行政院國家科學委員會專題研究計畫 成果報告

探討蒟蒻纖維抵禦高脂低纖維飲食誘發之氧化壓力及大腸腫瘤之作用及其細胞內相關路徑之初步探討(第 2 年)
研究成果報告(完整版)

計 畫 類 別 ： 個別型
計 畫 編 號 ： NSC 99-2320-B-040-014-MY2
執 行 期 間 ： 100 年 08 月 01 日至 101 年 07 月 31 日
執 行 單 位 ： 中山醫學大學營養學系（所）

計 畫 主 持 人 ： 陳曉鈴
共 同 主 持 人 ： 蔡崇弘

報 告 附 件 ： 出席國際會議研究心得報告及發表論文

公 開 資 訊 ： 本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 101 年 10 月 31 日

中文摘要： 1.本研究目的是以 Sprague-Dawley 雄性大鼠為實驗模式，探討(1) 高脂(玉米油)飲食是否惡化大腸癌發生的潛在危險因子；(2) 蒟蒻葡甘聚醣是否能調降大腸表皮細胞病變之危險指標及促進預防因子。五週齡大鼠(n=8/組)隨機分為四組: 低脂(5% 玉米油, w/w)無纖維組、高脂(25% 玉米油, w/w)無纖維組、以及高脂飲食加入蒟蒻葡甘聚醣(5%, w/w)或菊糖(作為益生質控制組)。第四週收集所有排出之糞便以測定大腸癌發生的潛在危險因子: 糞便細菌酵素刍-glucuronidase、mucinase 和膽酸；以及保護因子: 糞便菌相和盲腸短鏈脂肪酸。收集第四週排出之所有糞便，分析糞便刍-glucuronidase、mucinase 活性及膽酸；犠牲動物後收集盲腸內容物分析短鏈脂肪酸。結果顯示，相較於低脂無纖維組，高脂無纖維組糞便刍-glucuronidase 及 mucinase 活性顯著升高；並且增加糞便二級膽酸佔總膽酸的比例，證實高玉米油飲食促進大腸癌潛在危險因子上升高。然而，於高脂肪飼料中補充 5%蒟蒻聚葡甘露醣後，不但明顯降低糞便刍-glucuronidase、mucinase 活性及二級膽酸的比例，而且顯著增加每天糞便中 bifidobacteria 和 lactobacilli 菌數以及盲腸內容物乙酸濃度，而且蒟蒻葡甘聚醣的這些作用與菊糖相似。因此，補充蒟蒻葡甘聚醣或菊糖這兩種益生質有益於改善腸道環境、降低高脂肪飲食引起之糞便細菌酵素活性及二級膽酸，具有降低高脂飲食造成之大腸直腸癌危險性的潛力。

2.本研究探討蒟蒻纖維及菊糖寡醣調節高脂無纖維飲食誘發之氧化壓力、腸道表皮細胞及肝臟抗氧化酵素基因表現、血液抗氧化分子的變化。五週齡大鼠(n=8/組)隨機分為四組: 低脂(5% 玉米油, w/w)無纖維組、高脂(25% 玉米油, w/w)無纖維組、以及高脂飲食加入蒟蒻葡甘聚醣(5%, w/w)或菊糖(作為益生質控制組) ，餵食第四周收集糞便樣品，之後犠牲。測量糞便菌相、短鏈脂肪酸濃度、體內氧化壓力指標(如血液 MDA 及淋巴球 DNA 傷害程度)、抗氧化酵素(glutathione peroxidase, superoxide dismutase, catalase)基因表現及血漿抗氧化分子(a-tocopherol、ascorbic acid)濃度。結果顯示，於高脂無纖維飼料中添加蒟蒻聚葡甘露醣及菊糖有效降低大腸及肝臟脂質過氧化產物及白血球 DNA 傷害，同時這兩種纖維提高大腸及肝臟抗氧化酵素基因表現以及血液維生素 E 濃度。蒟蒻聚葡甘露醣及菊糖在大鼠大腸中發酵產生短鏈脂肪酸尤其是乙酸及丁酸。因此本研究建議體內利用蒟蒻聚葡甘露醣及菊糖產生腸道及全身系統性抗氧化作用。

中文關鍵詞： 蒟蒻聚葡甘露醣、糞便細菌酵素、短鏈脂肪酸、菌相、膽酸、氧化壓力

英 文 摘 要 ：

The aim of this study was to investigate the effects of KGM and inulin on the balance between pro-oxidative status and antioxidative defense systems in the colon, liver and plasma of rats fed a high-fat fiber-free diet. Male Sprague-Dawley rats were fed a high-fat (25% corn oil, w/w) fiber-free diet or that supplemented with KGM or inulin fiber (5%, w/w) for 4 weeks. The index of pro-oxidative status, malondialdehyde (MDA) and blood lymphocyte DNA damage, and the antioxidative defense, that is, antioxidant enzymes (glutathione peroxidase, superoxide dismutase, catalase) in the colonic mucosa and liver, and the plasma antioxidant levels were determined. Incorporation of KGM and inulin into the high-fat fiber-free diet beneficially reduced the MDA levels of the colon and liver and DNA damage in blood lymphocytes. On the other hand, both fibers enhanced the antioxidative defense systems by up-regulating the gene expressions of glutathione peroxidase and catalase in the colonic mucosa, and the superoxide dismutase and catalase in the liver. Furthermore, KGM and inulin promoted antioxidative status in the blood by elevating the a-tocopherol level. KGM and inulin were well-fermented in rats and increased the concentration and daily excretion of fecal short-chain fatty acids, especially acetate and butyrate. These results suggest that in vivo utilization of KGM and inulin stimulated both local and systemic antioxidative defense systems in rats.

英文關鍵詞： konjac glucomannan, inulin, malondialdehyde, DNA damage, antioxidant enzymes, short-chain fatty acid, bile acid

行政院國家科學委員會補助專題研究計畫
期末報告

探討蒟蒻纖維抵禦高脂低纖維飲食誘發之氧化壓力及大腸腫瘤之作用及其細胞內相關路徑之初步探討
（通過 2 年補助）

計畫類別：☑個別型計畫　□整合型計畫
計畫編號：NSC99-2320-B-040-014-MY2
執行期間：99 年 8 月 1 日至 101 年 7 月 31 日
執行機構及系所：中山醫學大學營養系

計畫主持人：陳曉鈴
共同主持人：
計畫參與人員：吳文慈

本計畫除繳交成果報告外，另含下列出國報告，共 _1_ 份：
□移地研究心得報告
☑出席國際學術會議心得報告
□國際合作研究計畫國外研究報告

處理方式：除列管計畫及下列情形者外，得立即公開查詢
☑涉及專利或其他智慧財產權，□一年☑二年後可公開查詢

中　華　民　國 101 年 10 月 20 日

目　　　錄

第一年成果	p. 2-10
第二年成果	p. 11-25
成果報告自評表	p. 26-27

蒟蒻地下球莖富含D-葡萄糖和D-甘露醣以β-1,4醣苷鍵鍵結而成的水溶性膳食纖維--葡甘聚醣(glucomannan)。本研究室近幾年積極開發其保健功效，例如降低第二型高血膽固醇病患之血糖及血膽固醇濃度、調節排便習慣、促進益生菌生長及降低糞便水萃物毒性等，並推測在低纖維高脂肪飲食中添加蒟蒻葡甘聚醣或菊糖寡醣具有調降糞便中大腸癌病變前危險因子的潛力，並且這些纖維於大腸代謝後具有提高大腸細胞以及全身系統的抗氧化機制。研究成果已經發表於兩篇論文如下:

1. Wen-Tzu Wu, **Hsiao-Ling Chen***. 2011. Effects of konjac glucomannan on putative risk factors for colon carcinogenesis in rats fed a high-fat diet. J Agric Food Chem 59:989-994. **(SCI)** ***Agriculture, multidisciplinary 2/55=3.6%, IF=2.816, 3*5*5=75***

2. Wen-Tzu Wu and **<u>Hsiao-Ling Chen</u>***. 2011. Konjac glucomannan and inulin systematically modulate antioxidant defense in rats fed a high-fat fiber-free diet. J Agric Food Chem 59:9194-9200 **(SCI)** ***Agriculture, multidisciplinary 2/55=3.6%, IF=2.816, 3*5*5=75***

第一年成果報告:

Wen-Tzu Wu, **Hsiao-Ling Chen***. 2011. Effects of konjac glucomannan on putative risk factors for colon carcinogenesis in rats fed a high-fat diet. J Agric Food Chem 59:989-994. **(SCI)** ***Agriculture, multidisciplinary 2/55=3.6%, IF=2.816, 3*5*5=75***

中文摘要

本研究目的是以 Sprague-Dawley 雄性大鼠為實驗模式，探討(1) 高脂(玉米油)飲食是否惡化大腸癌發生的潛在危險因子; (2) 蒟蒻葡甘聚醣是否能調降大腸表皮細胞病變之危險指標及促進預防因子。五週齡大鼠(n=8/組)隨機分為四組: 低脂(5% 玉米油, w/w)無纖維組、高脂(25% 玉米油, w/w)無纖維組、以及高脂飲食加入蒟蒻葡甘聚醣(5%, w/w)或菊糖(作為益生質控制組)。第四週收集所有排出之糞便以測定大腸癌發生的潛在危險因子: 糞便細菌酵素β-glucuronidase、mucinase和膽酸；以及保護因子: 糞便菌相和盲腸短鏈脂肪酸。收集第四週排出之所有糞便，分析糞便β-glucuronidase、mucinase 活性及膽酸；犧牲動物後收集盲腸內容物分析短鏈脂肪酸。結果顯示，相較於低脂無纖維組，高脂無纖維組糞便β-glucuronidase 及 mucinase 活性顯著升高；並且增加糞便二級膽酸佔總膽酸的比例，證實高玉米油飲食促進大腸癌潛在危險因子上升高。然而，於高脂肪飼料中補充 5%蒟蒻聚葡甘露醣後，不但明顯降低糞便β-glucuronidase、mucinase 活性及二級膽酸的比例，而且顯著增加每天糞便中 bifidobacteria 和 lactobacilli 菌數以及盲腸內容物乙酸濃度，而且蒟蒻葡甘聚醣的這些作用與菊糖相似。因此，補充蒟蒻葡甘聚醣或菊糖這兩種益生質有益於改善腸道環境、降低高脂肪飲食引起之糞便細菌酵素活性及二級膽酸，具有降低高脂飲食造成之大腸直腸癌危險性的潛力。

關鍵字：蒟蒻聚葡甘露醣、糞便細菌酵素、短鏈脂肪酸、菌相、膽酸

Abstract

Konjac glucomannan (KGM), a soluble dietary fiber, is rich in glucomannan polysaccharides which is composed of D-glucose and D-mannose by β-1,4 glycosidic bond. The aim of study was to determine effects of KGM in a high fat corn oil diet on risk factors of colon carcinogenesis, that is, fecal β-glucuronidase, mucinase and bile acids, and on preventive factors, that is, fecal microflora and cecal short-chain fatty acids (SCFAs). Sprague-Dawley rats (n = 8 animals per group) were fed a normal-fat fiber-free (5% corn oil, w/w), or high-fat (25% corn oil, w/w) diets containing no fiber, KGM (5%, w/w) or inulin (5%, w/w, as a prebiotic control) for 4 weeks. Results indicated that the high-fat fiber-free diet significantly elevated the fecal β-glucuronidase and mucinase activities, total bile acid concentration, and decreased cecal SCFA contents, as compared with its normal-fat counterpart. The incorporation of KGM, as well as inulin, into the high-fat fiber-free diet beneficially reduced the fecal β-glucuronidase and mucinase activities, and lithocholic acid (secondary bile acid) concentration. Although KGM elevated the daily fecal total bile acid excretion, the change was due to the increase in the primary, instead of the secondary, bile acids. In addition, KGM beneficially promoted the daily fecal excretion of bifidobacteria and lactobacilli, and cecal SCFA contents, as compared with the high-fat fiber-free diet. Therefore, the present study suggests that KGM potentially attenuated the high fat-induced risk in colon carcinogenesis.
Key words：Konjac glucomman; β-glucuronidase; mucinase; short-chain fatty acid; microflora; bile acid

Introduction

Colorectal cancer is among the leading causes of cancer mortality in developed countries *(1)*. The protective role of dietary fiber-containing foods was proposed, however, mechanisms where by dietary fiber modulate the colorectal cancer remain to be illustrated. It is generally accepted that dietary fiber may decrease incidence of colorectal cancer by increasing colonic bulk, reducing the transit time *(1, 2)* and promoting the production of short-chain fatty acids (SCFAs) that have been shown to induce apoptosis, cell cycle arrest and differentiation of colon cancer cells *(3, 4)*. Recent studies also suggest that probiotics, such as bifidobacteria and lactobacilli, may reduce the colon carcinogensis *(5, 6)*. The anticarcinogenic effect of soluble dietary fibers may be partially mediated by their prebiotic effects. However, effects of dietary fibers on gut microflora-associated risk factors of colorectal cancer *(7)* such as β-glucuronidase that hydrolyzes pro-carcinogen into carcinogen *(8)*, and mucinase that hydrolyzes the protective mucin coat in the intestinal wall and thus exposes the underlying colonocytes to the luminal carcinogens *(9)* are not fully investigated.

