alginate powder (ALG) were used. Five different types of hydrocolloid mixture were prepared, namely CWY, CLY, ALG, mixtures of CWY and ALG (CWY/ALG) and CLY and ALG (CLY/ALG) at a ratio of 1:1. The biscuits (BIS) formula were incorporated with 2.5% of these hydrocolloid mixtures and biscuit without addition of hydrocolloid (NON-BIS) was used as control. A meal tolerance test (MTT) was carried out by feeding each biscuit to alloxan-induced hyperglycemia rats respectively, and the blood glucose levels were monitored for the first 120 min. A long term antihyperglycemic effect was monitored weekly for their blood glucose levels and changes in body weight for 4 weeks, followed by the determination of the short chain fatty acids (SCFAs) profile from the caecum digesta at the end of feeding treatment. As a comparison, standard feed was respectively fed to a group of hyperglycemia rats (HYP-STD) and a group of normal rats (NOM-STD). The MTT results indicated that all hydrocolloid containing biscuits affected the absorption of glucose into the blood and were able to maintain it at a normal level. ALG-BIS was the most effective, followed by CWY/ALG-BIS, CLY/ALG-BIS, CWY-BIS and CLY-BIS. After 4 weeks, the glucose blood levels were significantly reduced, showing a pattern of reduction similar to that of the MTT. The magnitude of reduction was as follows: 57% (ALG-BIS), 50% (CWY/ALG-BIS), 49% (CLY/ALG-BIS), 39% (CWY-BIS), 35% (CLY-BIS) and 2% (NON-BIS) respectively. The hyperglycemic rats fed with biscuits gained about 7-11% in body weight and opposite results were found on hyperglycemic rats fed with standard feed. Despite the variation in composition, the order of concentration of the SCFAs was similar, i.e. acetic acid > propionic acid > butyric acid. It may be concluded that biscuits added with WSP, extracted from tubers of either wild or lesser yam, or their mixtures with alginate, have significant hypoglycemic effects.

Introduction

The tubers of yams (*Dioscorea* spp.) are not only known as source of carbohydrates, but bioactive compounds as well. Wild yam (*Dioscorea hispida* Dennts) and lesser yam (*Dioscorea esculenta* L) are members of the family widely grown in Indonesia. Previous studies on the tubers of *D. alata*, *D. batatas*, *D. bulbilfera*, and *D. opposite* revealed that they contains dioscorin (Hou *et al*., 2001; Chan *et al*., 2006; Liu *et al*., 2009), diosgenin (Chou *et al*., 2006; Braun, 2008; Yang and Lin, 2008), and water-soluble polysaccharides (WSP) (Liu *et al*., 2007).

Tubers of Dioscoreaceae contain a viscous, mucilage-like glycoprotein (Fu *et al*., 2008; Ohizumi *et al*., 2009), in which polysaccharide was bound with protein (Myoda *et al*., 2006), and is known as water soluble polysaccharide (WSP). It was found that crude WSP extracted from tubers of either lesser yam (Harijono *et al*., 2012) or wild yam (Estiasih *et al*., 2012) show hypoglycemic effect on alloxan-induced hyperglycemic rats. It was likely that crude WSP delays feed digestion in the intestine resulting in a lower absorption of glucose into the blood (Chen *et al*., 2003).

Hydrocolloids were able to increase the viscosity of digesta (Masdar *et al*., 1988; Torsdottir, 1991; Weickert and Pfeiffer, 2008). Viscous matrix of foods

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act as a physical barrier to the diffusion of nutrients in the intestinal mucosa (Lyon and Reichert, 2010) resulting in less absorption of glucose into the blood (Lunn and Buttriss, 2007). Physical forms of fiber affect on the ability to inhibit the absorption of glucose. Soy fiber in the form of powder was not able to inhibit the absorption of glucose, but if formulated in bakery products, these fibers inhibited the increase of glucose levels in the blood (Madar *et al*., 1988). When carbohydrates were not well digested and the absorption of sugars in the small intestine was inhibited, the digesta was transferred into the colon and then fermented by bacteria (Lunn and Buttriss, 2007) producing short chain fatty acids (SCFAs). The presence of dietary fiber in the consumed foods affected the metabolism and production of SCFAs in the colon (Marsman and McBurney, 1996). de Leeuw *et al.* (2004) presented the relationship between fiber, glucose, and insulin levels. Non digestible oligosaccharides contributed to health through fermentation and proliferation of beneficial bacterial species in the colon. Types of oligosaccharides affected the profile of SCFAs (Campbell *et al*., 1997), and gases (CO_2, CH_4, and H_2) produced (Henningsson *et al.*, 2002).

