行政院國家科學委員會專題研究計劃成果報告

台灣本土栽培山藥塊莖之腸道生理功能

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一、中文摘要

本研究由本省特有或推廣之山藥品系中，選擇三種塊莖：**基隆山藥(基藥), 台農二號(台藥)及民間長紅(紅藥)**，來進行其腸道功能及脂質代謝之探討。將山藥冷凍乾燥粉末以25% 及50% 取代澱粉方式加入半純化飼料配方，進行三週之動物實驗。在腸道功能方面結果顯示: 1. 攝食山藥減低體重增加速率，然各組老鼠盲腸及大腸重量大於控制組，攝食量大致以50%山藥組高於control組及25%山藥組。2. 25% 及50%台藥、基藥及紅藥明顯增加盲腸表皮細胞高度，25% 及50%台藥、基藥增加表皮柱狀細胞核數，50%台藥及基藥增加表皮柱狀細胞與增生指標(deoxy-uridine)結合之最大高度 (labeled crypt height)。3. 在大腸方面，所有山藥飼料明顯增加表皮細胞寬度，50%山藥飼料皆明顯增加labeled crypt height及與增生指標(deoxy-uridine)結合之細胞數，顯示50%山藥飼料促進細胞分裂。4.在盲腸短鏈脂肪酸含量上，基藥效果高於台藥，而紅藥無明顯作用。5. 在大腸上，則50%紅藥明顯增加短鏈脂肪酸含量，基藥及台藥則無作用。6. 所有山藥飼料明顯增加每日糞便濕重及乾重，台藥最能增加糞便濕份。7. 以PCR定量腸道細菌發現台藥明顯抑制*Clostridium perfringens*濃度，顯示台藥具有保禦腸道之作用。在調節血脂功能方面，1. 25%紅藥及基藥組顯著降低血漿三酸甘油脂濃度至控制組之75%, 65%左右，但50%山藥組皆無此效應。2. 在血膽固醇方面，50% 紅藥及台藥顯著降低血漿總膽固醇及低密度脂蛋白膽固醇，25%山藥則皆無此效應。除25%台藥組外，高密度脂蛋白膽固醇濃度皆受到各山藥組之抑制。低密度脂蛋白膽固醇: 高密度脂蛋白膽固醇比值則各組皆相似。3. 紅藥組之肝臟三酸甘油脂濃度顯著降低。4. 紅藥及基藥組顯著增加糞便膽固醇及膽酸排出量。5. 山藥組不影響小腸lipase比活性，但紅藥及50%基藥使脂質吸收率明顯下降。因此，本土山藥，尤其是民間長紅有非常顯著之調節三酸甘油脂及

膽固醇代謝之能力。

在調節小腸黏膜白胺酸胺基側胜 酵素(leucine aminopeptidase)、麥芽糖及蔗糖酶活性方面：50%紅藥及台藥組之小腸黏膜細胞白胺酸胺基側胜 酵素高於控制組，而50%基藥及台藥組各自增加麥芽糖酶及蔗糖酶。

Abstract

The present study was aimed to evaluate effects of three types of Taiwanese yam tubers, *Dioscorea alata* L. var. *purpurea* M. Pouch (Purpurea), *Dioscorea alata* L. cv. Tainung No. 2 (Tainung), and *Dioscorea japonica* Thunb var. *pseudojaponica* Yamamoto (Japonica) on the bowel physiology and liid metabolism. Adult (6 wk old) male Balb/c mice were fed on control or either level (25% or 50%) of yam-enriched experimental diet for three weeks *ad libitum*. All yam diets elevated the lower gut weights, except for the 25% Purpurea diet. All experimental diets, among which Purpurea was least effective, promoted the crypt depth for 40-60% and cell density for 20-50% in the cecum. The labeled crypt height was raised only in mice fed on 50% Tainung and Japonica diets. In the distal colon, all experimental diets promoted the width of crypt columns, but only 50% yam-enriched diets increased the labeled crypt height and the labeled cell density. The SCFA production demonstrated type and dose effects. Tainung and Japonica diets greatly enhanced SCFA production in the cecum. Purpurea diet caused extensive fermentation in the colon, maybe by sparing that in the cecum. Taiwanese yams exerted trophic effects in the cecum, which was possibly mediated by luminal fermentation. All yam diets elevated fecal weight, while Tainung was the most effectice in raising fecal moisture. The PCR quantitation of fecal microbial indicated that Tainung reduced the concentration of *Clostridium perfringens*, which suggests Tainung could protect the immunity of GI. In terms of effects of yam on the lipid metabolism. Mice fed on 50% Purpurea and Tainung diets exerted the greatest hypocholesterolemic effects without significant alterations in the atherogenic indices. Dietary fiber, a major ingredient of yams, may mediate these effects by increasing fecal excretions of cholesterol and bile acid and decreasing the total cholesterol pool, with no alterations in hepatic cholesterol contents. In contrast, the increases in the daily fecal triacylglycerol outputs and the decreases in the hepatic triacylglycerol contents and the apparent absorption rates were the greatest in mice fed on Purpurea, and secondly in mice fed on Japonica diets. Therefore, the lipotropic effects

of Purpurea and Japonica may be due to the poor fat absorption caused by viscous dietary fiber. In summary, viscous dietary fiber was possibly involved in the hypocholesterolemic effects of Purpurea and Tainung and the lipotropic effects of Purpurea and Japonica in mice.