KGM, consisting of D-glucose and D-mannose units joined together with β-1,4 glycosidic bond linkages, is a fermentable soluble fiber derived from the tubers of *Amorphophallus konjac* C. koch *(10)*. KGM is shown to improve serum cholesterol and blood glucose levels *(10, 11)*, bowel movement and colonic microflora *(12, 13)*. Besides these beneficial functions, KGM may prevent the risk of colon carcinogenesis with a normal level of dietary fat by reducing fecal concentration of secondary bile acids in mice *(14)* and the toxicity of fecal water obtained from mice toward a model of colonocytes *(14, 15)*.

A high fat intake has been shown to increase fecal β-glucuronidase activity and

secondary bile acids *(16, 17)*. However, the role of fat on fecal mucinase, microflora and short chain fatty acids remains to be investigated. In addition, mechanisms underlying the anticarcinogenic potential of dietary fibers in the matrix of high-fat diet have not been clearly demonstrated. Therefore, the effects of dietary fat (25% vs. 5% corn oil, w/w) on potential precancerous risk factors of colon carcinogenesis, that is, fecal β-glucuronidase, mucinase, and secondary bile acids, and their effects on preventive factors, that is, fecal bifidobacteria and lactobacilli and cecal SCFAs, in rats fed a fiber-free diet, were determined in this study. We further determined effects of KGM or inulin (as a prebiotic control) in the high-fat fiber-free diet on these modulated factors of colon carcinogenesis.

Results

The energy intake, weight gain and feed efficiency were similar between rats fed the normal-fat fiber-free (NF) diet and its high-fat counterpart (**Table 2**). The incorporation of KGM and inulin into theHF diet significantly suppressed the energy intake by 10.3% and 11.6%, respectively, and the body weight gain by more than 30%. In agreement with that, the feed efficiencies of high-fat KGM and inulin diets were significantly lower than that of their fiber-free counterpart.

The HF diet significantly increased fecal β-glucuronidase and mucinase activity by 145% and 52% as compared with its normal-fat counterpart (**Figure 1**). The incorporation of KGM and inulin into the HF diet significantly reduced β-glucuronidase activity by 71% and 82%, respectively, to levels similar to that shown in the NF diet. The incorporation of KGM and inulin into the HF diet significantly reduced mucinase activity by 68% and 43%, respectively, to levels even lower than that shown in the NF diet.

The high-fat fiber-free diet increased the concentration of primary bile acids, cholic acid and chenodeoxycholic acid, and the total bile acids, but not the secondary bile acids (deoxycholic acid and lithocholic acid), as compared with its normal-fat counterpart (**Table 3**). KGM and inulin did not reduce the fecal concentration of total bile acids. However, the fecal cholic acid concentration was greater in the KGM and inulin group, respectively, than that in the HF group, while the lithocholic acid concentration was lower in the KGM and inulin group, respectively. Therefore, addition of KGM and inulin significantly reduced the proportion of secondary bile acids to 31.5 ± 3.5% and 31.0 ± 1.6%, respectively, as compared to that (42.6 ± 3.7%) in the HF group.

The daily fecal mass was similar between rats fed the NF diet (1.6 ± 0.2 g/d) and its high-fat counterpart (1.3 ± 0.1 g/d). The incorporation of KGM and inulin into the high-fat diet significantly elevated the fecal output to 1.9 ± 0.1 g/d and 1.9 ± 0.2 g/d, respectively. Therefore, the effects of various diets on daily fecal output of bile acids were determined (**Table 3**). The HF diet increased the daily fecal output of cholic acid and chenodeoxycholic acid and total bile acids, but not the secondary bile acids, as compared with its normal-fat counterpart. The incorporation of KGM and inulin further elevated the fecal output of cholic acid to 3.2- and 2.7-fold that in the HF group, respectively, and both elevated fecal output of total bile acids to ~1.5-fold. However, the daily fecal output of lithocholic acid, a secondary bile acids, tended to decrease by the addition of KGM as compared with its high-fat counterpart.

The concentration, the daily fecal output and proportion of individual genus of bacteria to total bacteria were not different between the normal-fat and the HF groups (**Table 4**). The incorporation of KGM decreased only the fecal clostridia concentration, and significantly increased the daily fecal output of bifidobacteria and lactobacilli, as compared with the HF diet, respectively. The incorporation of inulin into the high-fat fiber-free diet significantly increased the concentration of lactobacilli and decreased the concentration of clostridia. The inulin group also elevated the daily output of bifidobacteria and lactobacilli, respectively. Furthermore, KGM and inulin significantly decreased the relative ratio of clostridia, respectively, as compared with the HF diet.

The cecal concentration of propionate and cecal contents of each SCFA were significantly lower in the HF group as compared to those in the normal-fat counterpart (**Table 5**). The incorporation of KGM promoted only the cecal *i*-butyrate concentration, as compared with the high-fat fiber-free diet, and elevated the cecal content of each SCFA to the level similar to that shown in the NF group. Inulin diet did not significantly elevate the cecal concentration of any SCFA, but significantly elevated cecal contents of acetate and propionate, as compared with the HF diet.

Discussion

The current study showed that both KGM and inulin ameliorated the high fat-induced mutagenic load to colonocytes by reducing the colonic bacteria β-glucuronidase and mucinase activities and concentrations of lithocholic acid. In addition, KGM and inulin promoted the growth of colonic bifidobacteria and lactobacilli, in agreement with previous studies *(13, 18)*, and production of short chain fatty acids. Therefore, the current study suggested that addition of KGM or inulin into a high corn oil diet beneficially decreased the risk of colonic carcinogenesis partially by modulating the colonic bacteria enzyme activities.

The increased fecal bile acid excretion due to a high-fat diet is considered a major risk for colon carcinogenesis *(16, 17)*. The current study examined the fecal bile acid profile and indicated that dietary corn oil mainly increased fecal excretion of primary bile acids, instead of the secondary bile acids. The use of viscous dietary fibers on colon carcinogenesis prevention is of concern because they may increase the delivery of bile acids to the colon and increase the contact of carcinogenic bile acid to the colonocytes. Results of our study demonstrated that KGM increased the fecal output of total bile acids, in agreement with a previous study *(11)*. However, this increased output was due to the primary, instead of the secondary, bile acids. Although mechanisms remained unclear, we proposed that KGM may hinder the contact between primary bile acids and their metabolizing enzymes. Moreover, KGM has been shown to speed up the transit time, which may prevent the primary bile acids from subsequent transformation into toxic metabolites by the intestinal microflora. Furthermore, the increased cecal SCFA contents in the KGM group may acidify colon contents which would depress the 7α-dehydroxylase activity *(19)*.

The current study observed that incorporation of KGM and inulin into the high-fat fiber-free diet significantly decreased the fecal lithocholic acid concentration and slightly modulated the deoxycholic acid concentration. Previous studies have

shown that the secondary bile acids, such as lithocholic acid and deoxycholic acid, were cytotoxic for normal colonic mucosa cells and caused a compensatory mucosal proliferation, which is related to higher risk of colon carcinogenesis *(20)*. Therefore, the incorporation of KGM and inulin in the high-fat fiber-free diet has the potential to reduce the fecal toxicity toward colonocytes.

The principal metabolites of fermentable fibers are SCFAs, namely acetate, propionate and butyrate. The SCFAs, in particular *n*-butyrate, have been shown to be utilized as an energy source in normal intestinal epithelia *(21),* induce apoptosis and inhibit proliferation of transformed cells *(22)*. This study was the first to demonstrate that a high-fat diet resulted in a lower cecal SCFA production than did its normal-fat counterpart, which may lead to a greater risk of colonic carcinogenesis. However, the incorporation of KGM and inulin into this high-fat fiber-free diet almost diminished the fat-induced reduction in the cecal SCFA contents. Therefore, this result suggests that KGM and inulin may reduce the high fat diet-induced risk of colon carcinogenesis partially by offering the fermentation substrate for colonic microflora, supporting normal intestinal epithelia *(21)* and suppressing the neoplasia.

Conclusion

In conclusion, KGM, as well as its prebiotic control inulin, diminished the high corn oil-induced alterations in the fecal β-glucuronidase and mucinase activities and cecal short-chain fatty acid contents. These soluble fibers also reduced the fecal lithocholic acid concentration and enhanced the fecal output of bifidobacteria and lactobacilli. Therefore, KGM and inulin potentially prevent the risk of colon carcinogenesis associated with a high-fat intake.

References

(1) *Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective.* American Institute for Cancer Research: Washington, DC, 2007.
(2) Burkitt, D. P. Epidemiology of cancer of the colon and rectum. *Cancer* **1971**, *28*, 3-13.
(3) Coradini, D.; Pellizzaro, C.; Marimpietri, D.; Abolafio, G.; Daidone, M. G. Sodium butyrate modulates cell cycle-related proteins in HT29 human colonic adenocarcinoma cells. *Cell Prolif.* **2000**, *33*, 139-146.
(4) Emenaker, N. J.; Calaf, G. M.; Cox, D.; Basson, M. D.; Qureshi, N. Short-chain fatty acids inhibit invasive human colon cancer by modulating uPA, TIMP-1, TIMP-2, mutant p53, Bcl-2, Bax, p21 and PCNA protein expression in an in vitro cell culture model. *J. Nutr.* **2001**, *131*, 3041s-3046s.
(5) Reddy, B. S.; Rivenson, A. Inhibitory effect of *Bifidobacterium longum* on colon, mammary, and liver carcinogenesis induced by 2-amino-3-methylimidazo[4,5-f] quinoline, a food mutagen. *Cancer Res*. **1993**, *53*, 3914-3918.
(6) McIntosh, G. H.; Royle, P. J.; Playne, M. J. A probiotic strain of *L. acidophilus* reduces DMH-induced large intestinal tumors in male Sprague-Dawley rats. *Nutr. Cancer* **1999**, *35*, 153-159.
(7) Hambly, R. J.; Rumney, C. J.; Fletcher, J. M.; Rijken, P. J.; Rowland, I. R. Effects of high- and low-risk diets on gut microflora-associated biomarkers of colon cancer in human flora-associated rats. *Nutr. Cancer* **1997**, *27*, 250-255.
(8) Klinder, A.; Glei, M.; Pool-Zobel, B. Prebiotics and reduction of risk of carcinogenesis: review of experimental and human data. In *Handbook of*