Crude WSP of wild yam and lesser yam tubers may be prepared by different methods of extraction. One of those is a tempeh fermentation-assisted extraction, as described by Estiasih *et al.* (2012) and Harijono *et al.* (2012). They prove that an addition of such crude extracts into standard feed is able to reduce the level of blood glucose of rats. In addition, the crude WSP also produces SCFAs which help to improve the insulin response in the liver. According to Lunn and Burttiss (2007), a high intake of fiber is recommended for diabetic patients. The viscous properties of WSP and gel formation may inhibit macronutrient absorption and reduce postprandial glucose response. WSP fermentation in the colon produces SCFAs such as acetic, propionic and butyric acids. SCFAs were able to control body metabolism that lead to a number of effects including insulin sensitivity, modulation of hormone secretion in the digestive tract, and a variety of metabolic processes associated with the metabolic syndrome (Weickert and Pfeiffer, 2008).

The prevalence of people suffering from diabetes in the world increases with a projected rise from 171 million in 2000 to 366 million in 2030 (Wild *et al*., 2004). Diabetes is associated with diminished production of insulin or increasing resistance to its action resulting in an abnormal metabolism of carbohydrate, fat and protein. There are many groups of oral hypoglycemic drugs and insulin for clinical use, but exhibit side effects. Management of diabetes with minimum side effects is still a challenge to the medical system. Apart from medication, a diet of low fat and high in complex carbohydrates like hydrocolloid and insoluble fiber and regular exercise are usually recommended to patients with DM, either for non-insulin-dependent diabetes mellitus (NIDDM) or insulin-dependent diabetes mellitus (IDDM). Therefore, a regular consumption of hydrocolloid containing foods may reduce the use of hypoglycemic drugs. Torsdottir (1991) developed hypoglycemic bread by an addition of alginate in the bread formula. The researcher reported that hypoglycemic effect was observed on rats fed with such bread. A development of medicinal foods for type 2 diabetic patients is an interesting approach as it will reduce the spending on medicines. This study was carried out to examine antihyperglicemic effects of biscuits enriched with hydrocolloid (WSP) extracted from tubers of either wild yam or lesser yam, in a combination with alginate or not.

Materials and Methods

Materials

Tubers of wild yam and lesser yam were obtained from local markets in the province of East Java, Indonesia. Crude WSP was obtained by tempeh fermentation-assisted extraction method (Estiasih *et al*., 2012; Harijono *et al*., 2012). Standard feed of AIN 93M (Reeves *et al*., 1993) and commercial alginate powder were used in the study. All chemicals are analytical grade unless otherwise stated. The basic formula of biscuit consists of wheat flour (50%), sugar (9%), margarine and butter (34%), fresh egg yolk (4%), and flavouring materials (0,5%). The formula was made to 100% by adding single or a mixture of hydrocolloids.

Hydrocolloids and production of biscuits

Crude WSP, respectively extracted from tubers of Indonesia's wild yam (CWY) and from Indonesia's lesser yam (CLY), and commercial alginate powder (ALG) were used in the experiment. Five kinds of hydrocolloid were prepared, i.e. CWY, CLY, ALG and a mixture at a ratio of 1:1 between CWY and ALG (CWY/ALG) and between CLY and ALG (CLY/ALG). Each kind of hydrocolloid was incorporated separately into the biscuit recipe at 2.5% final concentration and non-hydrocolloid containing biscuit (NON-BIS) was used as control. Proximate analysis on the crude WSP obtained was based on AOAC methods (1996) and the colour measurement on the samples was carried out using the L-a-b colorimetry method.

Experimental animals

Selected male Wistar rats (*Ratus novergicus* strain Wistar) 2-3 months of age, weighing 150-225 g were used in the experiment. Each rat was placed in a separate cage housed in air-conditioned animal house with temperatures maintained at 20-25°C and fed standard feed of AIN 93M (Reeves *et al*., 1993) and boiled water *ad libitum.* Acclimatization was conducted for seven days.