Keywords: yam, *Dioscorea*, cecum, hypertrophy, proliferation, short chain fatty acids, cholesterol, triacylglycerol

二、緣由與目的

山藥(Dioscorea spp.)為薯蕷科(*Dioscoreaceae*)薯蕷屬(*Dioscorea*)蔓性多年生草本植物，原產我國、日本及亞洲熱帶地區。山藥依其塊莖之形狀可分為塊狀山藥及長形山藥，塊狀山藥如本省品種如台農一號、巴西山藥及民間長紅等，一般均做為食用。長形山藥之地下莖如台農二號、台北懷山藥、基隆山藥、及恆春山藥等。在我國最早之本草藥典記載之藥用山藥與現今淮山藥 *Dioscorea opposita* Thunb. (*D. batatas* Decne.) 之外型一致，具有補脾、養胃、生津、益肺等功能，常用來治療腹瀉咳嗽等病症，民間亦應用於治療高血糖症。然而本土山藥品種繁多，至今尚未釐清其生理效應。

山藥塊莖含不可溶性纖維及水溶性黏液質，皆屬於膳食纖維。良好的非水溶性膳食纖維來源，如小麥麩等，一般具有促進腸道蠕動，縮短腸道通過時間(Gastrointestinal transit time)及減緩便秘等功能。而水溶性膳食纖維的良好來源，如燕麥麩，果膠等，則主要調節小腸部位之消化吸收，有助於控制飯後血糖、減少脂肪吸收、增加膽酸的排泄，及降低血中膽固醇及三酸甘油脂，水溶性膳食纖維並可能促使腸道微生物生長，產生短鏈脂肪酸。因此本研究以 Balb/c 小鼠為模式，以評估塊狀山藥：民間長紅(*D. alata* L. (Roxb) M. Pouch)及長形山藥：基隆山藥(*D. japonica* Thunb. Var. Pseudojaponica (Hay.) Yamamoto)之腸道及脂質代謝效應。

三、 結果與討論

Results- Bowel function

Dietary fiber intakes and weights of the lower gut walls

The composition of Purpurea, Tainung and Japonica is shown in Table 2. Purpurea had the least carbohydrate content, 2% (dry wt) lower than the other two yam types. Japonica obtained the least protein content, ~1% less than Purpurea and Tainung, respectively. Tainung had the highest crude fiber, total dietary fiber and soluble dietary fiber contents among three types of yams. The difference in total dietary fiber content between Purpurea (4.4%) and Tainung (5.3%) was due to the difference in the soluble dietary fiber fraction.

Japonica obtained equal dietary fiber content as Purpurea but its soluble dietary fiber was only 0.5%, far less than Tainung (1.9%), and almost half of the level in Purpurea (0.9%). The ratio of soluble dietary fiber to total dietary fiber was quite different among types. Soluble dietary fiber accounts for 1/3, 1/5 and 1/10 the total dietary fiber in Tainung, Purpurea and Japonica, respectively.

The total fiber and soluble fiber intakes and effects of diets on weights of the cecum and colon walls are shown in Table 2. The total dietary fiber content in the 25% and 50% Purpurea, Tainung, Japonica-enriched diets were 6.10% and 7.21%, 6.33% and 7.65%, and 6.10% and 7.19%, respectively, compared with 5% in control diet. The mice fed on 25% Purpurea-, Tainung-, Japonica-enriched diets therefore ingested 48%, 35% and 57% more dietary fiber than mice fed on the control diet, respectively. In addition, the soluble fiber contents in the 25% Purpurea, Tainung, Japonica-enriched diets were 0.23%, 0.47% and 0.14%, respectively. Mice fed on 25% Japonica diet consumed the least amount of soluble fiber (8 mg/d) as compared to mice fed on any yam-containing diets, and was nearly one third of the amounts consumed by mice fed on 25% Tainung diet (23 mg/d).

Morphological and proliferative indices in the cecum

The measurements of morphology and proliferation of the epithelial cells in the cecum is shown in Table 3. For the 25%-enriched yam diets, Japonica and Tainung similarly promoted the crypt depth by >40% and Purpurea elevated the crypt depth for 36%, as compared to the control diet. Fifty% Tainung and Japonica diets further increased the crypt height for ~60% as compared to the control diet while 50% Purpurea diet only promoted the crypt height for 44%. In contrast to increases in the crypt depth, the crypt width was not altered by any experimental diets at the cecum. The labeled crypt height, and the maximum height of the labeled cell in a crypt column, was significantly increased only in mice fed on 50% Tainung and 50% Japonica diets for 79% and 71%, respectively. Twenty five % Tainung and Japonica diets tended to increase this measurement for 45%, respectively, but these changes were not statistically significant. The labeling zone (the ratio of labeled crypt height to crypt depth) was not different among groups. In addition, 13% of the crypt cells were labeled with 5-bromo-2'-deoxyuridine in mice fed on the control diet, which was not altered significantly by any yam-containing diets.