Prebiotics; Gibson, G., Roberfroid, M., Eds.; CRC Press: Boca Raton, FL, 2008; pp 295-328.
(9) Miller, R. S.; Hoskins, L. C. Mucin degradation in human colon ecosystems. Fecal population densities of mucin-degrading bacteria estimated by a "most probable number" method. *Gastroenterology* **1981**, *81*, 759-765.
(10) Doi, K. Effect of konjac fibre (glucomannan) on glucose and lipids. *Eur. J. Clin. Nutr.* **1995**, *49*, s190-s197.
(11) Chen, H.-L.; Sheu, W.-H.; Tai, T.-S.; Liaw, Y.-P.; Chen, Y.-C. Konjac supplement alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects--a randomized double-blind trial. *J. Am. Coll. Nutr.* **2003**, *22*, 36-42.
(12) Chen, H.-L.; Cheng, H.-C.; Liu, Y.-J.; Liu, S.-Y.; Wu, W.-T. Konjac acts as a natural laxative by increasing stool bulk and improving colonic ecology in healthy adults. *Nutrition* **2006**, *22*, 1112-1119.
(13) Chen, H.-L.; Cheng, H.-C.; Wu, W.-T.; Liu, Y.-J.; Liu, S.-Y. Supplementation of konjac glucomannan into a low-fiber Chinese diet promoted bowel movement and improved colonic ecology in constipated adults: a placebo-controlled, diet-controlled trial. *J. Am. Coll. Nutr*. **2008**, *27*, 102-108.
(14) Chen, H.-L.; Lin, Y.-M.; Wang, Y.-C. Comparative effects of cellulose and soluble fibers (pectin, konjac glucomannan, inulin) on fecal water toxicity toward Caco-2 cells, fecal bacteria enzymes, bile acid, and short-chain fatty acids. *J. Agric. Food Chem.* **2010**, 58, 10277-10281.
(15) Yeh, S.-L.; Lin, M.-S.; Chen, H.-L. Inhibitory effects of a soluble dietary fiber from *Amorphophallus konjac* on cytotoxicity and DNA damage induced by fecal water in Caco-2 cells. *Planta Med.* **2007**, *73*, 1384-1388.
(16) Reddy, B. S.; Mangat, S.; Sheinfil, A.; Weisburger, J. H.; Wynder, E. L. Effect of type and amount of dietary fat and 1,2-dimethylhydrazine on biliary bile acids, fecal bile acids, and neutral sterols in rats. *Cancer Res.* **1977**, *37*, 2132-2137.
(17) Reddy, B. S. Dietary fat and its relationship to large bowel cancer. *Cancer Res.* **1981**, *41*, 3700-3705.
(18) Chen, H.-L.; Fan, Y.-H.; Chen, M.-E.; Chan, Y. Unhydrolyzed and hydrolyzed konjac glucomannans modulated cecal and fecal microflora in Balb/c mice. *Nutrition* **2005**, *21*, 1059-1064.
(19) Christl, S. U.; Bartram, H. P.; Paul, A.; Kelber, E.; Scheppach, W.; Kasper, H. Bile acid metabolism by colonic bacteria in continuous culture: effects of starch and pH. *Ann. Nutr. Metab.* **1997**, *41*, 45-51.
(20) Bernstein, H.; Bernstein, C.; Payne, C. M.; Dvorakova, K.; Garewal, H. Bile acids as carcinogens in human gastrointestinal cancers. *Mutat. Res.* **2005**, *589*, 47-65.
(21) Roediger, W. E. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* **1982**, *83*, 424-429.
(22) Scharlau, D.; Borowicki, A.; Habermann, N.; Hofmann, T.; Klenow, S.; Miene, C.; Munjal, U.; Stein, K.; Glei, M. Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. *Mutat. Res.* **2009**, *682*, 39-53.

Table 1. Composition of experimental diets[1]

	Normal-fat	High-fat		
	Fiber-free	Fiber-free	KGM	Inulin
Ingredients	- g/kg diet -			
Corn starch	700	500	437.5	441.5
Casein	200	200	200	200
Corn oil	50	250	250	250
Inulin	-	-	-	58.5
KGM	-	-	62.5	-
Methioine	3	3	3	3
Choline	2	2	2	2
AIN Mineral Mix 76-A	35	35	35	35
AIN Vitamin Mix 76-A	10	10	10	10
Total energy (MJ/kg diet)	17.0	21.1	20.1	20.1

[1]The diets were modified from AIN-76

Table 2. Effects of normal-fat fiber-free diet and high-fat diets on energy intake, weight gain, and feed efficiency of rats[1]

	Normal-fat	High-fat		
	Fiber-free	Fiber-free	KGM	Inulin
Feed intake (g/day)	20.0 ± 0.1[b]	15.1 ± 0.6[a]	14.7 ± 0.4[a]	14.2 ± 0.4[a]
Energy intake (kJ/day)	335.9 ± 0.9[b]	328.4 ± 9.0[b]	294.7 ± 7.3[a]	290.4 ± 7.0[a]
Weight gain (g/day)	6.5 ± 0.3[b]	6.2 ± 0.3[b]	4.6 ± 0.1[a]	4.5 ± 0.2[a]
Feed efficiency[2] (%)	1.9 ± 0.1[b]	1.8 ± 0.1[b]	1.6 ± 0.1[a]	1.6 ± 0.1[a]

[1]Data are expressed as mean ± SE. Different letters across a row denote significant differences between treatments according to one-way ANOVA followed by LSD test ($p < 0.05$).
[2]Feed efficiency (%) = [Daily body weight gain (g) / Daily feed intake (kJ)] × 100%.

Table 3. The concentration and daily fecal excretion of bile acids in rats fed various diets[1-2]

	Normal-fat	High-fat		
	Fiber-free	Fiber-free	KGM	Inulin
Concentration (nmol/g wet feces)				
CA	46.4 ± 7.1[a]	194.2 ± 22.6[b]	431.5 ± 59.3[d]	333.8 ± 15.5[c]
CDCA	38.5 ± 3.5[a]	332.4 ± 46.2[b]	250.9 ± 24.3[b]	333.6 ± 51.0[b]
DCA (a)	275.1 ± 21.3	290.0 ± 55.9	270.6 ± 28.3	239.2 ± 21.1
LCA (b)	79.2 ± 14.4[ab]	112.5 ± 27.6[b]	36.7 ± 10.1[a]	55.6 ± 21.2[a]
Total[3]	439.2 ± 36.3[a]	929.2 ± 110.5[b]	989.7 ± 77.2[b]	962.1 ± 70.1[b]
a + b	354.4 ± 30.1	402.5 ± 66.0	307.3 ± 35.8	294.7 ± 20.5
Daily fecal excretion (nmol/day)				
CA	74.1 ± 10.2[a]	253.9 ± 28.3[b]	821.4 ± 91.8[c]	695.7 ± 68.3[c]
CDCA	61.9 ± 8.1[a]	437.7 ± 51.4[b]	504.1 ± 54.1[bc]	704.6 ± 127.8[c]
DCA (a)	455.1 ± 55.3	384.9 ± 71.3	495.1 ± 67.5	452.3 ± 81.2
LCA (b)	126.6 ± 19.4	149.5 ± 37.7	74.4 ± 22.2	105.7 ± 36.3
Total	717.7 ± 69.8[a]	1226.1 ± 125.9[b]	1895.1 ± 144.7[c]	1958.2 ± 231.9[c]
a + b	581.6 ± 65.9	534.5 ± 83.4	569.6 ± 84.4	558.0 ± 85.7

[1]Data are presented as mean ± SE. Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by LSD test ($p < 0.05$).
[2]CA: cholic acid, CDCA: chenodeoxycholic acid, DCA: deoxycholic acid, LCA: lithocholic acid.
[3]The sum of bile acids determined.

Table 4. The concentration and daily output of fecal microflora in SD rats fed various diets[1]

	Normal-fat	High-fat		
	Fiber-free	Fiber-free	KGM	Inulin
$\log_{10}$ counts/g feces				
Bifidobacteria	9.67 ± 0.09[a]	9.72 ± 0.14[ab]	9.89 ± 0.08[ab]	10.03 ± 0.08[b]
Lactobacilli	9.65 ± 0.06[a]	9.68 ± 0.10[a]	9.85 ± 0.11[ab]	9.97 ± 0.08[b]
Clostridia	9.86 ± 0.05[bc]	10.05 ± 0.08[c]	9.63 ± 0.13[ab]	9.46 ± 0.10[a]
Total[2]	10.71 ± 0.03[a]	11.09 ± 0.09[b]	11.00 ± 0.09[b]	10.92 ± 0.08[ab]
$\log_{10}$ counts/day feces				
Bifidobacteria	9.88 ± 0.07[ab]	9.84 ± 0.15[a]	10.15 ± 0.08[bc]	10.32 ± 0.05[c]
Lactobacilli	9.86 ± 0.05[a]	9.77 ± 0.09[a]	10.15 ± 0.09[b]	10.27 ± 0.07[b]
Clostridia	10.07 ± 0.06[b]	10.16 ± 0.09[b]	9.88 ± 0.14[ab]	9.72 ± 0.13[a]
Total	10.92 ± 0.01[a]	10.87 ± 0.22[a]	11.30 ± 0.06[b]	11.22 ± 0.03a[b]
% Total bacteria				
Bifidobacteria	9.5 ± 1.6[ab]	6.5 ± 2.0[a]	10.0 ± 2.0[ab]	13.7 ± 0.5[b]
Lactobacilli	8.8 ± 1.1[ab]	5.2 ± 0.8[a]	9.1 ± 1.3a[b]	11.7 ± 2.0[b]
Clostridia	14.6 ± 1.9[b]	12.6 ± 1.7[b]	5.2 ± 1.1[a]	4.6 ± 1.0[a]

[1]Data are presented as mean ± SE. Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by LSD test ($p < 0.05$).
[2]Total bacteria were quantified by DAPI.

Table 5. The concentration and total output of cecal short-chain fatty acids in Sprague-Dawley rats fed with various diets[1]

	Normal-fat	High-fat		
	Fiber-free	Fiber-free	KGM	Inulin
μmol/g cecal content				
Acetate	43.8 ± 3.7	39.2 ± 3.2	49.9 ± 6.7	45.3 ± 4.2
Propionate	15.9 ± 0.6[b]	11.2 ± 1.5[a]	13.3 ± 1.2[ab]	13.0 ± 1.3[ab]
i-Butyrate	1.3 ± 0.2[ab]	0.9 ± 0.1[a]	1.7 ± 0.5[b]	0.8 ± 0.1[a]
n-Butyrate	6.0 ± 0.3	5.2 ± 0.8	4.7 ± 0.4	4.7 ± 0.7
Total SCFA[b]	67.1 ± 4.4	56.4 ± 5.0	69.6 ± 7.5	69.3 ± 5.7
μmol/cecum				
Acetate	97.7 ± 19.5[b]	51.3 ± 9.1[a]	106.3 ± 8.1[b]	102.1 ± 15.1[b]
Propionate	35.1 ± 5.6[b]	13.8 ± 2.6[a]	31.8 ± 4.9[b]	30.3 ± 6.1[b]
i-Butyrate	3.2 ± 0.5[b]	1.1 ± 0.2[a]	3.2 ± 0.6[b]	2.1 ± 0.5[ab]
n-Butyrate	12.9 ± 1.5[b]	6.0 ± 1.0[a]	11.9 ± 2.2[b]	11.5 ± 2.8[ab]
Total SCFA	148.9 ± 26.7[b]	72.1 ± 12.3[a]	153.2 ± 15.1[b]	146.1 ± 24.2[b]

[1]Data are presented as mean ± SE. Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by LSD test ($p < 0.05$).
[2]Sum of acetate, propionate, *i*-butyrate and *n*-butyrate.

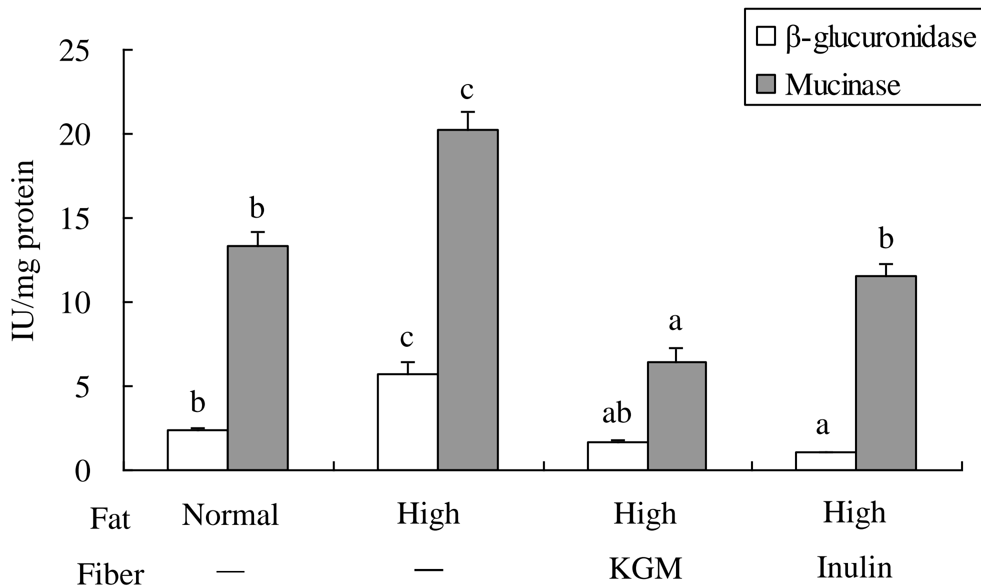


Figure 1. Fecal β-glucuronidase and mucinase activities in rats fed various diets. Different letters denote significant differences across groups for each individual enzyme as analyzed by one-way ANOVA followed by LSD test ($p < 0.05$).

