Effects of Dehulled Adlay on the Culture Count of Some Microbiota and Their Metabolism in the Gastrointestinal Tract of Rats

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Experiments were conducted to study the effect of a dietary supplement of dehulled adlay (*Coix lachryma-jobi* L. var. ma-yuen Stapf) on the culture counts of some important groups of intestinal bacteria and their metabolism in the gastrointestinal (GI) tract of Sprague−Dawley rats. Rats were divided into four groups, and each group was fed a diet containing different levels of dehulled adlay for 30 days as follows: 0% (control), 5%, 20%, and 40%. All animals fed with adlay had normal healthy intestinal walls and no pathogenic signs whatsoever. There were no significant differences in body weight gain or the cecal pH between different groups of rats. Both the 20% and 40% groups had lower culture counts of enterics in their feces than the 5% and control groups, whereas the culture counts of fecal lactic acid bacteria were higher in feces of rats fed with adlay than in the control group. Cecal total short-chain fatty acid (SCFA) content and fecal SCFA were significantly higher in the 20% and 40% groups than in the control and 5% groups. All the adlay-fed rats had a higher fecal butyric acid concentration than the control rats. It is concluded that adlay has a significant influence on the growth of intestinal bacteria, which may ultimately affect the physiology and other functions of GI tracts of rats.

Keywords: *Adlay; lactic acid bacteria; short chain fatty acids*

INTRODUCTION

Coix lachryma-jobi L. var. ma-yuen Stapf, commonly called adlay (Job's tears), is an annual crop. It has long been consumed both as a food supplement and as a herbal medicine. The ancient Chinese medical book *Pen-tsao kang mu* (Li, 1596) described it as an efficient remedy for a number of maladies and particularly beneficial to the digestive system. It is widely planted in Taiwan, China, and Japan, where people consider it a healthy food supplement.

Ukita and Tanimura (1961) reported that the growth of Ehrlich ascites sarcoma was inhibited by adlay and identified the active component to be coixenolide (Tanimura, 1961). Nagao et al. (1985) isolated from coix seed a number of benzoxazinones that showed anti-inflammatory activity. Takahashi et al. (1986) also reported that coixans A, B, and C isolated from adlay seed had hypoglycemic activity in rats. Park et al. (1988) discovered that lipid components in plasma and feces would decrease in rats fed with coix seed. Hidata et al. (1992) demonstrated that ingestion of coix seed tablets could increase the activities of cytotoxic T-lymphocytes and natural killer cells. Check and K'Ombut (1995) also showed a decrease in fibrinolytic activities of blood plasma of Wistar rats on a coix mixed diet. Numerous other reports have indicated that the consumption of coix seed is beneficial to human health (Kondo et al., 1988; Otsuka et al., 1988, 1989; Tsai et al., 1999; Yang and Tsai, 1998; Yang et al., 1998). Results from our laboratory also showed that some extracts of adlay seed had anti-allergic and antimutagenic activities (Huang and Chiang, 1999; Shyu et al., 1998). However, the effect of coix seed on the growth of different microorganisms and their metabolism in the GI tract has not been reported.

When adlay seed is ingested, the initial contact is the GI tract, which is one of the most active metabolic sites in the human body (Chung, 1996). Microorganisms in the GI tract may play a role in many disease processes, including cancer (Gibson and Robertfroid, 1995; Chung, 1996). How adlay components interact with the intestinal microbiota and affect the metabolism and physiology of the GI tract may be of importance to health. In this paper, we investigated the effect of adlay on the culture counts of certain important microbial groups and on their metabolism.

MATERIALS AND METHODS

Source of Adlay. Adlay was purchased from a farmer who planted the Taichung Shuenyu No.4 (TCS4) of *Coix lachryma-jobi* L. var. ma-yuen Stapf in Taichung, Taiwan in March of 1997 and harvested it in July of the same year. The air-dried adlay seed was dehulled, blended into powder, and screened through a 20-mesh sieve (aperture 0.94 mm). The powder was then used for a dietary supplement as described below.