Morphological and proliferative indices in the colon

The morphological and proliferation indices in the colon are shown in Table 4. The crypt depth was significantly increased only in mice fed on 50% Japonica (for 36%) as compared to mice fed on the control diet. Similarly, the cell density was not altered in any yam-enriched diets. In contrast, crypt width was increased by all experimental diets among which 50% Purpurea diet exerted the greatest effect (~100% increase). The magnitude of increases in the crypt width was 70% for either Japonica diet, 77% for 25% Tainung and 85% for 50% Tainung diet, respectively, as compared to the control level.

Short chain fatty acids content in the cecum

The short chain fatty acid content in the cecum digesta is shown in Table 5. Total SCFA content in the cecum was significantly raised for 3.5 folds by consuming 50% Tainung and 50% Japonica diets, respectively, and for 2 folds by consuming 25% Japonica diets. The acetate content was also elevated to ~4 folds of the control level by 50% Tainung and 50% Japonica diets, respectively, and to ~2 folds by 25% Japonica diet. Propionate contents were not significantly increased by experimental diets as compared to the control diet, except that 25% Japonica diet caused ~3 folds increase over the control level. Butyrate (sum of *i*-butyrate and *n*-butyrate) production was greatly enhanced by approximately 3- and 6- folds in mice fed on 25%, and 50% Tainung diet or either Japonica diets, respectively.

Short chain fatty acid content in the colon

The daily fecal short chain fatty acid excretion is shown in Table 6. Ingestion of 50% Purpurea diet, not the other diets, significantly increased daily excretion of acetate, propionate, butyrate and total SCFA. Fecal acetate and total SCFA contents were increased by nearly 6 folds, propionate by nearly 7 folds, and butyrate by 8 folds, over the control level. Acetate was the dominant SCFA in feces. The molar percentage of acetate in the feces was approximately 90% for the control and experimental groups except that 50% Tainung diet decreased the acetate ratio to be ~80% by increasing the ratio of butyrate to be ~11%.

Discussion-Bowel function

Since yam has been used as an important herb to normalize bowel function, it may obtain the functionality to improve the epithelial cell structure even in healthy men. The present study demonstrated that when a 5% cellulose diet was consumed, replacement of starch with yam tubers further increased the cell proliferation in the lower gut. The roles of yam-enriched diets in the epithelial cells structure and proliferation were site-specific. Furthermore, these trophic effects were not determined by the amount of yam fibers, but probably by their fermentability in the lower gut.

Previous studies have supported the regulatory roles of soluble fibers such as pectin (Jacob et al, 1984, Lupton et al. 1988, Zhang and Luptoan 1994), guar (Jacob et al, 1984, Lupton et al. 1988), konjac glucomannan (Oku 1995), oat bran (Zhang and Lupton 1994) and wheat bran (Gestel et al. 1994) on epithelium cell morphology and proliferation. Our findings agreed with these studies since Tainung and Japonica diets exerted their trophic effects as early as in the cecum, and the their effects continued in the distal colon. Purpurea that resulted in lower SCFA production exerted less trophic effects in the cecum than Tainung and Japonica did. The present study further demonstrated that consumption of Taiwanese yams as part of a staple modulated the morphology and proliferation indices site-specifically. In the cecum, most experimental diets promoted crypt height and cell density but labeled crypt height was increased only in mice fed on diets contained 50% Tainung and Japonica. Therefore, the present study suggested that enrichment of yam mainly caused increases in epithelial cells and cell migration rate in the cecum. Tainung and Japonica, as compared with Purpurea, were stronger promotors for cell proliferation in the cecum. On the other hand, diets enriched with yams exerted significant effects on the crypt width, rather than on the crypt height in the distal colon. This site-specific change in the structure of mucosa cells was also observed in a previous study in which high fiber diet increased crypt depth in the ileum (proximal to the ileal-cecal valve) but increased crypt width in the colon (Jin et al. 1994).

The labeling zone in the cecum and colon were 36% for mice consumed the control 5% cellulose diet, which agreed with previous studies. This measurement in both cecum and distal colon was not altered by any experimental diet, which indicated the turnover rate of the mucosa cells were independent on the diet consumed even the total surface area of the mucosa layer was increased. The labeled cell density (proportion of labeled to total epithelial cells) was not altered in the cecum by any yam diet. But, this measurement was promoted in the distal colon by all of 50% yam diets, which indicated that yam-enriched diets promoted the relative proliferative activity.

Result-Lipid metabolism

Body weights, feed intakes and energy intakes

The growth and food intake conditions are shown in Table 3. Mice were in good health with no differences in the initial and final body weights among groups. Purpurea and Japonica diets exerted greater effects in food intake than Tainung diet. Independent of yam types, feed and energy intakes increased as dose of yam increased. When compared with mice of the control group, feed and energy intakes were significantly increased in all yam groups except for the 25% Tainung group. Mice fed on 50% Purpurea diet consumed the greatest amount of feed and energy, approximately 60% more than those consumed by mice fed on the control diet.

Plasma lipids levels

Table 4 shows effects of different diets on plasma cholesterol and triacylglycerol concentrations, and the atherogenic index [percentage ratio of (LDL+VLDL)-cholesterol to HDL-cholesterol]. The plasma total, (LDL+VLDL)-, and HDL-cholesterol levels, and atherogenic index were not influenced by types, but significantly affected by the dose of yam. The interaction of type and dose effects on plasma total cholesterol and HDL cholesterol concentrations was also significant.