Meal tolerance test (MTT)

MTT was conducted using a modified method of Madar *et al.* (1988) to determine the changes of blood glucose level increase short after 120 min of consuming the feed. After seven days of acclimatization, the rats were weighed and divided into six groups of three animals each. Diabetes was induced by intraperitoneal injection of freshly-prepared alloxan at a dose of 80 mg/kg body weight. The rats were further acclimatized for three days and followed by fasting for 16 hrs before feeding with biscuits. A retro-orbital plexus blood sample was collected on non-fasting rats. Each of the six biscuit recipes, namely CWY-BIS, CLY-BIS, ALG-BIS, CWY/ALG-BIS and NON-BIS, was used separately to feed each group of diabetic rats. Retro-orbital plexus blood sample were taken at 30 min intervals for the first 120 min after feeding. A total of 5 ml the retro orbital plexus blood sample was collected and centrifuged at 4,000 rpm for 10 minutes. Supernatant was then collected for serum glucose measurement by the Glucose Oxydase-Peroxidase (GOD/POD) method.

Hypoglycemic effect assay

The assay, modified from the one described by Chen *et al.* (2003), was intended to determine the ability of above-described biscuits in reducing blood glucose levels of alloxan-induced hyperglycemic rats. A total of 32 rats were employed in the assay. They were divided into 8 groups, seven of which were made hyperglycemia by alloxan induction. One group was kept as the non-hyperglycemic one. Freshly-made alloxan solution was induced at a dose of 175 mg/kg body weight. Only rats with glucose level of above 200 mg/dL were used in the study. Feeding treatments similar to MTT were carried out. Each kind of the above-mentioned biscuits (CWY-BIS, CLY-BIS, ALG-BIS, CWY/ALG-BIS, CLY/ALG-BIS and NON-BIS) was respectively used to feed a group of hyperglycemic rats. At the same time, a group of hyperglycemic rats (HYP-STD) and a group of non-hyperglycemia or normal rats (NOM-STD) both were fed standard feed of AIN 93M and considered as negative and positive controls respectively. The rats were fed ad libitum (20 g/day) for a period of 4 weeks. The blood glucose levels and body weight of rats were determined weekly i.e. week 1, 2, 3 and 4, while the feed residue was weighed daily. Postprandial glucose blood levels were measured after fasting for 16 hours. A retro orbital plexus blood sample was collected and determined by the GOD/POD method.

Determination of short chain fatty acids (SCFAs)

The profile of SCFAs was determined by a modified method of Henningsson *et al.* (2002). As many as 2 rats per group were taken and anaesthetized with ether before the caecum was taken. The caecum digesta was removed and then centrifuged at 14,000 rpm for 15 minutes. The level of SCFAs from supernatant was measured by gas chromatography (GC Shimadzu Serie GC 8A). Supernatant of 1 µL was injected into the gas chromatography, equipped with a 2 m-length column of GP 10% SP 1200 1% HPP30 and FID detector, at a temperature of 130°C. The injector and detector temperatures were maintained at 230°C. The carrier gas was N_2 at a pressure of 1.25 kg/cm^2.

Experimental design and data analysis

A nested design experiment with two factors was employed for the experiment. The response to the given treatment was then measured accordingly and the data obtained were analyzed using analysis of variance and continued with Least Significant Difference Test (α = 5%).

Results and Discussions

Composition of CWY and CLY

The characteristics of CWY and CLY are shown in Table 1. It is obvious that the chemical compositions and the color of hydrocolloids are similar, but the yield of CWY (13.53%) was much higher than that of CLY (4.16%). The yields are slightly different from previous studies, i.e. 11.05% on CWY (Estiasih *et al*., 2012) and 3.43% on CLY (Harijono *et al*., 2012). This observation can be attributed to the much higher levels of crude fiber (3.19% on in CWY and 3.07% in CLY) of the WSP extracts obtained from this study, compared with the previous reports: 0.72% (CWY) (Estiasih *et al*., 2012) and 0.35% (CLY) (Harijono *et al*., 2012).