第二年成果報告:

Wen-Tzu Wu and **Hsiao-Ling Chen***. 2011. Konjac glucomannan and inulin systematically modulate antioxidant defense in rats fed a high-fat fiber-free diet. J Agric Food Chem 59:9194-9200 **(SCI)** ***Agriculture, multidisciplinary 2/55=3.6%, IF=2.816, 3*5*5=75***

中文摘要

本研究探討蒟蒻纖維及菊糖寡醣調節高脂無纖維飲食誘發之氧化壓力、腸道表皮細胞及肝臟抗氧化酵素基因表現、血液抗氧化分子的變化。五週齡大鼠(n=8/組)隨機分為四組: 低脂(5% 玉米油, w/w)無纖維組、高脂(25% 玉米油, w/w)無纖維組、以及高脂飲食加入蒟蒻葡甘聚醣(5%, w/w)或菊糖(作為益生質控制組) ，餵食第四周收集糞便樣品，之後犠牲。測量糞便菌相、短鏈脂肪酸濃度、體內氧化壓力指標(如血液 MDA 及淋巴球 DNA 傷害程度)、抗氧化酵素(glutathione peroxidase, superoxide dismutase, catalase)基因表現及血漿抗氧化分子(α-tocopherol、ascorbic acid)濃度。結果顯示，於高脂無纖維飼料中添加蒟蒻聚葡甘露醣及菊糖有效降低大腸及肝臟脂質過氧化產物及白血球 DNA 傷害，同時這兩種纖維提高大腸及肝臟抗氧化酵素基因表現以及血液維生素 E 濃度。蒟蒻聚葡甘露醣及菊糖在大鼠大腸中發酵產生短鏈脂肪酸尤其是乙酸及丁酸。因此本研究建議體內利用蒟蒻聚葡甘露醣及菊糖產生腸道及全身系統性抗氧化作用。

關鍵字：蒟蒻聚葡甘露醣、糞便細菌酵素、短鏈脂肪酸、菌相、膽酸

ABSTRACT

The aim of this study was to investigate the effects of konjac glucomannan (KGM) and inulin on the balance between pro-oxidative status and antioxidative defense systems in the colon, liver and plasma of rats fed a high-fat fiber-free diet. Male Sprague-Dawley rats (*n* = 8 animals per group) were fed a high-fat (25% corn oil, w/w) fiber-free diet or that supplemented with KGM or inulin fiber (5%, w/w) for 4 weeks. The index of pro-oxidative status, malondialdehyde (MDA) and blood lymphocyte DNA damage, and the antioxidative defense, that is, antioxidant enzymes (glutathione peroxidase, superoxide dismutase, catalase) in the colonic mucosa and liver, and the plasma antioxidant levels were determined. Incorporation of KGM and inulin into the high-fat fiber-free diet beneficially reduced the MDA levels of the colon and liver and DNA damage in blood lymphocytes. On the other hand, both fibers enhanced the antioxidative defense systems by up-regulating the gene expressions of glutathione peroxidase and catalase in the colonic mucosa, and the superoxide dismutase and catalase in the liver. Furthermore, KGM and inulin promoted

1 antioxidative status in the blood by elevating the α-tocopherol level. KGM and inulin were
2 well-fermented in rats and increased the concentration and daily excretion of fecal
3 short-chain fatty acids, especially acetate and butyrate. These results suggest that *in vivo*
4 utilization of KGM and inulin stimulated both local and systemic antioxidative defense
5 systems in rats.
6
7 **Keywords:** konjac glucomannan, inulin, malondialdehyde, DNA damage, antioxidant
8 enzymes, short-chain fatty acid
9
10 **INTRODUCTION**
11
12 Free radical damage is believed to cause cell and tissue damage that ultimately results in
13 morbidities such as cancer and cardiovascular disease *(1, 2)*. Studies have been conducted to
14 explore the antioxidative capacities of phytochemicals such as flavanols, flavonols, and
15 anthocyanidins *(3-5)*. However, the role of a major component of plant, dietary fiber, in
16 eliminating the free radicals and ultimately defending oxidative stress has been rarely studied.
17
18 Dietary fibers are metabolized in the colon where they beneficially modulate the
19 microflora profile and bowel habit *(6)*. In addition, some dietary fibers are considered to exert
20 beneficial physiological functions beyond the digestive system, such as improving the lipid
21 metabolism in the liver *(7)*, and reducing blood inflammation indices in the diabetic patients
22 *(8)*. The colon, the interface between the large population of microflora and the colonic
23 content, is constantly challenged either by the diet-derived or the endogenous reactive
24 oxidative substances (ROS). An imbalance in the cellular redox system leading to elevated
25 ROS level in the colon causes gastrointestinal tract dysfunction and even the colorectal
26 cancer *(9, 10)*. Recent studies have shown that polysaccharides derived from a tropical fruit
27 litchi (*Litchi chinensis* Sonn.) exerted *in vitro* free radical-scavenging effects *(11)*. Xanthan
28 gum, a soluble dietary fiber, has been shown to prevent the autoxidation of soybean oil and to
29 exert the iron-chelating ability *(12)*. These studies suggest an antioxidative capacity for
30 dietary fibers. However, the antioxidative capacity derived from fermentation of dietary fiber
31 is not well established.
32
33 Konjac glucomannan (KGM), consisting of D-glucose and D-mannose units joined
34 together with β-1,4 glycosidic bond linkages, is a highly polymerized viscous fiber derived
35 from the tubers of *Amorphophallus konjac* C. Koch *(13)*. The KGM has been made into jelly
36 and noodles that are commonly consumed in Japan and Taiwan. Inulin, a mixture of
37 fructo-oligosaccharides, derived from the tuber of chicory (*Cichorium intybus*), is another
38 purified dietary fiber that is widely used as a supplement in foods. We previously reported
39 that fermentation of KGM, as well as oligofructose, by several lactic acid bacteria exerted *in*
40 *vitro* antioxidative capacities *(14)*, and that supplementation of KGM and inulin into a
41 fiber-free diet reduced the DNA damaging effect of fecal water towards Caco-2 cells, a model
42 of human colonocytes *(15, 16)*. These studies suggest that KGM and inulin may reduce the
43 oxidative stress in the colon. Further, since the fermentation products of soluble dietary fibers
44 may be carried to the liver and the circulation system, KGM and inulin may modulate the
45 redox status beyond the digestive system, which has never been investigated.
46
47 The aim of the present study was to determine the effects of KGM and inulin on the

balance between pro-oxidative status and antioxidative defense system in Sprague-Dawley rats fed a high-fat fiber-free diet. Lymphocyte DNA damage and the lipid oxidation product, malondialdehyde (MDA), in the colonic tissues, liver, and plasma reflect pro-oxidative status. Antioxidative defense systems were assessed by measuring plasma antioxidant levels and through expression of antioxidant enzymes in the proximal and distal colonic mucosa cells and in the liver.

Materials and Methods

Animals. Five-week-old male Sprague-Dawley rats (BioLASCO Taiwan Co., Ltd, Yi-Lan, Taiwan) were housed in plastic cages in an animal room maintained on a 12 h light-dark cycle at 24 ± 1°C and 50% humidity. Animals were allowed free access to water and food throughout the study. Animal care followed the guidelines of the National Research Council *(17)* and the experiment was approved by the Institutional Animal Care and Use Committee at Chung Shan Medical University.

Experimental Design. Initially, rats were fed with a standard rodent diet (Rodent Laboratory chow diet 5001, Purina Co., St. Louis, MO) during one week of acclimatization. Afterwards, animals were randomly assigned to the high fat (25% corn oil, w/w) modified AIN-76 diets *(18)* (n = 8 animals per group) containing no dietary fiber, or 5% (w/w) konjac glucomannan fiber (Fukar Co., Taipei, Taiwan) or inulin fiber (Sentosa Co., Taipei, Taiwan). The compositions and caloric densities of experimental diets were shown in Table 1. Fresh feces were collected during the last week of the experiment as described previously *(16)*. After 4 weeks, rats were anaesthetized by carbon dioxide after an overnight fast and blood samples collected from the abdominal aorta were placed into heparinized tubes, from which an aliquot (~0.3 mL) was used to isolate lymphocytes for analysis of DNA damage; the remaining blood samples were centrifuged at 550g for 10 min to obtain the plasma for analysis of MDA and antioxidants. For determination of plasma ascorbic acid, an aliquot of plasma was mixed with equal volume of 5% (w/v) meta-phosphoric acid (Sigma Chemical Co., St. Louis, MO) to remove the protein and the sample were analyzed immediately *(19)*. The remaining plasma was stored at -80°C for analyses of α-tocopherol and MDA within a month. The liver was dissected into 5 pieces and frozen immediately. Colons were removed, cut longitudinally, flushed with saline to remove the contents and blotted dry with a paper towel. A 0.5-cm^2 tissue from the proximal and distal colon was dissected for determination of MDA. The mucosa of the remaining colon was scraped with a glass slide immediately and placed into the RNAase-free phosphate buffered saline. The tissue and mucosa samples were stored at -20°C for subsequent analysis.

MDA levels in the Colon, Liver, and Plasma. MDA levels were determined using 1,1,3,3-tetraethoxypropane (Sigma) as the standard using the method described by Lee and Csallany *(20)*. The proximal and distal colon tissue samples and hepatic tissues (0.2 g) were homogenized in 9 fold volumes of 50 mM potassium phosphate buffer containing 1.15% (w/v) potassium chloride (pH 7.0) and centrifuged at 12000g for 10 min to obtain the supernatant. An aliquot (0.1 mL) of the supernatant or plasma was mixed with 25 μL of 0.2% (w/v) butylated hydroxytoluene and 12.5 μL of 10 N NaOH at 60°C for 30 min. After incubation with a trichloroacetic acid solution (7.2% w/v, containing 1% w/v potassium iodide) in ice for 10 min, samples were centrifuged at 12000g for 10 min. An aliquot of this supernatant (0.5 mL) was mixed with 1 mL of 0.6% (w/v) thiobarbituric acid (TBA) at 95°C for 1 h, and MDA-TBA adduct was extracted with n-butanol. The MDA-TBA adduct was eluted with a mixture of phosphate buffer (50 mM) and methanol (65:35, v/v) at 0.8 mL/min in an HPLC

system (Jasco, Tokyo, Japan) equipped with a C18 reverse phase column (LiChroCART 250-4, Merck, Darmstadt, Germany) capped with a guard column (LiChrospher 100 RP-18e, Merck) at 532 nm and quantified with a standard curve. The tissue MDA content was further calibrated with the protein measured by the method of Bradford using a commercial reagent (Life Science Research, Hercules, CA).

Plasma α-Tocopherol and Ascorbic Acid levels. Plasma α-tocopherol was extracted and analyzed according to the method described by Catignani and Bieri *(21)* with the HPLC system described above. In brief, an aliquot (0.2 mL) of plasma was mixed with an internal standard (0.1 mL of α-tocopheryl acetate, 105.7 μM, Sigma) and extracted twice with *n*-hexane twice. After removal of the hexane under a stream of nitrogen, samples were dissolved in 99% (v/v) methanol immediately before being eluted with 98% (v/v) methanol (1.2 mL/min) from a C18 reverse phase column (LiChroCART 250-4, Merck). The absorbance of α-tocopherol was determined at 290 nm and quantified with a standard curve. The plasma α-tocopherol levels were calibrated with the total lipid level determined by the method of Frings and Dunn *(22)*. Plasma ascorbic acid was determined according to the method described previously *(19)* with a HPLC system described above. The sample was eluted with potassium phosphate buffer (0.1 M, pH 3.5) with a flow rate of 0.8 mL/min. The ascorbic acid was detected at 245 nm and quantified with a standard curve.