Plasma total cholesterol concentrations in mice fed on 50% Purpurea and 50% Tainung diets were reduced to 79% and 77%, respectively, of the level observed in mice fed on the control diet. These decreases in the plasma total cholesterol level were due to decreases in both (LDL+ VLDL)-cholesterol and HDL-cholesterol concentrations. Regardless of the changes in cholesterol profile, mice fed on any diet had similar atherogenic index values.

Mice fed on 25% Purpurea and Japonica diets, not on other yam-containing diets, showed approximately 25% and 33% lower plasma triacylglycerol levels, respectively, than mice fed on the control diet. Nevertheless, 50% Purpurea and Japonica did not depressive the plasma triacylglycerol levels as compared to the control diet.

Hepatic weights and lipid contents

Table 5 shows effects of different diets on hepatic weights, cholesterol and triacylglycerol contents. Mice from different groups obtained similar liver weights and relative liver weights. The hepatic cholesterol contents tended to decrease for 10-20% in mice consuming yam diets. However, the differences were not statistically significant. Type, instead of dose, was a significant factor for hepatic triacylglycerol content. The effect of Purpurea on hepatic triacylglycerol content was more pronounced than the other two yams. The hepatic triacylglycerol levels of mice fed on both 25% and 50% Purpurea diets were only 70% and 50% of the levels in mice fed on the control diet, respectively. Although 50% Tainung, 25% and 50% Japonica diets also showed a trend in decreasing hepatic triacylglycerol content, effects of these diets were not statistically significant.

Fecal lipids and bile acid concentrations and excretions

Table 6 shows the fecal concentrations and daily fecal outputs of cholesterol, bile acid and triacylglycerol. Mice fed on Tainung diet had lower fecal cholesterol, bile acid and triacylglycerol concentrations than mice fed on the control and the other two types of yams. The fecal cholesterol concentration was the greatest in mice fed on the control diet. The fecal cholesterol concentration in mice fed on 25%Purpurea, Tainung and Japonica was only 44%, 33%, and 44% of the level exerted in mice of the control group. Mice fed on 50% Tainung diet had the lowest fecal cholesterol concentration, only ~ 30% of the level in mice fed on the control diet. When the daily fecal mass was accounted, the dose effect was significant for the daily cholesterol output. Lower level (25%) of yam diet tended to increase the cholesterol output. Higher level of Tainung tended to increase fecal cholesterol output. In contrast, mice fed on 50% Purpurea and 50% Japonica diet elevated the cholesterol outputs enormously, ~3.8 and 4.6 folds over the level observed in mice fed on the control diet, respectively.

Tainung had the strongest role in decreasing the fecal bile acid concentration (78% lower than the control level) among all types of yams investigated in the present study. In contrast, Purpurea and Japonica were strong stimulators for daily fecal bile acid excretion. Similar to cholesterol output, the dose of yam was positively associated with the level of daily output. Mice fed on 25% yam diets tended to excrete bile acid 2-3 folds of the levels in mice fed on the control diet, although these increments were not statistically significant. Among the 50% yam diets, mice fed on the Purpurea and Japonica excreted approximately 6 folds of the levels seen in the control-fed mice.

Fecal triacylglycerol concentrations in mice fed on 25% Purpurea, 50% Purpurea and 25% Japonica were elevated to 190%, 180% and 172% of the level observed in mice fed on the control diet. The fecal triacylglycerol output in 25% and 50% Purpurea were ~ 2 and 14 folds of the level excreted by the control mice, respectively. Either dose of Japonica diets increased the daily triacylglycerol output to 7 folds of the level seen in mice fed on the control diet. Either dose of Tainung diets increased the triacylglycerol output slightly (to 4 folds of the control), and these increases were not statistically significant.

Apparent triacylglycerol absorption and Lipase activity on the brush border

Table 7 reveals effects of various diets on apparent triacylglycerol absorption and lipase activity on the brush border of the small intestine. The apparent triacylglycerol absorption decreased as the dose increased. The mean absorption rate was 95% for control-fed mice, which was reduced to around 75% in mice fed on 25% of Purpurea diet, and to below 70% in mice fed on the 50% Purpurea and 50% Japonica diets.

Discussion-Lipid metabolism

In the present study, we observed reduction in blood cholesterol below the normal level in mice fed on 50% Purpurea and Tainung diets, which was in accordance with increases in fecal cholesterol and bile acid outputs in mice. The dietary fibers could possibly contribute to the hypocholesterolemic effects and inflated fecal cholesterol and bile acid outputs. Apart from starch, dietary fiber was the second predominant components, 4.4% in Purpurea and Japonica, and 5.3% in Tainung, respectively. Tainung especially contained rich amount of soluble fiber (1.88%), almost 1/3 of the total dietary fiber in the soluble fraction. Purified viscous fibers such as psyllium have been shown to effectively reduced blood total and LDL cholesterol levels in human (Anderson *et al.* 1988; Davidson *et al.* 1998) or animal studies (Frias & Sgarbieri 1998; Levrat-Verny *et al.* 2000). However, several reports indicated that along with the decreases in blood total cholesterol concentrations, HDL-cholesterol levels were also reduced by ingestion of soluble fibers (Abraham & Mehta 1988; Gupta *et al.* 1994). The present study confirmed the simultaneous decreases in total, LDL and HDL-cholesterol concentrations.