Preparation of Animal Diets. Compositions of animal diet are shown in Table 1. Diets were supplemented with 0%, 5%, 20%, or 40% adlay. The chemical composition of adlay seed powder is crude protein, 17.5%; crude fat, 8.3%; crude fiber, 2.6%; and non-nitrogen extract, 69.3% (Hung et al., 1994). These

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Table 1. Compositions of the Various Experimental Diets

diet ingredients	group (g/kg diet) control (0%)	5% adlay	20% adlay	40% adlay
dehulled adlay[a]		50.0	200.0	400.0
casein (protein from adlay)	200.0	191.2 (8.8)	165.0 (35.0)	130.0 (70.0)
corn starch (carbohydrate from adlay)	650.0	615.3 (34.7)	511.4 (138.6)	372.8 (277.2)
soybean oil (oil from adlay)	50.0	45.8 (4.2)	33.4 (16.6)	16.8 (33.2)
cellulose (fiber from adlay)	50.0	48.7 (1.3)	44.8 (5.2)	39.6 (10.4)
methionine	3.0	3.0	3.0	3.0
AIN-76 mineral mixture	35.0	35.0	35.0	35.0
AIN-76 vitamin mixture	10.0	10.0	10.0	10.0
choline bitartrate	2.0	2.0	2.0	2.0

[a] Composition of dehulled adlay: crude protein 17.5%, crude fat 8.3%, fiber 2.6%, carbohydrates 69.3% (wt %, dried weight) (Hung et al., 1994).

percentages were used for calculating the percent of substitution of casein, soybean oil, cellulose, and corn starch (Table 1).

Casein, methionine, and choline bitartrate were purchased from Sigma Chemical Co. (St. Louis, MO), soybean oil was from President Enterprises Corp. (Tainan, Taiwan), corn starch was from Samyang Genex Company (Seoul, Korea), and cellulose was from J. Rettenmaier and Sohne Faserstaff-Werks (Germany). An AIN-76 mineral mixture and an AIN-76 vitamin mixture were obtained from ICN Biochemicals, Inc. (Costa Mesa, CA).

Different components of the diet were added as shown in Table 1 and mixed homogeneously. Soybean oil was added last. The diets were mixed again, meshed, then sealed and stored at −20 °C.

Animals. Twenty-eight Sprague−Dawley male rats at 4 to 5 weeks old were purchased from the Animal Breeding and Research Center of the National Science Council of the Republic of China (Taipei, Taiwan). They were kept in an animal room at 22 ± 2 °C and under light for 12 h/d (06:00−18:00 light, 18:00−06:00 dark). They were given Lab. rodent chow diet (PMI Nutrition International, Inc., Brentwood, MO) for 10 days, then divided into four groups and fed with the prepared diets described above ad libitum. Feed consumption and weight gains were recorded every 2−3 days for 30 days.

Culture Counts. After three weeks of feeding the designed diet, fecal samples were randomly taken from at least 3 rats from each group. After weighing, sterile water was added to give one-tenth dilution, and the samples were stomached for 5 min in a Stomacher 400 (Tekmar, Inc., Cincinnati, OH). Suspensions were then serially diluted in 9-mL sterile Butterfield phosphate buffer (FDA, 1992) to the appropriate concentration and surface-plated on MacConkey agar (Difco Laboratories, Detroit, MI) and Lactobacillus Selective agar (Merck Company, Darmstadt, Germany). MacConkey agar plates were incubated aerobically at 37 °C, and Lactobacillus Selective agar plates were incubated at 37 °C in a Gas Pak Anaerobic chamber (BBL Co., MD) for 48 h. Colony forming units were the average of duplicate plates that showed 25 to 300 colonies for each trial. Data presented in Table 3 were the average of five experiments.

Short-Chain Fatty Acid (SCFA) Determination. Fresh fecal samples were collected 3 days before sacrifice and stored in a −70 °C deep freezer. Cecal samples were collected during sacrifice and stored the same way. Five volumes (v/v) of physiological saline solution (0.9% NaCl + 0.02% NaN_3) were added to the weighed sample, and the samples were pulled and homogenized with a Waring blender for 3 min. The mixtures were then centrifuged at 11000*g* at 4 °C for 15 min. One milliliter of the supernatant was mixed with 10 mL of H_2SO_4 (50% v/v) and extracted with 1 mL of diethyl ether. After thorough shaking, the samples were centrifuged at 11000*g* at 4° C for 5 min. The ether layer was pipetted out, and a small amount of anhydrous magnesium sulfate was added to remove any water. The ether samples were analyzed for short-chain fatty acids using gas chromatography (GC).