Blood Lymphocyte DNA Damage. An aliquot (3 mL) of whole blood was gently layered to the top of equal volume of a density medium (Histopaque-1077, Sigma) and centrifuged at 1000*g* for 10 min according to the method described previously *(23)*. The lymphocyte layer was carefully transferred to a tube, and gently washed twice with 5 mL RPMI1640 medium (Gibco Life Technologies, Grand Island, NY) *(24, 25)*. The viability of lymphocytes was determined using the trypan blue assay *(26)*. With ≥ 98% cell viability, lymphocytes (5×10^5/mL) were suspended in 1% (w/v) low-melting-point agarose and then layered onto a layer of normal-melting-point agarose (1%, w/v) on a frosted glass microscopy slide. After application of a top layer of normal-melting-point agarose, the slides were immersed in a cold lysing solution (10 mM Tris, 1% w/v sodium N-laurylsarcosine, 0.1 mM Na_2EDTA, 2.5 M NaCl, 1% v/v Triton X-100, 10% v/v DMSO, pH 10) for 1 h at 4°C. The slides were further treated with either 50 μL of saline or formamidopyrimidine glycosylase (FPG, 1 μg/mL, Sigma) at 37°C for 30 min for quantification of the oxidized nucleic acids. The FPG is a specific enzyme that nicks the DNA at sites of oxidized purine *(27)*. After being washed with a saline solution, the slides were allowed to unwind for 20 min in a alkaline solution (0.3 M NaOH, 1 mM Na_2EDTA), followed by electrophoresis at 25 V and 300 mA for 20 min. Duplicate slides were prepared from each animal and the DNA breakages from at least 100 lymphocytes per slide were determined. The image was analyzed using Interactive Image Analysis Comet Assay III (Perceptive Instrument, Haverhill, UK). The DNA damage was denoted as tail moment (% of DNA in tail × tail length).

Relative Gene Expressions of Antioxidant Enzymes in Colonic Mucosa and Liver. The expression of glutathione peroxidase, superoxide dismutase and catalase genes were determined using quantitative real-time PCR. The tissue RNA was extracted with REzol™ C&T reagent (PROtech Technology, Taipei, Taiwan) according to the method provided. Briefly, colonic mucosa or hepatic tissue (50 mg) was homogenized in 1 mL REzol™ C&T. After addition of 0.2 mL chloroform, the samples were vigorously mixed for 15 sec, followed by centrifugation 12,000*g* for 15 min at 4°C. The supernatant (aqueous phase) was mixed with an equal volume of isopropanol (J. T. Baker, Deventer, Netherlands) and the RNA pellet was precipitated with centrifugation, 12,000*g* for 10 min at 4°C. After washing with 75%

1 ethanol, the RNA was dissolved in RNA-free ultrapure water and stored at -70°C until
2 analysis. The concentration and quality of RNA was determined by the 260/280 nm
3 absorbance. The complementary DNA (cDNA) was synthesized using random primers
4 (Applied Biosystems Life Technologies, Foster City, CA) in a thermal cycler (TaKaRa
5 Biomedical, Shuzo, Japan) at 25°C for 10 min, 37°C for 2 h, and 85°C for 5 min.
6
7 The quantitative real-time PCR for messenger RNA (mRNA) levels were performed
8 using TaqMan gene expression assays (Applied Biosystems) with the StepOne Real-Time
9 PCR System (Model 7700, Applied Biosystems). The assay identification (accession number
10 of NCBI gene reference shown in parenthesis) of primers for the target genes, glutathione
11 peroxidase, superoxide dismutase and catalase, and the internal reference
12 glyceraldehydes-3-phosphate dehydrogenase (GAPDH), were Rn0057799_g1
13 (NM_030826.3), Rn01477289_m1 (NM_017050.1), Rn00560930_m1 (NM_012520.1), and
14 Rn99999916_s1 (NM_017008.3), respectively. The exact primer and probe sequences used
15 for real-time PCR in this study were not provided due to the proprietary issue and policy of
16 the supplier. The PCR reaction was performed at 50°C for 2 min, 95°C for 10 min, and 40
17 cycles of 95°C for 15 s and 60°C for 1 min. The relative gene expression of each enzyme was
18 normalized to that of GAPDH, and was furthered calculated relative to their fiber-free
19 counterpart using the comparative threshold cycle (C_t) method. The fold difference in gene
20 expression was calculated as $2^{-\Delta\Delta Ct}$.
21
22 **Fecal Short-Chain Fatty Acids.** Fecal acetate, propionate and butyrate were extracted
23 with methyl ether with 4-methyl-*n*-valeric acid (Sigma) as an internal standard according to
24 the method described previously *(16)*. The fecal short-chain fatty acids were dissolved in
25 10% phosphate solution before they were injected into a gas chromatography (GC-14B;
26 Shimadzu Corp., Kyoto, Japan) fitted with a glass capillary column (0.25 mm × 30 m,
27 Stabilwax-DA, Restek Corp., Bellefonte, PA) and a flame ionization detector. The
28 temperature of injector port and detector were 250°C, and the initial oven temperature was
29 100°C, increasing to 200°C at 6°C/min. The flow rate of N_2 was 1 mL/min. Peak areas were
30 analyzed with a C-R6A Chromatopac (Shimadzu Corp.).
31
32 **Statistical Analysis.** Data are presented as means ± SE and analyzed using SPSS
33 (version 12.0, SPSS Inc., Chicago, IL). The diet effects were determined using one-way
34 ANOVA followed by the post hoc analysis using Duncan test. A p value ≤ 0.05 was
35 considered to be statistically significant.
36
37 **RESULTS**
38
39 The MDA content of the proximal colon was significantly lowered by the inulin diet for
40 43% ($p < 0.001$) while that of the distal colon was reduced by KGM diet for ~30% ($p =$
41 0.011), as compared with the high-fat fiber-free counterpart (**Table 2**). The reductions by the
42 KGM diet of the MDA content in the proximal colon (~15%, $p = 0.1$) and that of the inulin
43 diet in the distal colon (~20%, $p = 0.077$) were not statistically significant. The MDA content
44 in the liver was significantly decreased by the KGM and inulin diets by 34% ($p = 0.008$) and
45 43% ($p = 0.001$), respectively, as compared with the fiber-free diet.
46
47 Plasma α-tocopherol levels were significantly increased with the KGM and inulin diets
48 by 66% ($p = 0.002$) and 41% ($p = 0.044$), respectively, as compared with the high-fat
49 fiber-free diet (**Table 3**). Plasma ascorbic acid and MDA levels were not affected by the

incorporation of either fiber into the diet.

The KGM and inulin diets reduced DNA damage (denoted as tail moment) of blood lymphocytes to approximately half ($p < 0.001$, respectively) of that shown in the fiber-free counterpart (**Figure 1**). The FPG treatment increased the tail moment of blood lymphocytes in all three groups of rats. KGM and inulin diets significantly reduced the tail moment in combination with FPG treatment by 43% ($p < 0.001$) and 54% ($p < 0.001$), respectively, as compared to the fiber-free counterpart. The FPG-induced tail moment in the fiber-free group was 3.1 ± 0.1, which was decreased by KGM for 31% ($p < 0.001$), and by inulin for 56% ($p < 0.001$).

The transcriptions of glutathione peroxidase, superoxide dismutase and catalase in the colonic mucosa and the liver were evaluated by quantitative real-time PCR normalized by GAPDH as an internal standard (**Figure 2A**-**C**). In the proximal colonic mucosa, the incorporation of KGM and inulin into the high-fat fiber-free diet similarly and significantly increased the glutathione peroxidase gene expression to ~4 fold ($p = 0.033$ and 0.037, respectively), but did not modulate the superoxide dismutase and catalase transcript levels (**Figure 2A**). In the distal colonic mucosa, KGM and inulin significantly increased the glutathione peroxidase gene expression to 2.6 ± 0.6 fold ($p = 0.017$) and 2.4 ± 0.3 fold ($p = 0.033$), respectively, and significantly increased the catalase gene expression to 2.2 ± 0.2 fold ($p = 0.016$) and 2.3 ± 0.4 fold ($p = 0.011$), respectively (**Figure 2B**). However, gene expression of superoxide dismutase in the colonic mucosa was not modulated by either fiber (**Figure 2A**-**B**). In the liver, the KGM and inulin diets significantly increased superoxide dismutase gene expression to 1.7 ± 0.2 fold ($p = 0.05$), and 2.0 ± 0.2 fold ($p = 0.005$), respectively (**Figure 2C**). In addition, KGM and inulin significantly increased the hepatic catalase gene expression to 1.5 ± 0.1 fold ($p = 0.012$) and 1.8 ± 0.2 fold ($p < 0.001$), respectively. The gene expression of glutathione peroxidase in the liver was similar among groups.

KGM diet significantly increased fecal acetate ($p = 0.01$), butyrate ($p = 0.004$), and total short-chain fatty acid ($p = 0.09$) concentrations, as compared with the high-fat fiber-free diet (**Table 4**). In contrast, the inulin diet slightly elevated the fecal acetate concentration ($p = 0.069$) and significantly caused a 1.6-fold increase ($p < 0.001$) in the butyrate concentration, as compared with the fiber-free counterpart. In addition, the KGM diet elevated the daily fecal output (μmole/day) of acetate to 2.5 fold ($p < 0.001$) and approximately tripled that of propionate ($p = 0.027$) and butyrate ($p < 0.001$), respectively, as compared with the fiber-free counterpart, while the inulin diet elevated the daily fecal acetate excretion by approximately 57% ($p = 0.022$) and almost tripled ($p = 0.001$) the daily butyrate excretion. The proportion of butyrate was significant higher in the inulin diet as compared with the fiber-free counterpart ($p = 0.005$).

DISCUSSION

This was the first study to indicate that two purified soluble dietary fibers, KGM and inulin, effectively alleviated the oxidative stress in rats fed a high-fat fiber-free diet. KGM and inulin effectively reduced the lipid peroxidative product, MDA, in either the distal or proximal colon. This reduced oxidative stress was likely to be mediated by the enhanced gene expression of glutathione peroxidase in the proximal and distal colonic mucosa, and the catalase gene expression in the distal colonic mucosa. Furthermore, KGM and inulin diets reduced the MDA levels in the liver, the site of metabolism of many compounds absorbed

from the gastrointestinal tract. These beneficial effects of fibers in the liver were related to the enhanced hepatic gene expression of superoxide dismutase and catalase. Besides, KGM and inulin improved the plasma antioxidative status, α-tocopherol/total lipid level, which may protect the blood cells from the oxidative stress and further reduce the oxidative damage of lymphocyte DNAs.

The effect of feeding a purified dietary fiber, in the absence of other antioxidant phytochemicals, on the intestinal redox system has not been well-documented. A recent study demonstrated that a mixture of grape fiber and polyphenol induced a glutathione redox system in the proximal colonic mucosa of rats *(28)*. Another study indicated that fermented wheat aleurone induced antioxidative defenses, such as the expression of glutathione *S*-transferase pi in the human colon cells, and the expression of both catalase and glutathione *S*-transferase pi in the HT-29 cells derived from a human adenocarcinoma *(29)*. However, the role of dietary fiber *per se* can not be concluded from these studies. Results of the current study determined that KGM and inulin effectively enhanced the gene expression of glutathione peroxidase involved in the glutathione recycling, as well as the expression of catalase involved in the elimination of hydroxyl radicals, in the colon. We suggest that the modulatory roles of these dietary fibers in gene expression of colonocytes are independent of any other dietary components and are likely to be mediated by their fermentation products, the short-chain fatty acid, and the intestinal microflora.