Table 1. Characteristics of wild yam and lesser yam water soluble polysaccharides (WSP) extracts

Component	Wild Yam WSP	Lesser Yam WSP
Water (%)	7.96	7.39
Ash (%)	0.49	1.62
Protein (%)	5.59	4.47
Fat (%)	2.55	3.19
Carbohydrate *by diff* (%)	83.40	83.34
Crude Fiber (%)	3.19	3.07
Starch (%)	14.61	15.81
Yield (%)	13.53	4.16
Color:		
• Lightness (L)	71.93	71.37
• Redness (a)	13.57	14.37
• Yellowness (b)	17.07	14.07

WSP=water soluble polysaccharide

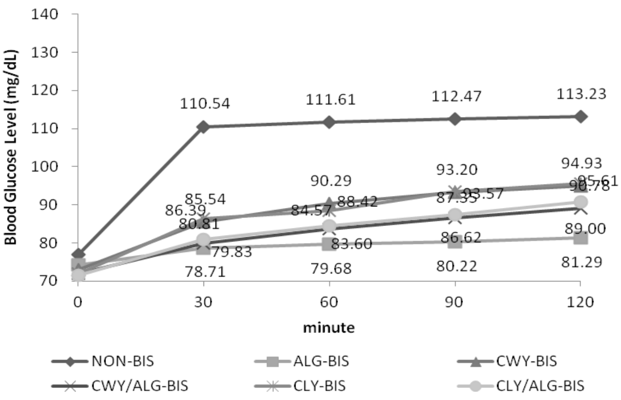

Figure 1. Response on MTT of rats fed with hydrolloids containing biscuits

Composistion of biscuits

Proximate analysis on the biscuits showed that their compositions varied slightly. They were composed of 4.5-5% water, 1.2-1.6-% ash, 0.8-1% crude fiber, 7.4-7.7% crude protein, 37-38% crude fat, and 48-50% carbohydrates. It contained high levels of fat and simple carbohydrates and 560-570 Cal/100 g.

Meal tolerance test (MTT)

The results of MTT indicates that all hydrocolloid containing biscuits affects the absorption of glucose into the blood and are able to maintain the blood glucose at normal levels (Figure 1). ALG-BIS is the most effective one, followed by CWY/ALG-BIS, CLY/ALG-BIS, CWY-BIS and CLY-BIS. After 2 hrs of feeding, the blood level of rats fed with the NON-BIS increases sharply from about 77 mg/dL to 110 mg/dL within 30 min. After 120 min, it achieved 113.23 mg/dL or an increase of 47%, much higher than those fed with CWY/ALG-BIS (24%), CLY/ALG-BIS (27%), CWY-BIS (30%), and CLY-BIS (33%). These observations may indicate that the hydrocolloids used have potential hypoglycemic effects. High level of starch (ca 15%) present in the crude WSP extracts possibly contribute to the lower hypoglycemic effect. Moreover, the presence of fat at a high level in biscuit probably reduces the strength of the gel. On the other hand, the ALG may form more viscous or gel matrix in the digestive tract resulting in more sugars absorbed or entrapped in the matrix. It is probably true since any addition of ALG to CWY or CLY into biscuit recipes, i.e. CWY/ALG or CLY/ALG, resulted in a better hypoglycemic effect, compared with the use CWY or CLY alone.

Hypoglycemic effect of biscuits containing biscuits

The results of the long feeding treatments concurred with the MTT results. At week 0, the level of hyperglycemia rats ranges from 206 mg/dL to 210 mg/dL. Those fed with hydrocolloids containing biscuits shows gradual reductions in the glucose blood level depending on hydrocolloid added into the biscuits. The best effect is shown by the use of ALG, similar to that found on the MTT. The degrees of reduction resulted from the use of ALG-BIS, CWY/ALG-BIS, CLY/ALG-BIS, CWY-BIS, CLY-BIS and NON-BIS are 57%, 50%, 49%, 39%, 35%, and 2%, respectively.