A Hewlett-Packard 5890 GC with flame ionization detection and a Stabilwax-DA column (Restek, No. 10823, length 30 m, i.d. 0.25 mm, and df 0.25 mm) was used. The column temperature was kept at 145 °C, and the injector and detector were at 250 °C. Helium was used as carrying gas. Pure acetic acid, propionic acid, and *n*-butyric acid (Sigma) were co-injected as standards. Calculation of the concentration of SCFA was the same as previously published (Whitehead et al., 1976).

Table 2. Effect of Test Diets on Body Weight and Feed Intake of Rats[a]

wt	group (g/kg diet) control (0%)	5% adlay	20% adlay	40% adlay
initial body wt (g/rat)	217 ± 18	215 ± 17	216 ± 17	217 ± 18
final body wt (g/rat)	425 ± 29	429 ± 22	418 ± 26	411 ± 26
body wt gain (g/rat)	208 ± 29	214 ± 22	202 ± 20	194 ± 22
feed intake (g/rat/day)	26.0 ± 1.5	26.4 ± 1.4	26.1 ± 1.1	26.4 ± 1.6

[a] Each value represents the mean ± SD ($n = 7$).

Table 3. Fecal Bacterial Counts and Cecal pH of Rats after Feeding Test Diets for Three Weeks[a]

parameter	group (g/kg diet) control (0% adlay)	5% adlay	20% adlay	40% adlay
cecal pH	6.52 ± 0.27	6.56 ± 0.24	6.79 ± 0.28	6.65 ± 0.24
enterics	7.65 ± 0.17[a]	7.51 ± 0.23[a]	6.55 ± 0.38[b]	6.68 ± 0.33[b]
lactic acid bacteria	9.33 ± 0.14[b]	9.72 ± 0.32[a]	9.77 ± 0.21[a]	9.87 ± 0.06[a]

[a] Each value represents the mean ± SD ($n = 5$). Bacterial culture count expressed as log CFU/g feces (wet weight). [b] Values not sharing the same superscript letter in a horizontal row are significantly different from each other by Duncan's multiple range test ($P < 0.05$).

RESULTS

Animals on the adlay diet appeared healthy, showing no pathological signs or abnormalities during the feeding period. Table 2 shows the body weight gain of rats. There was no significant difference between any treatments. The feed intake (g/day/rat) was the same in all groups tested. The rate of growth was also similar in all groups tested (data not shown).

There were no significant differences in cecal pH between different groups (Table 3). However, the number of enteric bacteria was significantly less in feces of rats on 20% and 40% adlay than in the 5% and control groups. The number of fecal lactic acid bacteria was significantly higher in the feces of rats on adlay diets (5%, 20%, or 40%) than in the control group (Table 3).

There was a higher total concentration of short-chain fatty acids (SCFA) in the cecal contents than in the fecal

Table 4. Contents of SCFA in Cecum and Feces of Rats Fed Different Diets[b]

concentration (μmol/g wet feces)	group (g/kg diet) control (0% adlay)	5% adlay	20% adlay	40% adlay
cecal SCFA				
acetic acid	40.2 ± 2.3	40.9 ± 2.8	54.1 ± 1.5	52.9 ± 2.9
propionic acid	13.5 ± 13.5	14.5 ± 0.9	20.0 ± 0.5	20.8 ± 1.2
butyric acid	11.2 ± 1.2	7.1 ± 0.4	10.9 ± 0.3	12.6 ± 0.8
total	64.8 ± 4.3	62.4 ± 4.1	85.0 ± 2.2	86.3 ± 4.8
fecal SCFA				
acetic acid	30.2 ± 0.9	29.0 ± 1.7	34.2 ± 0.8	32.7 ± 0.8
propionic acid	8.6 ± 0.2	7.2 ± 0.4	10.1 ± 0.4	7.2 ± 0.4
butyric acid	3.6 ± 0.1	4.8 ± 0.3	5.1 ± 0.2	4.8 ± 0.3
total	42.4 ± 1.2	41.0 ± 2.4	49.4 ± 1.3	44.7 ± 1.0

[a] Each value represents the mean ± SD ($n = 5$).

contents (Table 4). Acetic acid was predominant, followed by propionic acid and butyric acid in the feces of all the rats. Both cecal and fecal SCFA contents were significantly higher in feces of rats on 20% and 40% adlay. All the adlay-fed rats had a higher fecal butyric acid concentration than in the control.

DISCUSSION

Adlay is believed to be beneficial to the human digestive systems (Li, 1596). Adlay caused neither detrimental damage to the intestinal walls, as demonstrated by electron microscopy (data not shown) nor weight gain or cecal pH changes, as shown with rats in this study. Animals fed with adlay did not have any symptoms of diarrhea or other abnormalities.