Short-chain fatty acids play an important role in colon physiology *(30)*. Butyrate is considered particularly essential for colonocytes as it is a preferred energy source, a potent controller of cell growth and differentiation and an inducer of apoptosis *(31)*. Apart from its role in cell growth, butyrate also has been shown to modulate antioxidant defense of colonocytes *(29, 32, 33)* and to protect the hydrogen peroxide-induced DNA damage in isolated human colonocytes and HT29 cells *(32)*. Rectal administration of butyrate in healthy humans also has been shown to increase the glutathione level in the colonic mucosa *(33)*. However, the sole effect of butyrate may not be as great as the whole mixture of fermentation products on antioxidative enzymes *(29)*. Therefore, it is likely that butyrate mediates part of the antioxidant effects from dietary fiber fermentation. Our previous study has shown that both KGM and inulin promoted the cecal short-chain fatty acid production in the same animal model as the current study *(16)*. Furthermore, the current study demonstrated that both KGM and inulin significantly promoted the concentration and daily excretion of fecal short-chain fatty acids, especially acetate and butyrate. Therefore, results from these two studies support a role for fermentation of KGM and inulin in the elevation of antioxidant enzyme expression in the epithelium throughout the colon, and the subsequent reduction in the formation of lipid peroxidation product MDA.

The mechanisms by which KGM and inulin modulated the balance between pro- and anti-oxidation are not known. Several *in vitro* studies have demonstrated that many species of bifidobacteria and lactobacilli reduce the lipid peroxidation, increase the free radical scavenging ability, and chelate free ferrous ions *(34, 35)*. Both KGM and inulin supplementations are known to increase the amounts of fecal bifidobacteria and lactobacilli in mice fed a normal-fat fiber-free diet *(15)* and in rats fed a high-fat fiber-free diet *(16)*. Therefore, it is suggested that the antioxidative capacity of KGM and inulin is partially mediated via its prebiotic role.

Soluble dietary fibers may also exert its antioxidative capacity through its fermentation products *(14, 36)*. An *in vitro* study has demonstrated that fermentation products of KGM and

fructo-oligosaccharide by several colonic lactic acid bacteria exerted free radical-scavenging effects *(14)*. In addition, fecal soluble material from rats supplemented with KGM had been shown to enhance the iron-chelating capacity *(36)*, thus reduce the initiation of free radical cascades. Furthermore, feeding KGM and inulin to mice reduced the DNA damaging effect of fecal soluble substances toward Caco-2 cells, a model of colonocyte *(36)*. All these results suggest that KGM and inulin may reduce the oxidative challenge to the colonocytes due to the antioxidant capacity of their fermentation products.

The effects of dietary fibers on hepatic antioxidant enzymes have been rarely examined. As the oxidative stress induced by a cystine-rich fiber-free diet, pectin effectively retained the total superoxide dismutase and Cu, Zn-superoxide dismutase activities in the liver *(37)*. A recent study indicated that fructo-oligosaccharide, similar to vitamin E, effectively reduced the alterations in the hepatic superoxide dismutase and glutathione peroxidase activities in mice subcutaneously administered with D-galactose *(38)*. Our study demonstrates that hepatic MDA level (nmole/mg protein) was effectively reduced by more than 30% with either KGM or inulin, which was in agreement with the elevated gene expressions of the hepatic superoxide dismutase and catalase. Therefore, previous and current studies support that the utilization of dietary fiber in the large intestine exerts antioxidative effects in the liver.

The effect of fiber consumption on the blood antioxidative status also has not been well studied. Oligofructoses have been shown to reduce the plasma thiobarbituric acid-reactive substances (TBARS) and promote the plasma vitamin E/triglycerol levels in rats fed a high-fructose diet *(39)*. A placebo-controlled and diet-controlled trial indicated that fructo-oligosaccharide supplement beneficially reduced the plasma TBARS in constipated nursing-home residents *(40)*. In agreement with these previous studies, the current study indicates that both KGM and inulin diets reduced the index of plasma oxidative stress, the DNA breakage of lymphocytes, and increased the plasma α-tocopherol/total lipid level, as compared with the fiber-free counterpart. Although the mechanism is unclear, the prebiotic effect of KGM and inulin may partially contribute to the reduced blood oxidative status, as a previous study showing decreased oxidative stress markers in human blood and urine with probiotic-fermented milk consumption *(41)*.

In conclusion, addition of 5% (w/w) KGM or inulin into a high-fat fiber-free diet effectively up-regulated the gene expressions of antioxidant enzymes throughout the colon, and in the liver. The *in vivo* antioxidative effects of dietary fibers may reach the whole body by reducing the oxidative damage towards the blood lymphocytes and increasing plasma antioxidant levels. The local and systemic antioxidative effects of KGM and inulin are likely to be related to their fermentation in the cecum and colon, and their prebiotic effects.

ABBREVIATIONS USED

KGM, konjac glucomannan; MDA, malondialdehyde; ROS, reactive oxidative substances; FPG, formamidopyrimidine glycosylase; PCR, polymerase chain reaction.

ACKNOWLEGEMENT

The research was supported by grants from the National Science Council (NSC-99-2320-B-040-014-MY2), Republic of China.

Literature Cited

(1) Kehrer, J. P. Free radicals as mediators of tissue injury and disease. *Crit. Rev. Toxicol.* **1993**, *23*, 21-48.

(2) Halliwell, B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* **1994**, *344*, 721-724.
(3) Noda, Y.; Kaneyuki, T.; Mori, A.; Packer, L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, and pelargonidin. *J. Agric. Food Chem.* **2002**, *50*, 166-171.
(4) Henning, S. M.; Niu, Y.; Lee, N. H.; Thames, G. D.; Minutti, R. R.; Wang, H.; Go, V. L.; Heber, D. Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. *Am. J. Clin. Nutr.* **2004**, *80*, 1558-1564.
(5) Jaramillo, K.; Dawid, C.; Hofmann, T.; Fujimoto, Y.; Osorio, C. Identification of antioxidative flavonols and anthocyanins in Sicana odorifera fruit peel. *J. Agric. Food Chem.* **2011**, *59*, 975-983.
(6) Cummings, J. H. The effect of dietary fiber on fecal weight and composition. In *Dietary fiber in human nutrition*; Spiller, G., Ed.; CRC Press, New York, 2001; pp 183-252.
(7) Beylot, M. Effects of inulin-type fructans on lipid metabolism in man and in animal models. *Br. J. Nutr.* **2005**, *93*, s163-s168.
(8) Qi, L.; van Dam, R. M.; Liu, S.; Franz, M.; Mantzoros, S.; Hu, F. B. Whole-grain, bran, and cereal fiber intakes and markers of systemic inflammation in diabetic women. *Diabetes Care* **2006**, *29*, 207-211.
(9) Babbs, C. F. Free radicals and the etiology of colon cancer. *Free Radic. Biol. Med.* **1990**, *8*, 191-200.
(10) Seril, D. N.; Liao, J.; Yang, G.Y.; Yang, C. S. Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. *Carcinogenesis* **2003**, *24*, 353-362.
(11) Kong, F.; Zhang, M.; Liao, S.; Yu, S.; Chi, J.; Wei, Z. Antioxidant activity of polysaccharide-enriched fractions extracted from pulp tissue of *Litchi Chinensis* Sonn. *Molecules* **2010**, *15*, 2152-2165.
(12) Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* **1992**, *40*, 945-948.
(13) Doi, K. Effect of konjac fibre (glucomannan) on glucose and lipids. *Eur. J. Clin. Nutr.* **1995**, *49*, s190-s197.
(14) Wang, C.-H.; Lai, P.; Chen, M.-E.; Chen, H.-L. Antioxidative capacity produced by *Bifidobacterium*- and *Lactobacillus acidophilus*-mediated fermentations of konjac glucomannan and glucomannan oligosaccharides. *J. Sci. Food Agric.* **2008**, *88*, 1294-1300.
(15) Chen, H.-L.; Lin, Y.-M.; Wang, Y.-C. Comparative effects of cellulose and soluble fibers (pectin, konjac glucomannan, inulin) on fecal water toxicity toward Caco-2 cells, fecal bacteria enzymes, bile acid, and short-chain fatty acids. *J. Agric. Food Chem.* **2010**, *58*, 10277-10281.
(16) Wu, W.-T.; Chen, H.-L. Effects of konjac glucomannan on putative risk factors for colon carcinogenesis in rats fed a high-fat diet. *J. Agric. Food Chem.* **2011**, *59*, 989-994.
(17) *Guide for the Care and Use of Laboratory Animals*; Publication 85-23, rev.; National Research Council, National Institute of Health: Bethesda, MD, 1985.
(18) Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. *J. Nutr.* **1977**, *107*, 1340-1348.
(19) Ching, S. Y.; Prins, A. W.; Beilby, J. P. Stability of ascorbic acid in serum and plasma prior to analysis. *Ann. Clin. Biochem.* **2002**, *39*, 518-520.
(20) Lee, H. S.; Csallany, A. S. Measurement of free and bound malondialdehyde in vitamin E-deficient and -supplemented rat liver tissues. *Lipids* **1987**, *22*, 104-107.

1 (21) Catignani, G. L.; Bieri, J. G. Simultaneous determination of retinol and alpha-tocopherol
2 in serum or plasma by liquid chromatography. *Clin. Chem.* **1983**, *29*, 708-712.
3 (22) Frings, C. S; Dunn, R. T. A colorimetric method for determination of total serum lipids
4 based on the sulfo-phospho-vanillin reaction. *Am. J. Clin. Pathol.* **1970**, *53*, 89-91.
5 (23) Chuang, C.-H.; Hu, M.-L. Use of whole blood directly for single-cell gel electrophoresis
6 (comet) assay in vivo and white blood cells for in vitro assay. *Mutat. Res.* **2004**, *564*,
7 75-82.
8 (24) Singh, N. P.; McCoy, M. T.; Tice, R. R.; Schneider, E. L. A simple technique for
9 quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.* **1988**, *175*,
10 184-191.
11 (25) Wu, W.-T.; Cheng, H.-C.; Chen, H.-L. Ameliorative effects of konjac glucomannan on
12 human faecal β-glucuronidase activity, secondary bile acid levels and faecal water
13 toxicity towards Caco-2 cells. *Br. J. Nutr.* **2010**, *105*, 593-600.
14 (26) Phillips, H. J. Dye exclusion tests for cell viability. In *Tissue Culture. Method and*
15 *Applications*; Kruse, P. F.; Patterson, M. K., Eds.; Academic Press, New York, 1973; pp
16 2973.
17 (27) Collins, A. R.; Ma, A. G.; Duthie, S. J. The kinetics of repair of oxidative DNA damage
18 (strand breaks and oxidised pyrimidines) in human cells. *Mutat. Res.* **1995**, *336*, 69-77.
19 (28) Lopez-Oliva, M. E.; Agis-Torres, A.; Goñi, I.; Muñoz-Martinez, E. Grape antioxidant
20 dietary fibre reduced apoptosis and induced a pro-reducing shift in the glutathione redox
21 state of the rat proximal colonic mucosa. *Br. J. Nutr.* **2010**, *103*, 1110-1117.
22 (29) Stein, K.; Borowicki, A.; Scharlau, D.; Glei, M. Fermented wheat aleurone induces
23 enzymes involved in detoxification of carcinogens and in antioxidative defence in human
24 colon cells. *Br. J. Nutr.* **2010**, *104*, 1101-1111.
25 (30) Roediger, W. E., Utilization of nutrients by isolated epithelial cells of the rat colon.
26 *Gastroenterology* **1982**, *83*, 424-429.
27 (31) Scharlau, D.; Borowicki, A.; Habermann, N.; Hofmann, T.; Klenow, S.; Miene, C.;
28 Munjal, U.; Stein, K.; Glei, M. Mechanisms of primary cancer prevention by butyrate and
29 other products formed during gut flora-mediated fermentation of dietary fibre. *Mutat. Res.*
30 **2009**, *682*, 39-53.
31 (32) Rosignoli, P.; Fabiani, R.; De Bartolomeo, A.; Spinozzi, F.; Agea, E.; Pelli, M. A.;
32 Morozzi, G. Protective activity of butyrate on hydrogen peroxide-induced DNA damage
33 in isolated human colonocytes and HT29 tumour cells. *Carcinogenesis* **2001**, *22*,
34 1675-1680.
35 (33) Vanhoutvin, S. A.; Troost, F. J.; Kilkens, T. O.; Lindsey, P. J.; Hamer, H. M.; Jonkers, D.
36 M.; Venema, K.; Brummer, R. J. The effects of butyrate enemas on visceral perception in
37 healthy volunteers. *Neurogastroenterol Motil.* **2009**, *21*, 952-e76.
38 (34) Kullisaar, T.; Zilmer, M.; Mikelsaar, M.; Vihalemm, T.; Annuk, H.; Kairane, C.; Kilk, A.
39 Two antioxidative lactobacilli strains as promising probiotics. *Int. J. Food Microbiol.*
40 **2002**, *72*, 215-224.
41 (35) Sun, J.; Hu, X. L.; Le, G. W.; Shi, Y. H. Lactobacilli prevent hydroxy radical production
42 and inhibit *Escherichia coli* and *Enterococcus* growth in system mimicking colon
43 fermentation. *Lett. Appl. Microbiol.* **2010**, *50*, 264-269.
44 (36) Yeh, S.-L.; Lin, M.-S.; Chen, H.-L. Inhibitory effects of a soluble dietary fiber from
45 *Amorphophallus konjac* on cytotoxicity and DNA damage induced by fecal water in
46 Caco-2 cells. *Planta Med.* **2007**, *73*, 1384-1388.
47 (37) He, G.; Aoyama, Y. Effects of adding some dietary fibers to a cystine diet on the
48 activities of liver antioxidant enzymes and serum enzymes in rats. *Biosci. Biotechnol.*
49 *Biochem.* **2003**, *67*, 617-621.
50 (38) Chen, H.-L.; Wang, C.-H.; Kuo, Y.-W.; Tsai, C.-H. Antioxidative and hepatoprotective