The use of CWY or CLY added to the biscuits fed ad libitum results in lower hypoglycemic effect than the use of its corresponding crude extract given by forced feeding at a dose of 400 mg/kg body weight, similar to reports in previous studies (Estiasih *et al.*, 2012; Harijono *et al.*, 2012). After 4 weeks, the blood glucose level is reduced to a normal level of about 84.40 mg/dL by forced feeding of CWY or CLY but the effect is less when the corresponding hydrocolloid is incorporated into biscuit given ad libitum. Previous research reported that instant noodles (low fat and low sugars) contaning crude WSP extract from wild yam and alginate at a level of 10%, given at a dose of 15 g feed/rat/day on hyperglycemic rats was able to bring the blood glucose level to a normal level (101.28 mg/dL) within 4 weeks (Rachmawati, 2011). It reveals that lower level of hydrocolloids added to product with high levels of fat and sugars is less effective.

It was likely that alginate, given in the form of biscuits, had a better chronical hypoglycemic effect than that of either crude WSP extract of either wild yam or lesser yam. It possibly indicated that alginate when mixed with simple sugars (from biscuits) in acidic condition (in the gastric) formed more viscous gel that inhibited more to the transport of nutrients and prevented the contact between nutrients and the mucosal surface. As a result, the peristaltic movement was decreased minimizing the contact between enzyme and substrates. As a consequent, the

Table 2. Weight changes of rats fed with hydrocolloid containing biscuits

Feed	Group of Rats	Acclimt[1] (g)	Week 0[2] (g)	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	Weight gain (g)[3]	Weight gain (%)
NOM-STD[4]	Normal[4]	219.75	222.25	232.00	240.50	249.25	258.75	36.50	16.42
HYP-STD[5]	Hypergl[6]	246.00	242.25	237.50	234.00	232.75	230.75	(11.50)[7]	(4.75)[7]
NON-BIS	Hypergl	*147.75*	*144.25*	*142.50*	*146.50*	*149.50*	*155.75*	*11.50*	*7.97*
CWY-BIS	Hypergl	165.00	162.25	163.50	168.50	173.75	180.25	18.00	11.09
CLY-BIS	Hypergl	175.00	172.75	174.50	179.25	183.75	190.25	17.50	10.13
ALG-BIS	Hypergl	239.75	237.00	238.50	241.75	247.50	253.75	16.75	7.07
CWY/ALG-BIS	Hypergl	205.50	203.00	205.00	208.75	213.25	219.00	16.00	7.88
CLY/ALG-BIS	Hypergl	222.50	219.50	221.00	225.25	229.75	236.25	16.75	7.63

1) At the end of 7 days acclimatization
2) 3 days after alloxan induction
3) Weight different between Week 4 and Week 0
4) Normal rats fed with standard feed of AIN 93M as a positive or untreated control
5) Hyperglycemia rats fed with standard feed as a negative control
6) Alloxan-induced hyperglycemia rats
7) Negative weight gain

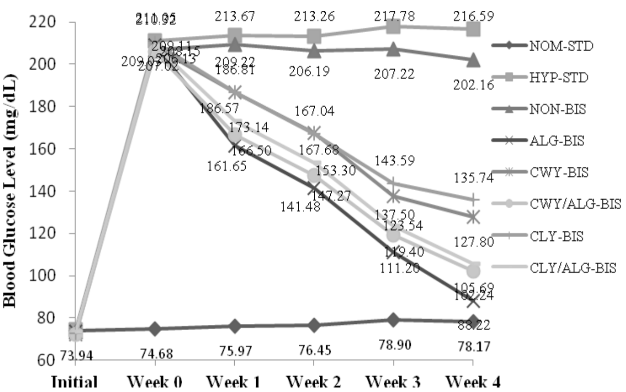

Figure 2. Changes of blood glucose level of rats during 4 weeks feeding with hydrocolloids containing biscuits

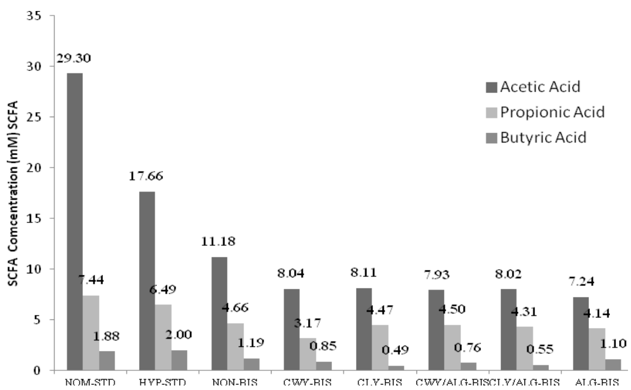

Figure 3. SCFAs level in *caecum* digesta of rats fed with hydrocolloids containing biscuits

absorption of nutrients, including glucose molecules, was slowed down. Zhang *et al*. (2006) explained that soluble fiber particularly galactomannan was able to form viscous gel in the gut and thereby reducing the absorption of sugars and fats.