The dissociation between fecal bile acid output and plasma cholesterol level found in Japonica-fed mice may be due to the expansion in bile pool. Diosgenin, an important starting material for the manufacture of sexual hormone (Vasil'eva & Paseshnichenko 1996; Araghiniknam *et al.* 1996), has been suggested to increase biliary cholesterol and bile acid output but not necessarily decrease blood cholesterol level (Roman *et al.* 1995, Thewles *et al.* 1993; Juarez *et al.* 1987). The presence of diosgenin in Japonica, possibly not in the other two types (personal communication), could cause increased cholesterol and bile synthesis. This hypothesis explained why fecal cholesterol and bile acid outputs increased dose-dependently in mice fed on Japonica diet, but blood cholesterol level did not decrease accordingly.

The plasma triacylglycerol levels were found reduced in mice ingesting 25%, but not 50% Purpurea and Japonica diets. This observation suggested that reduced fat absorption was not the only mechanism for the changes in the plasma triacylglycerol level. In fact, plasma triacylglycerol level did not always reflect the amount of triacylglycerol absorbed. Instead, many studies have observed that blood triacylglycerol levels were elevated under the conditions of malabsorption, such as in chylomicron-deficient mice (Jung *et al.* 1999) or in men with celiac disease (Ciampolini & Bini 1991). The switch from a high fat diet to a low fat, high carbohydrate, high fiber diet also causes greater blood triacylglycerol level (Mensink & Katan 1987). Although the mechanism for the hypotriglyceridemic effects remained to be clarified, it is suggested that malabsorption resulted in increased *de no* triacylglycerol synthesis by using free fatty acid released from the adipose tissue as a substrate (Jung *et al.* 1999). Besides, low fat high carbohydrate diet may stimulate *de no* lipogenesis using carbohydrate as the substrate (Hudgins *et al.* 1996).

In summary, three different types of Taiwanese yams, Purpurea, Tainung and Japonica, modulated lipid metabolism in different aspects. High dose (50%) of Tainung reduced plasma cholesterol levels by reducing the reabsorption of biliary cholesterol and bile acid. In contrast, high dose (50%) of Purpurea may exert the hypocholesterolemic effects through the poor triacylglycerol absorption and poor re-uptake of cholesterol in the enterohepatic cycle. Purpurea and Japonica exerted lipotropic effect that was associated with reduced fat absorption. However, plasma triacylglycerol level rebounded when fat absorption was depressed to be below 70% in mice fed on high level of Purpurea and Japonica.

四、參考文獻

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Table 1. Composition and caloric density of control and diets incorporated with 25% or 50% of *D. alata,* Purpurea (Purpurea), *D. alata,* Tainung No. 2 (Tainung), and *D. japonica* (Japonica)

	Control	25% yam	50% yam
Ingredients (g/kg)			
Corn oil	70	70	70
Corn starch	529.49	279.49	29.49
Sucrose	100	100	100
Casein	200	200	200
Cellulose	50	50	50
AIN 93 mineral mix	35	35	35
AIN 93 vitamin mix	10	10	10
L-cysteine	3	3	3
Choline salt	2.5	2.5	2.5
TBHQ*	0.014	0.014	0.014
Yam powder	0	250	500
Caloric density (MJ/kg)			
Control	16.56	—	—
Purpurea	—	16.41	16.10
Taichung	—	16.51	16.30
Japonica	—	16.46	16.19

* TBHQ denotes for Tert-butyl hydroquinone.

Table 2. Composition of the lyophilized powders prepared from peeled tubers of *D. alata*, Purpurea (Purpurea), *D. alata,* Tainung No. 2 (Tainung), and *D. japonica* (Japonica)*

Type...	Purpurea	Tainung	Japonica
Ingredients (g/kg)			
Carbohydrate	840.4	861.6	860.3
Protein	69.7	68.9	58.0
Fat	6.7	7.5	8.0
Ash	4.4	3.6	3.3
Crude fiber	23.2	41.0	15.1
Total dietary fiber	44.1	53.1	43.8
Soluble dietary fiber	9.2	18.8	5.5

*Analyses were done according to the methods of the Association of Official Analytical Chemists (1997).

Table 3. The total and soluble fiber intakes and weights of the cecum and colon walls in mice fed on control diet or diets enriched with 25% or 50% *D. alata*, L.var. Purpurea or *D. alata L.* cv. Tainung No. 2, or *D. japonica* Thumb. var. *pseudojaponica* (Hay.) Yamamoto

	Dietary fiber intake (g/d)		Weights of organ walls (g)	
	Total	Soluble	Cecum	Colon
Control	0.23	0	0.07	0.15
D. alata, Purpurea				
25%	0.34**	0.013**	0.10	0.23*
50%	0.53**	0.034**	0.13**	0.26**
D. alata, Tainung No. 2				
25%	0.31**	0.023**	0.14**	0.26**
50%	0.43**	0.052**	0.14**	0.26**
D. japonica				
25%	0.36**	0.008**	0.12**	0.25**
50%	0.48**	0.018**	0.13**	0.28**
Pooled SEM	0.012	0.002	0.004	0.007

[1] Data were expressed as means and pooled SEM, N=11.