effects of fructo-oligosaccharide in D-galactose-treated Balb/cJ mice. *Br. J. Nutr.* **2011**, *105*, 805-809.

(39) Busserolles, J.; Gueux, E.; Rock, E.; Demigné, C.; Mazur, A.; Rayssiguier, Y. Oligofructose protects against the hypertriglyceridemic and pro-oxidative effects of a high fructose diet in rats. *J. Nutr.* **2003**, *133*, 1903-1908.

(40) Yen, C.-H.; Kuo, Y.-W.; Tseng, Y.-H.; Lee, M.-C.; Chen, H.-L. Beneficial effects of fructo-oligosaccharides supplementation on fecal bifidobacteria and index of peroxidation status in constipated nursing-home residents—A placebo-controlled, diet-controlled trial. *Nutrition* **2011**, *27*, 323-328.

(41) Kullisaar, T.; Songisepp, E.; Mikelsaar, M.; Zilmer, K.; Vihalemm, T.; Zilmer, M. Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. *Br. J. Nutr.* **2003**, *90*, 449-456.

Figure Legend

Figure 1. DNA damage of lymphocytes with basal condition or in combination with 1 μg/mL FPG (basal + FPG). Lymphocytes were isolated from Sprague-Dawley rats fed a high corn oil diets containing no fiber (white bar), 5% (w/w) KGM (gray bar) or inulin (black bar). The DNA damage was determined by comet assay and expressed as tail moment. Bars are presented as mean ± SE ($n = 8$ animals per group). Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by Duncan test ($p < 0.05$). KGM, konjac glucomannan; FPG, formamidopyrimidine DNA-glycosylase.

Figure 2. The gene expression of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in the (A) proximal, (B) distal colonic mucosa, and (C) liver in Sprague-Dawley rats fed a high corn oil diet containing no fiber, 5% (w/w) KGM or inulin. Data (mean ± SE, colonic mucosa, $n = 4$ animals per group; liver, $n = 8$ animals per group) are reported as fold difference with respect to fiber-free diet after normalization for GAPDH. Different superscript letters denote significant differences across groups as analyzed by one-way ANOVA followed by Duncan test ($p \leq 0.05$). KGM, konjac glucomannan.

1 **Table 1.** Composition of Experimental Diets[a]

	Fiber-free	KGM	Inulin
Ingredients		— g/kg diet —	
Corn starch	500	437.5	441.5
Casein	200	200	200
Corn oil	250	250	250
Inulin[b]	-	-	58.5
KGM[c]	-	62.5	-
Methioine	3	3	3
Choline	2	2	2
AIN Mineral Mix 76-A	35	35	35
AIN Vitamin Mix 76-A	10	10	10
Total energy (MJ/kg diet)	21.1	20.1	20.1

2 [a]The diets were modified from AIN-76 *(18)*.
3 [b]The purity of inulin was 85.5%.
4 [c]The purity of KGM was 94.8%.
5
6
7 **Table 2.** MDA Levels in the Proximal Colon, Distal Colon, and Liver of
8 Sprague-Dawley Rats Fed a High Corn Oil Diet Containing no Fiber, 5% (w/w)
9 KGM or Inulin[a]

	Fiber-free	KGM	Inulin
		— nmole/mg Protein —	
Colon			
Proximal	2.72 ± 0.19b	2.31 ± 0.15b	1.56 ± 0.16a
Distal	3.79 ± 0.33b	2.65 ± 0.24a	3.03 ± 0.28ab
Liver	1.03 ± 0.12b	0.68 ± 0.04a	0.59 ± 0.06a

10 [a]Data are presented as means ± SE (n = 8 animals per group). Different
11 superscript letters denote significant differences across groups as analyzed by one
12 way ANOVA followed by Duncan test ($p < 0.05$)

13
14
15 **Table 3.** Plasma α-Tocopherol, Ascorbic Acid and MDA Levels in Sprague-Dawley
16 Rats Fed a High Corn Oil Diet Containing no Fiber, 5% (w/w) Konjac Glucomannan
17 or Inulin[a]

	Fiber-free	KGM	Inulin
α-tocopherol (μmole/g Plasma Lipid)	1.87 ± 0.23a	3.10 ± 0.24b	2.63 ± 0.28b
Ascorbic acid (mM)	0.20 ± 0.04	0.21 ± 0.03	0.19 ± 0.03
MDA (μM)	8.2 ± 0.6	7.9 ± 0.5	7.4 ± 0.5

18 [a]Data are expressed as mean ± SE (n = 8 animals per group). Different superscript

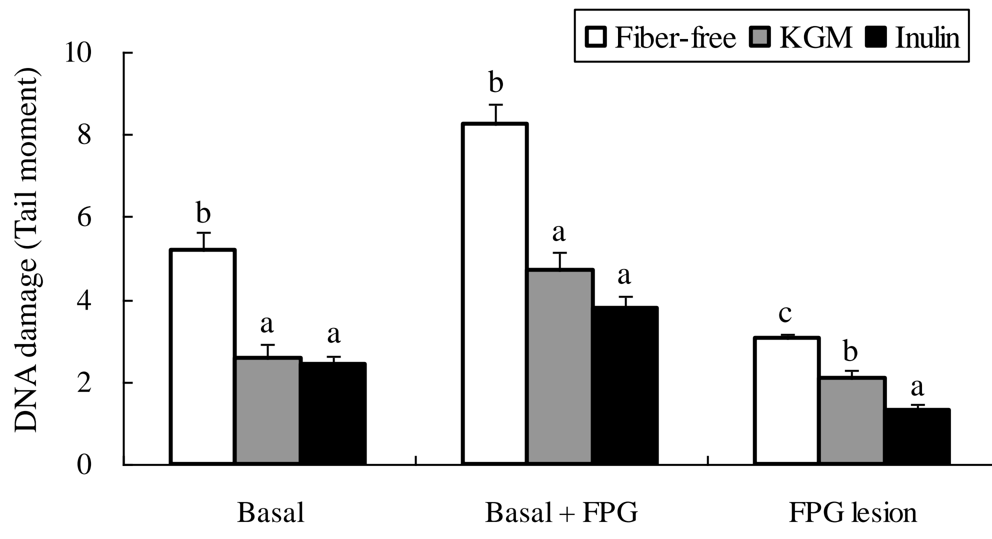
1 letters denote for significant differences across groups as analyzed by one-way
2 ANOVA followed by Duncan test ($p < 0.05$).

3

4 **Table 4.** Concentration, Daily Excretion and Relative Proportion of Fecal
5 Short-Chain Fatty Acids in Sprague-Dawley Rats Fed a High Corn Oil Diet
6 Containing no Fiber, 5% (w/w) KGM or Inulin[a]

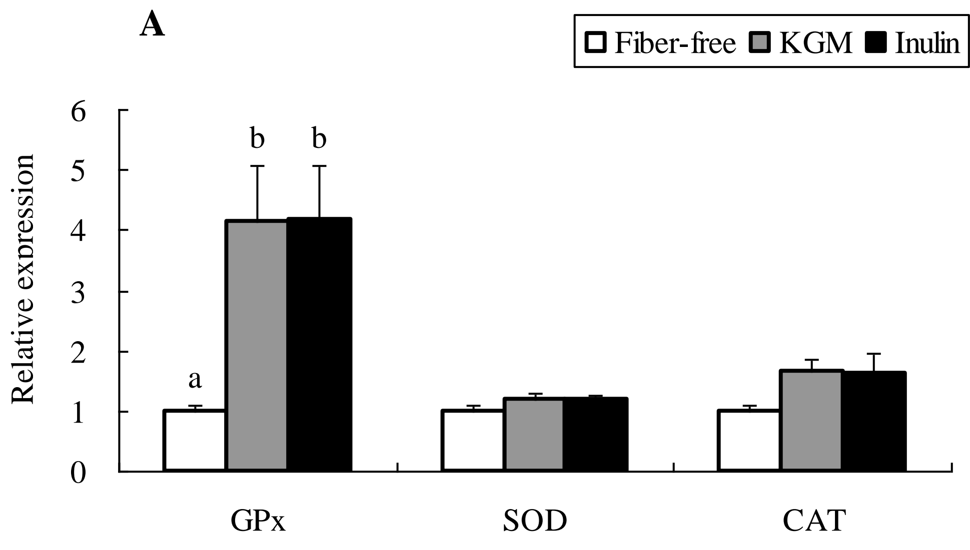
	Fiber-free	KGM	Inulin
	Concentration (μmole/g of Wet Feces)		
Acetate	64.3 ± 7.9a	104.4 ± 6.8b	90.9 ± 11.0ab
Propionate	10.9 ± 2.2	22.4 ± 4.9	12.2 ± 3.3
Butyrate	5.1 ± 1.1a	9.9 ± 0.8b	13.2 ± 0.9c
Total SCFA[b]	80.3 ± 10.5a	136.7 ± 11.7b	116.2 ± 13.9ab
	Daily Fecal Excretion (μmole/Day)		
Acetate	155.2 ± 18.6a	389.9 ± 16.8c	244.7 ± 30.0b
Propionate	26.4 ± 6.0a	84.4 ± 25.1b	29.6 ± 5.6a
Butyrate	12.3 ± 2.9a	38.8 ± 4.9b	35.5 ± 2.0b
Total SCFA	193.8 ± 26.0a	513.2 ± 40.7c	309.8 ± 30.6b
	Relative Ratio (% total)		
Acetate	80.9 ± 2.8	77.3 ± 2.0	78.0 ± 2.1
Propionate	13.1 ± 1.9	15.5 ± 2.1	9.5 ± 1.7
Butyrate	6.1 ± 0.9a	7.3 ± 0.3a	12.5 ± 2.0b