Changes of body weight

Table 2 shows that hydrocolloids containing biscuits or NON-BIS given for 4 weeks resulted in slight increases in body weight of the hyperglycemic rats. The highest level of weight gain was observed on the control fed with the standard feed (NOM-STD). On the contrary, an adverse effect was observed on the hyperglycemic rats fed with standard feed (HYP-STD). Diabetes generally causes glycation of body proteins (Sharma, 1993) and thus on the long term affect the body weight. It is likely that hydrocolloids containing biscuits are still able to maintain a normal metabolism of macromolecules.

SCFAs of caecum

The decline in blood glucose levels was not solely due to the ability of the hydrocolloids to inhibit the absorption of glucose in the intestine. It was likely that other mechanism such as the increase in insulin response to glucose in the liver due to the formation of SCFAs. It was shown that the body weight of rats fed with hydrocolloids-containing biscuits and the group of normal rats increased. The increase was much higher on the normal rats fed with the standard feed, but the same feed resulted in significant reduction on the body weight of hyperglicemic rats (Table 2). The results indicated that feeding with biscuits mixed with crude hydrocolloids of either CWY or CLY, combined with or without alginate, may contribute to the different body metabolism in rats. It was found that feeding of rats with various feed formulas produced different caecum's SCFAs profiles (Figure 3). Similar to the report of Lunn and Buttriss (2007), the SCFAs profile was mainly dominated by acetic acid, followed by propionic acid and butyric acid. The concentration of each acid was dependent on the types of hydrocolloids added to the feed.

The levels of SCFAs produced by hyperglycemic rats fed with hydrocolloids-containing biscuits were lower than that fed with either non-WSP biscuits or a standard feed. The highest level was shown by the group of normal rats fed with a standard feed.

The findings contradicts of Estiasih *et al.* (2012) and Harijono *et al.* (2012), who reported that higher levels of caecum SCFAs were found on the mice fed with the standard feed mixed with either crude WSP extracts of wild yam or lesser yam than those fed with the standard feed alone. It is possibly due to the alteration of the hydrocolloids resulted from extensive heating during the processing of biscuit. However, the exact cause and mechanism was still not well understood. Henningsson *et al.* (2002) stated that the physical properties of dietary fiber affected fermentation characteristics. Highly soluble dietary fiber such as pectin and guar gum were generally fermented in the colon rapidly. Metabolism pathways and fermentation products varied with microorganisms and the state of substrates. Therefore, the SCFAs' profiles were different.

Luthana (2009) explained that SCFAs were absorbed in the colon and transported to the liver via the enterohepatic circulation, a system connecting the liver and the intestine. The SCFAs in the liver were used in the alteration of monosaccharides from the bloodstream into glycogen, and in the oxidation process of glucose to CO_2 and H_2O. SCFA also reduced the hepatic glucose production (Weickert and Pfeiffer, 2008). Increasing SCFA concentration in vena porta activated hepatic AMPK (activated protein kinase) which functioned to regulate energy production in cells and metabolic homeostatic condition (Hu *et al*., 2010). Despite the contradictory results with the previous reports related to the SCFAs profiles, crude WSP extracts from either wild yam or lesser yam without or in a combination with alginate, given in the form of biscuits, are effective in lowering blood glucose level in a long-term consumption.

Conclusion

Crude WSP extracts from either wild or lesser yams, without or in a combination with alginate, incorporated into biscuits at a level of 2.5% effectively reduce blood glucose levels of rats in the long-term (4 weeks) feeding experiments. The effect varied with the types of hydrocolloids used. The most effective one was the alginate-containing biscuit, followed by that containing a mixture of crude WSP extracts from either wild or lesser yams used with alginate. They are able to reduce the levels of blood glucose from hyperglicemia state to a normal one. The caecum's SCFAs profiles varied with the hydrocolloids added. However, the order of caecum's SCFAs' concentration was similar, i.e. acetic acid > propionic acid > butyric acid.

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