*, **Mean value was significant different from the control as analyzed by one-way ANOVA followed by Dunnett's test, *$0.05 > P > 0.01$, **$P < 0.01$.

TABLE 4. Effects of yam-enriched diets on the morphological and proliferative indices of the mucosa cells in the cecum of Balb/c mice[1]

	Crypt depth (μm)	Crypt width (μm)	Cell density[2]	Labeled crypt height[3] (μm)	Labeling zone[4]	Labeled cell density[5]
Control	53.3	45.8	10.7	19.1	0.36	0.13
D. alata, Purpurea						
25%	72.8*	46.9	13.0	24.1	0.32	0.12
50%	77.0**	50.6	15.0*	30.3	0.39	0.11
D. alata, Tainung No. 2						
25%	75.5**	53.6	15.9**	27.7	0.37	0.12
50%	86.0**	51.6	15.5**	34.2*	0.39	0.14
D. japonica						
25%	76.0**	44.6	15.6**	27.6	0.36	0.11
50%	87.5**	49.2	16.0**	32.8*	0.36	0.17
Pooled SEM	1.7	1.0	0.3	1.3	0.01	0.01

[1]Data were expressed as means and pooled SEM, 10 crypts from each mice, n=8.

[2]The number of cells in one side of the crypt.

[3]The distance of the labeled cell from the bottom of the crypt (μm).

[4]Defined as the ratio of labeled crypt height to the crypt depth.

[5]Defined as the ratio of the number of labeled cells to the cell density.

*, ** Mean value was significantly different from the control as analyzed using 2-sided Dunnett's test; * $0.05 > P \geq 0.01$, ** $P < 0.01$.

TABLE 5. Effects of yam-enriched diets on the morphological and proliferative indices of the mucosa cells in the distal colon of Balb/c mice[1]

	Crypt depth (μm)	Crypt width (μm)	Cell density[2]	Labeled crypt height[3] (μm)	Labeling zone[4]	Labeled cell density[5]
Control	78.5	25.8	12.6	26.5	0.36	0.13
D. alata, Purpurea						
25%	81.2	42.9**	11.8	33.5	0.41	0.21
50%	99.5	51.7**	14.2	42.4**	0.43	0.23*
D. alata, Tainung No. 2						
25%	91.4	45.6**	14.5	34.5	0.38	0.17
50%	98.8	47.7**	14.2	43.5**	0.45	0.22*
D. japonica						
25%	101.0	43.0**	13.0	33.6	0.33	0.21
50%	106.8*	44.8**	14.6	45.4**	0.41	0.23*
Pooled SEM	2.4	1.7	0.3	1.3	0.02	0.01

[1]Data were expressed as means and pooled SEM, 10 crypts from each animals, n=8.

[2]The number of cells in one side of the crypt.

[3]The distance of the labeled cell from the bottom of the crypt (μm).

[4]Defined as the ratio of labeled crypt height to the crypt depth.

[5]Defined as the ratio of the number of labeled cells to the cell density.

*, **Mean value was significantly different from the control as analyzed using 2-sided Dunnett's test; *: $0.05 > P > 0.01$, **: $P < 0.01$.

Table 6. Effects of yam-enriched diets on cecal short chain fatty acid content in Balb/c mice[1]

	Acetate	Propionate	Butyrate	Total
		μmol/cecum		
Control	35.97	5.06	6.27	47.31
D. alata, Purpurea				
25%	63.01	5.93	10.10	79.04
50%	62.33	5.58	11.69	79.60
D. alata, Tainung No. 2				
25%	86.21	11.81	22.57*	120.59
50%	159.75**	14.52	42.33**	216.60**
D. japonica				
25%	104.40*	22.71*	17.36*	144.48*
50%	159.95**	15.33	33.36**	208.63**
Pooled SEM	8.8	1.5	0.24	46.5
		Molar percentage (%)		
Control	77.7	11.2	11.1	-
D. alata, Purpurea				
25%	79.3	7.6	13.1	-
50%	78.2	7.0	14.7	-
D. alata, Tainung No. 2				
25%	73.8	9.0	16.9	-
50%	73.8	6.2	20.0**	-
D. japonica				
25%	78.3	7.0	14.7	-
50%	79.3	7.6	13.1	-
Pooled SEM	0.80	0.66	0.74	

[1]Data were expressed as means and pooled SEM, N=8.

**Mean value was significant different from the control as analyzed by one-way ANOVA followed by Dunnett's test; *$0.05 > P \geq 0.01$, **$P<0.01$.