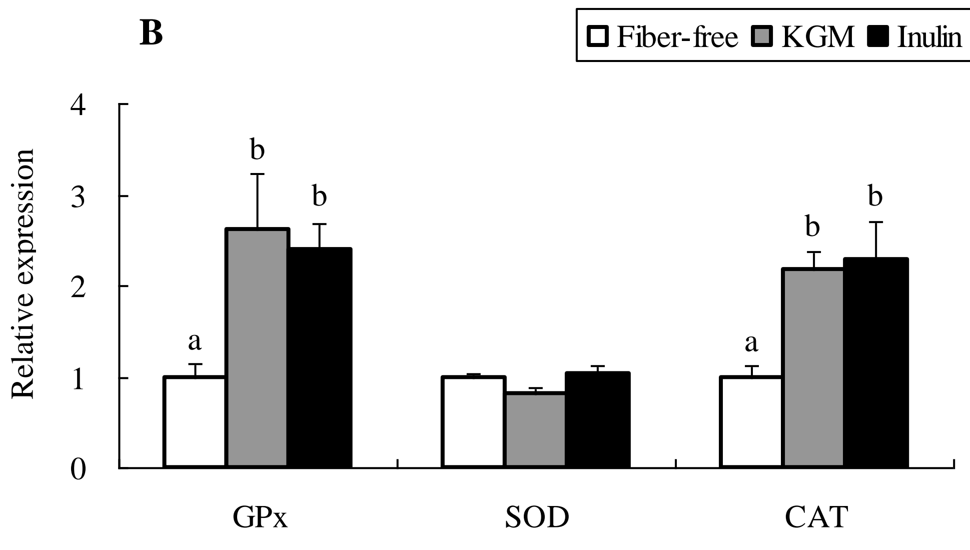
7 [a]Data are presented as mean ± SE ($n = 8$ animals per group). Different superscript
8 letters denote significant differences between treatments as analyzed by one-way
9 ANOVA followed by Duncan test ($p < 0.05$).
10 [b]Sum of acetate, propionate, and butyrate
11
12
13



1

Figure 1





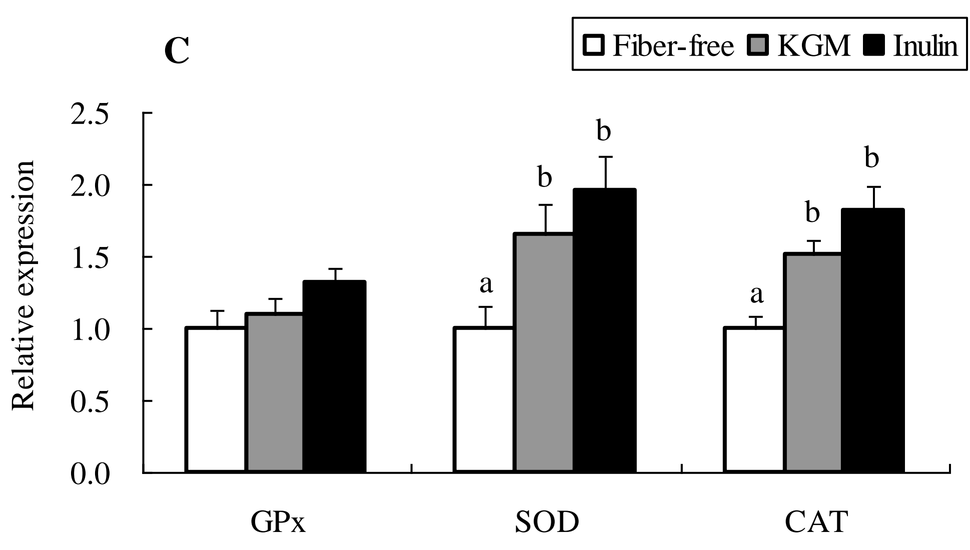


Figure 2

附件二

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

☑達成目標

☐ 未達成目標（請說明，以 100 字為限）

☐ 實驗失敗

☐ 因故實驗中斷

☐ 其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：☑已發表 ☐未發表之文稿 ☐撰寫中 ☐無

專利：☐已獲得 ☐申請中 ☐無

技轉：☐已技轉 ☐洽談中 ☐無

其他：（以 100 字為限）

已發表之論文:

1. Wen-Tzu Wu, **Hsiao-Ling Chen***. 2011. Effects of konjac glucomannan on putative risk factors fo colon carcinogenesis in rats fed a high-fat diet. J Agric Food Chem 59:989-994. **(SCI)** ***Agriculture, multidisciplinary 2/55=3.6%, IF=2.816, 3*5*5=75***

2. Wen-Tzu Wu and **Hsiao-Ling Chen***. 2011. Konjac glucomannan and inulin systematically modulate antioxidant defense in rats fed a high-fat fiber-free diet. J Agric Food Chem 59:9194-9200 **(SCI)** ***Agriculture, multidisciplinary 2/55=3.6%, IF=2.816, 3*5*5=75***

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限）

流行病學研究發現多攝取富含膳食纖維食物有助於降低大腸癌發生率，但是受限於富含膳食纖維的食物往往同時含有植物素等癌症預防因子干擾對膳食纖維作用的判斷，因此目前未能歸納膳食纖維對大腸癌的預防作用。本研究**突破瓶頸**，以國人常食用之純化蒟蒻纖維為材料，探討對腸道內容物中大腸直腸癌危險因子及預防因子的調節作用，尤其是在高脂肪飲食中黏稠性膳食纖維對膽酸之吸附以及轉換作用非常具有**創新性**。此研究採用**國內首創之螢光 S16 RNA probe** 來定量腸道細菌，並以 gas chromatography 分析極為複雜之糞便膽酸代謝物，**技術上為全國創新發展**。此成果得以證實蒟蒻纖維預防大腸癌發生之潛力及透過腸道細菌發酵以及益生菌之可能機制。**研究投稿很快就獲得接受及刊登足見本論文之重要性。**以現代人普遍攝取不足量的膳食纖維並且飲食呈現精緻化現象，相信本篇的研究成果，可進一步應用於人體實驗上，達到促進健康的目標。

國科會補助專題研究計畫項下出席國際學術會議心得報告

日期：100 年 7 月 30 日

<table>
<tr><td>計畫編號</td><td colspan="3">NSC 99-2320-B-040-014-MY2</td></tr>
<tr><td>計畫名稱</td><td colspan="3">探討蒟蒻纖維抵禦高脂低纖維飲食誘發之氧化壓力及大腸腫瘤之作用及其細胞內相關路徑之初步探討</td></tr>
<tr><td>出國人員姓名</td><td>陳曉鈴
吳文慈</td><td>服務機構及職稱</td><td>中山醫學大學營養系教授
中山醫學大學營養系博士班</td></tr>
<tr><td>會議時間</td><td>101 年 7 月 13 日至 101 年 7 月 16 日</td><td>會議地點</td><td>新加坡</td></tr>
<tr><td>會議名稱</td><td colspan="3">XI Asian Congress of Nutrition</td></tr>
<tr><td>發表論文題目</td><td colspan="3">1.The acute apoptotic responses to carcinogen in mice fed a high-fat low-fiber diet: regulation by dietary fiber
2. Effects of konjac glucomannan, inulin and cellulose on the azoxymethane-induced colonocytes DNA damages in mice fed a high-fat low-fiber diet</td></tr>
</table>

一、參加會議經過

每四年舉辦一次的 Asian Congress of Nutrition，第 11 屆於 2011 年 7 月 13-16 日在新加坡 Suntec Singapore International & Exhibition Center 展覽館舉辦。本實驗室於 7 月 14 日發表張貼 2 篇論文，第一篇是『The acute apoptotic responses to carcinogen in mice fed a high-fat low-fiber diet: regulation by dietary fiber』比較菊醣及纖維素對於對於單劑致癌藥物(azoxymethane)注射後，C57BL/6J 小鼠大腸上皮組織細胞凋亡的情形。結果顯示，纖維素組在 AOM 注射

6 小時後，近端結腸細胞凋亡情形較菊醣組低；然而遠端結腸在 0 及 6 小時後，纖維素組細胞凋亡情形則較菊醣組高。第二篇發表的是『Effects of konjac glucomannan, inulin and cellulose on the azoxymethane- induced colonocytes DNA damages in mice fed a high-fat low-fiber diet』；探討蒟蒻、菊醣及纖維素對於單劑 AOM 注射後，對 C57BL/6J 小鼠大腸上皮細胞的 DNA 傷害。結果顯示，相較於高脂低纖維組，補充三種纖維後增加 0 小時的大腸細胞 DNA 傷害；額外以 FPG 處理後，蒟蒻纖維組顯示 FPG lesion 較其他組別高。在 AOM 注射 6 小時後，蒟蒻纖維及纖維素組 FPG lesion 明顯較高；24 小時後，則菊醣組大腸細胞 DNA 傷害明顯比其他組低。不同纖維對於致癌藥物的急性反應不盡相同，有待更進一步的研究，瞭解膳食纖維在早期腸道損傷所扮演的角色。

二、與會心得

1. 感謝國科會此次補助本人及一名博士生參加營養界知名的學術研討會。
2. 在會場中同時與來自台灣以及鄰近亞洲地區學者交流，相信能促

進國內與亞洲地區交流發展。

3. 研究生透過國際會議的參與有效培養其聽力以及口語表達能力。
4. 這次研討會的主題『Nutritional Well-Being for a Progressive Asia Challenges and Opportunities』,共包括八個次議題,內容十分豐富,兩百多名國際知名學者，分享並討論營養在未來所面臨的挑戰，從基礎研究到政策的應用都能從會議中獲得寶貴的知識。

總之感謝國科會的補助，此次與會獲益良多。

三、建議

感謝國科會給予補助出席國際會議。經由參與此次亞洲營養盛會不難發現來自中國大陸的學者為數眾多,他們在科學研究上所下的功夫及政府的支持絕不亞於台灣；當然，台灣在營養研究上的成果雖也不容小覷,因此希望國家政府單位可以繼續於科學研究上的投機及獎勵。

四、攜回資料名稱及內容

會議手冊及論文發表張貼日程表手冊各一本、紀念背包一個、參加證明書。

國科會補助計畫衍生研發成果推廣資料表

日期:2012/10/31

<table>
<tr><td rowspan="3">國科會補助計畫</td><td>計畫名稱: 探討蒟蒻纖維抵禦高脂低纖維飲食誘發之氧化壓力及大腸腫瘤之作用及其細胞內相關路徑之初步探討</td></tr>
<tr><td>計畫主持人: 陳曉鈴</td></tr>
<tr><td>計畫編號: 99-2320-B-040-014-MY2　　學門領域: 保健營養</td></tr>
<tr><td colspan="2">無研發成果推廣資料</td></tr>
</table>

99 年度專題研究計畫研究成果彙整表

計畫主持人：陳曉鈴	計畫編號：99-2320-B-040-014-MY2
計畫名稱：探討蒟蒻纖維抵禦高脂低纖維飲食誘發之氧化壓力及大腸腫瘤之作用及其細胞內相關路徑之初步探討	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	1	1	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	轩 Marquis Who’s Who 2013 轩 國科會特殊優秀人才, 2012/8-2013/7 轩 台灣營養學會營養學術研究傑出獎, 2012/5/19 轩 獲邀於第 38 屆營養會擔任主講人，主題為[膳食纖維與大腸癌預防: 以蒟蒻纖維為例]。2012/5/19 轩 國科會特殊優秀人才, 2011/8-2012/7 轩 台灣營養學會(Zespri Taiwan Inc 贊助)第五屆【舒暢身心 果真健康】百萬論文大賞(與吳文慈同學共同獲獎), 2011

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：（以 100 字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限）

流行病學研究發現多攝取富含膳食纖維食物有助於降低大腸癌發生率，但是受限於富含膳食纖維的食物往往同時含有植物素等癌症預防因子干擾對膳食纖維作用的判斷，因此目前未能歸納膳食纖維對大腸癌的預防作用。本研究突破瓶頸，以國人常食用之純化蒟蒻纖維為材料，探討對腸道內容物中大腸直腸癌危險因子及預防因子的調節作用，尤其是在高脂肪飲食中黏稠性膳食纖維對膽酸之吸附以及轉換作用非常具有創新性。此研究採用國內首創之螢光 S16 RNA probe 來定量腸道細菌，並以 gas chromatography 分析極為複雜之糞便膽酸代謝物，技術上為全國創新發展。此成果得以證實蒟蒻纖維預防大腸癌發生之潛力及透過腸道細菌發酵以及益生菌之可能機制。研究投稿很快就獲得接受及刊登足見本論文之重要性。以現代人普遍攝取不足量的膳食纖維並且飲食呈現精緻化現象，相信本篇的研究成果，可進一步應用於人體實驗上，達到促進健康的目標。