The GI tract is one of the most active metabolic sites in the body. A large number of microorganisms are present in the GI tract. These microorganisms represent a source of great metabolic power and may play a role in many disease processes, including cancer (Chung, 1996; Gibson and Robertfroid, 1995; Mallett and Rowland, 1990; Wollowski et al., 1999). Maintaining the microbial balance is important for health. Microbial balance of the GI tract is affected by many factors, including diet (Chung et al., 1977; Gibson and Robertfroid, 1995). Inclusion of adlay seed in the diet increased the number of fecal lactic acid bacteria while decreasing the number of enterics, as illustrated in this paper (Table 3). Intestinal lactic acid bacteria are generally considered beneficial to health because they produce nutrients, prevent exogenous infections, contribute to alleviation of diarrhea, increase lactose tolerance in susceptible individuals, modulate the immune response, and decrease the incidence of colon cancer (Bartram et al., 1994; Jiang et al., 1996; Kaila et al., 1992; Marin, 1997; Perdigon et al., 1980; Van der Waaij, 1982; Wollowski et al., 1999). On the other hand, intestinal enterics are opportunistic pathogens, causing invasive infection and producing enterotoxins (Betley et al., 1986; Chu and Walker, 1993; Chung, 1996). Adlay may, therefore, be used as a prebiotic, defined by Gibson and Robertfroid (1995) as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon and thus improving host health.

There are many compounds present in adlay, including fibers. Many beneficial effects of dietary fiber have been reported (Cameron-Smith et al., 1994; Harris and Ferguson, 1993; Hillemeier, 1995). The increased lactic acid bacteria may be due to fiber-enhanced growth of the lactic acid bacteria. The growth of some pure cultures of lactic acid bacteria (i.e., *Bifidobacterium adolescents*, *B. longum* and *Lactobacillus acidophilus*) was significantly enhanced by inclusion of some of these adlay fibers in the medium, whereas the growth of some enterics was not affected (Cheng, 1998).

There are many biologically active compounds in adlay, and some of them have been isolated and identified (Chien, 1998; Otsuka et al., 1988; Takahashi et al., 1988; Tanimura, 1961). Ishiguro et al. (1993a,b) demonstrated that 3,5-dimethoxy-1-*H*-inden-1-one (coixinden A) and 1-acetyl-1-hydroxy-3,5-dimethoxy-1-*H*-inden (coixinden B) isolated from the methanol extracts of etiolated seedling of adlay had antimicrobial properties. Coixinden A had wide antimicrobial activity against bacteria, whereas coixinden B had antimicrobial activity against bacteria such as *Bacillus subtilis* IFO 3009 but not against yeasts such as *Saccharomyces cerevisiae* IFO 0304 and fungi such as *Aspergillus niger* JCM 5697 (Ishiguro et al., 1993c) There were other biologically active substances such as coixol, 2-hydroxy-7-methoxy-1,4(2*H*)-benzoxazin-3-one, 2-*o*-*β*-glycopyranosyl-4-hydroxy-7-methoxy-1,4(2*H*)-benzoxazin-3-one, 2-*o*-*β*-glycopyranosyl-4,7-dimethoxy-1,4(2*H*)-benzoxazin-3-one, and so forth in the adlay (Nagao et al., 1985; Otsuka et al., 1988, 1989). Whether some of these compounds would suppress the growth of some intestinal microbiota and thus regulate the microbial population in the GI tract remains to be investigated.

Animals fed with adlay had higher concentrations of SCFA in the GI tracts. Some of the fatty acids have important physiological functions. For example, butyric acid can be used as an energy source for intestinal walls. The growth of colon tumor cells was inhibited by butyric acid (Hague et al., 1993; Smith and German, 1995). Propionic acid has been reported to modulate the production of cholesterol (Chen et al., 1984; Smith and German, 1995).

The effects of the adlay diet on the culture counts of some intestinal microbiota and cecal and fecal short-chain fatty acids of rats are much more pronounced with 20% and 40% than with 5% adlay diets. There might be a threshold of adlay required to manifest these effects. The threshold seems to be larger than 5%, but the exact percentage remains to be determined.

In brief, animals on adlay diets (20% and 40%) were healthy, grew normally, and had higher populations of lactic acid bacteria and fewer enterics. The GI tract also contained higher concentrations of short-fatty acids, which are beneficial to health.

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