Table 7. Effects of yam-enriched diets on daily fecal excretion of short chain fatty acids in Balb/c mice[1]

	Acetate	Propionate	Butyrate	Total
		μmol/d		
Control	362.71	23.33	19.10	405.10
D. alata, Purpurea				
25%	583.01	38.39	36.44	657.88
50%	2465.41*	181.70*	186.46*	2833.58*
D. alata, Tainung No. 2				
25%	492.55	26.38	19.88	538.76
50%	510.32	59.47	60.03	632.40
D. japonica				
25%	599.63	42.67	27.04	669.30
50%	747.89	47.12	19.09	814.06
Pooled SEM	103.47	8.20	10.01	119.40
		Molar Percentage (%)		
Control	89.2	5.4	5.4	-
D. alata, Purpurea				
25%	89.2	6.0	4.8	-
50%	87.3	6.3	6.4	-
D. alata, Tainung No. 2				
25%	90.3	5.6	4.1	-
50%	79.7*	9.6	10.7*	-
D. japonica				
25%	90.5	6.0	3.5	-
50%	92.2	5.4	2.5	-
Pooled SEM	0.70	0.41	0.41	

[1]Data were expressed as means and pooled SEM, N=8.

*Mean value was significant different from the control as analyzed by one-way ANOVA followed by Dunnett's test; *$0.05>P>0.01$.

Table 8. Fecal characteristics and number of fecal pellets excreted from Balb/c mice fed control or diet containing 25% or 50% *D. alata*, L. var. Purpurea or *D. alata L.* cv. Tainung No. 2, or *D. japonica Thunb.* var. *pseudojaponica* (Hay.) Yamamoto. for 21 days [1,2]

	Daily wet wt	Daily dry wt	Moisture	Number of fecal pellet
	g/d		%	
Control	0.43 ± 0.19 [a]	0.23 ± 0.10 [a]	40.4 ± 9.5 [a]	53.6 ± 19.6 [a]
D. alata, Purpurea				
25%	1.60 ± 0.32 [b]	0.90 ±0.17 [b]	44.0±1.7 [bc]	91.6±18.8 [b]
50%	3.45±0.37 [d]	2.01±0.17 [d]	41.5±3.9 [ab]	135.3±21.9 [c]
D. alata, Taichung No. 2				
25%	1.76±0.19 [b]	0.90±0.09 [b]	49.8±3.9 [d]	87.3±26.1 [b]
50%	2.95±0.74 [c]	1.54±0.38 [c]	46.9±4.2 [cd]	117.9±30.1 [c]
D. japonica				
25%	1.80±0.32 [b]	0.96±0.18 [b]	46.6±3.6 [cd]	93.6±21.2 [b]
50%	3.79±0.53 [d]	2.07±0.32 [d]	45.5±1.9 [cd]	119.0±20.8 [c]

[1] mean ± SD. N=8.

[2] Different superscripts denotes for significant different ($P < 0.05$) between treatment groups as analyzed by one-way ANOVA followed by LSD test.

Table 9. Body weights, feed and energy intakes of mice fed on control or diets incorporated with 25% or 50% of *D. alata*, Purpurea (Purpurea), *D. alata*, Tainung No. 2 (Tainung), and *D. japonica* (Japonica) for 21 d (Mean values for eleven mice per group)

Experimental groups…	Control	Purpurea		Tainung		Japonica		SEM
		25%	50%	25%	50%	25%	50%	
Body weight (g)								
Initial	17.84	18.40	18.03	17.91	18.21	17.79	18.71	0.26
Final	22.05	22.63	21.77	23.06	22.86	22.29	22.84	0.20
Intake/d								
Feed (g)	4.5	5.6[b]	7.4[b]	4.9	5.6[b]	5.9[b]	6.7[b]	0.12
kJ	74.8	91.1[b]	119.5[b]	81.2	90.7[b]	96.6[b]	108.2[b]	1.93

[a,b] Mean value was significantly different from the control as analyzed using 2-sided Dunnett's test; [a]: 0.05 >P

> 0.01, [b]: $P < 0.01$.

TABLE 10. Plasma lipid levels and atherogenic index of mice fed on control or diets incorporated with 25% or 50% of *D. alata*, Purpurea (Purpurea), *D. alata*, Tainung No. 2 (Tainung), and *D. japonica* (Japonica) for 21 d

(Mean values for eleven mice per group)

Groups...	Control	Purpurea		Tainung		Japonica		
		25%	50%	25%	50%	25%	50%	Pooled SEM
Cholesterol (mmol/l)								
Total	3.96	3.70	3.14[b]	3.92	3.03[b]	3.58	3.56	0.07
(LDL+VLDL)	2.22	2.16	1.62[a]	2.23	1.63[a]	2.24	2.02	0.10
HDL	1.07	0.87[§]	0.82[b]	1.07	0.76[b]	0.92	0.91	0.06
TG (mmol/l)	1.37	1.02[†]	1.32	1.14	1.28	0.92[b]	1.39	0.05
Atherogenic index*	50	75	48	58	57	72	62	6.46

*Defined as (VLDL + LDL)-cholesterol × 100%/HDL-cholesterol.

[a,b] Mean value was significantly different from the control as analyzed using 2-sided Dunnett's test; [a]: $0.05 > P > 0.01$, [b]: $P < 0.01$.

TABLE 11. Hepatic weight, cholesterol and triacylglycerol contents (mg/liver) of mice fed on control or diets incorporated with 25% or 50% of *D. alata*, Purpurea (Purpurea), *D. alata*, Tainung No. 2 (Tainung), and *D. japonica* (Japonica) for 21 d

Groups...	Control	Purpurea		Taichung		Japonica		
		25%	50%	25%	50%	25%	50%	Pooled SEM
Weight (g)	1.04	0.96	1.01	1.00	0.99	1.00	0.97	0.02
(% body weight)	4.71	4.22	4.66	4.36	4.33	4.51	4.23	0.06
Cholesterol	22.4	17.2	19.2	20.8	20.7	22.1	15.8	2.0
Triacylglycerol	152.8	106.7[a]	66.0[b]	135.6	118.8	114.0	113.5	5.0

[a,b] Mean value was significantly different from the control as analyzed using 2-sided Dunnett's test; [a]: $0.05 > P > 0.01$, [b]: $P < 0.01$.

TABLE 12. Fecal content and daily excretions of cholesterol, bile acid and triacylglycerol of mice fed on control or diets incorporated with 25% or 50% of *D. alata*, Purpurea (Purpurea), *D. alata*, Tainung No. 2 (Tainung), and *D. japonica* (Japonica) for 21 d

(Mean values for eleven mice per group)

Experimental groups...	Control	Purpurea		Tainung		Japonica		
		25%	50%	25%	50%	25%	50%	Pooled SEM
Cholesterol								
mg/g wet feces	30.74	13.55[b]	13.64[b]	10.29[b]	9.30[b]	13.37[b]	14.10[b]	2.79
mg/d	9.17	17.23	44.53[a]	18.28	28.57	22.25	51.38[b]	3.30
Bile acids								
mg/g wet feces	0.99	0.66[b]	0.55[b]	0.44[b]	0.22[b]	0.47[b]	0.59[b]	0.07
mg/d	0.28	0.81	1.82[b]	0.72	0.82	0.77	1.68[a]	0.12
Triacylglycerol								
mg/g wet feces	13.17	25.10[a]	23.60	13.13	8.58	22.70	11.29	1.39
mg/d	5.42	39.23[a]	76.80[b]	23.61	24.87	39.97[a]	42.10[a]	4.22

[a,b] Mean value was significantly different from the control as analyzed using 2-sided Dunnett's test; [a]: $0.05 > P > 0.01$, [b]: $P < 0.01$.

TABLE 13. Apparent fat absorption and lipase activity in the intestinal brushborder of mice fed on control or diets incorporated with 25% or 50% of *D. alata*, Purpurea (Purpurea), *D. alata*, Taichung No. 2 (Taichung), and *D. japonica* (Japonica) for 21 d

(Mean values for eleven mice per group)

Groups...	Control	Purpurea		Taichung		Japonica		
		25%	50%	25%	50%	25%	50%	Pooled SEM
Apparent fat absorption (%)	94.3	74.4[a]	62.1[a]	80.9	77.9	86.8	69.1[a]	2.15
Lipase activity (U/mg protein)	188.8	272.3	271.9	247.3	239.6	205.5	189.6	12.8

[a,b] Mean value was significantly different from the control as analyzed using 2-sided Dunnett's test; [a]: $0.05 > P > 0.01$.

TABLE 14. Specific enzyme activities in the brush border of the small intestine in mice with free access to control, or diets incorporated with 25% or 50% yams (*D. alata*, Purpurea, *D. alata*, Tainung No. 2, and *D. japonica*) for 21 d [1]

	Control	*D. alata*, Purpurea		*D. alata*, Tainung No. 2		*D. japonica*	
		25%	50%	25%	50%	25%	50%
U/mg DNA							
Leucine aminopeptidase	0.96±0.48	1.52±0.42	2.02±0.8*	1.75±0.82	2.26±0.63*	1.72±0.42	1.46±0.52
Maltase	13.67±3.70	16.02±0.77	13.28±3.91	14.25±3.50	21.30±9.10	21.33±3.15	26.45±8.30*
Sucrase	31.23±12.47	44.89±11.22	33.27±9.27	41.08±11.85	52.08±20.80*	48.17±7.88	37.89±8.77
U/mg protein							
Leucine aminopeptidase	0.10±0.02	0.16±0.01	0.12±0.04	0.22±0.08*	0.21±0.04*	0.14±0.03	0.11±0.02
Maltase	1.76±0.47	1.72±1.13	2.21±0.80	1.10±0.20	1.99±1.59	1.65±0.22	3.56±1.57*
Sucrase	4.97±1.83	4.33±2.41	4.57±2.11	3.18±1.53	5.38±5.15	3.48±0.27	2.78±0.53

[1] Values are means ± SD, n=8.

* Significant different from the control as analyzed with one-way ANOVA followed by Dunnett's test, $P < 0.05$.

No.	Sample	Density
1	Marker	27216
2	Negative control	15770
3	Negative control	13890
4	50% 紅藥 diet	69750
5	Control diet	93240
6	25%紅藥 diet	88128
7	50%紅藥 diet	74375
8	50%台藥	45630
9	25%台藥	16632
10	25%基藥	81702
11	50%基藥	74520
12	C. Perfringens 10^{-1}	67284
13	C. Perfringens 10^{-2}	45175
14	C. Perfringens 10^{-2}	16380

Fig 1. Electrophoresis of PCR products against 16 S of *C. perfringens*