Effects of Two α -Glucosidase Inhibitors, Acarbose, and BAY-m-1099, on Intestinal Microflora, Cecal Properties, Body-Weight Gains, Serum Cholesterol and Serum Lipids of Rats

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Abstract Effects of two α -glucosidase inhibitors, Acarbose and BAY-m-1099, on the intestinal microflora, cecal weight, cecal ammonia concentration, cecal pH, body-weight gains, and levels of serum cholesterol and lipids of Fischer male rats were determined. The cecal microflora of rats fed Acarbose-supplemented diets showed a reduction of Enterobacteriaceae and increases of lecithinase-negative *Clostridium* spp. and *Peptostreptococcus* spp. by feeding day (FD) 14. By FD 28, the numbers of *Bifidobacterium* spp. in the Acarbose groups were higher than those in the control and BAY-m-1099 groups. Increasing numbers of *Staphylococcus* spp. were observed in the cecum of rats fed Acarbose (the volume of intake, 40 mg/100 g of feed)- and BAY-m-1099-supplemented diets. The weight of cecum in the Acarbose groups was significantly increased when compared with those in the other groups. The ammonia concentration and pH values in the cecum of rats fed Acarbose diets were significantly lower than those in the other groups. The body-weight gains in the Acarbose groups by FD 28 were significantly lower than those in the BAY-m-1099 and control groups. Significantly decreased levels of total cholesterol, LDL cholesterol, triglycerides, and phospholipids were observed in the Acarbose groups by FD 28. These findings indicated that inhibition of sugar absorption in the small intestine of rats influenced the composition of intestinal microflora, body-weight gains, differences in cecal weight, cecal pH, and cecal ammonia concentration, body-weight gains, and the levels of serum cholesterol and lipids.

Key words: α -glucosidase inhibitor; intestinal microflora; cecal ammonia; serum cholesterol and lipids

Pseudo-oligosaccharide (Acarbose, BAY-g-5421) and deoxynojirimycin derivative (BAY-m-1099) suppress the digestion of carbohydrates in parts of the small intestine by inhibiting α -glucosidase activity (11, 12). Such reductions of carbohydrate absorption, i.e. sucrose, maltose, and isomaltose, from the small intestine may cause an increased concentration of carbohydrates and change the composition of the microflora in the large intestine. Acarbose (BAY-g-5421) inhibited carbohydrate absorption in all parts of the small intestine, while BAY-m-1099 did not inhibit it in the ileum. Excretion of carbohydrates in the feces was increased by Acarbose, but not changed by BAY-m-1099. It is of interest to determine what effects, if any, these two α -glucosidase inhibitors exert on the intestinal microflora.

The present study was undertaken to define the different effects of Acarbose and BAY-m-1099 on the intestinal microflora, cecal weights, cecal water contents, cecal pH, cecal ammonia concentration, body-weight gains, serum cholesterol and serum lipids of rats.

MATERIALS AND METHODS

Animals. Eighty Fischer male rats (Charles River Japan Inc.) with an average initial weight of 188 g were used. The rats were kept in $34 \times 45 \times 19$ cm cages with five rats per cage. All animals were fed *ad libitum* a standard diet (MF, Oriental Yeast Co., Ltd., Japan) and water for 1 week before the experiment. The composition of MF consists of crude protein, 24%; fat, 5.1%; ash, 6.2%; crude fiber, 3.2%; soluble non-protein compounds, 54.5%; vitamins and others, 0.16%; and water, 7.0%. The rats were then assigned to four groups: group A fed MF diet alone as a control group; group B fed MF with Acarbose (20 mg/100 g of MF); group C fed MF with Acarbose (40 mg/100 g); and group D fed MF with BAY-m-1099 (40 mg/100 g). Acarbose and BAY-m-1099 were supplied by Bayer Yakuhin, Ltd., Osaka. Each group consisted of 20 rats. All experimental diets were given to each rat for 28 days. The animal room was kept at $24 \pm 1^\circ\text{C}$; relative humidity was $55 \pm 5\%$; light and dark periods were at 12-hr intervals. Cages and bedding were changed once a week. Body-weight gains were computed on a weekly basis.

Sampling procedures. After feeding days (FD) 14 and 28, the microflora of the small intestine (mainly the upper part of the ileum) and cecum of rats in each group was examined. Fresh samples of the small intestine and cecum were collected from 10 rats on FD 14 and 28, respectively.

Bacterial analysis. The method for bacterial analysis of the intestinal microflora in this study was essentially the same as that of Mitsuoka et al (6, 7). After thorough mixing, a series of 10-fold dilutions (10^{-1} to 10^{-7}) was made in anaerobic diluents (6). From appropriate dilutions, 0.05 ml aliquots were spread onto four non-selective agar plates [modified medium 10 (M10) with 10% cecal contents of rats for fastidious anaerobes (6); modified Eggerth-Gagnon (EG) agar for anaerobes (7); glucose blood liver (BL) agar for anaerobes (7); trypticase soy blood (TS) agar (BBL Microbiology System, Cockeysville, Md) for aerobes (7)] as well as eleven selective agar plates [bifidobacteria-selective (BS) agar for *Bifidobacterium* spp. (7); eubacteria-

selective (ES) agar for *Eubacterium* spp. (6); neomycin-brilliant green-taurocholate-blood (NBGT) agar *Bacteroides* spp. (7); neomycin-Nagler (NN) agar for lecithinase-positive *Clostridium* spp. (7); modified veillonella-selective (VS) agar for *Veillonella* spp. and *Megasphaera* sp. (7); modified lactobacilli-selective (LBS) agar for *Lactobacillus* spp. (7); triphenyltetrazolium chloride-acridine orange-thallosulfate aesculin crystal violet (TATAC) agar for *Enterococcus* spp. and *Streptococcus* spp. (7); phenyl-ethylalcohol egg yolk suspension (PEES) agar for *Staphylococcus* spp. (7); potato dextrose (PD) agar (Difco, Detroit) for yeasts and molds (7); deoxycholate hydrogen sulfide lactose (DHL) agar (Eiken, Tokyo) for Enterobacteriaceae (7); nalixidic acid cetrimide (NAC) agar (Eiken) for *Pseudomonas aeruginosa*. Modified medium 10 was used in the "Plate-in-bottle" method (8) at 37°C for 5 days. Eight agar plates of EG, BL, BS, NBGT, ES, NN, VS, and LBS were incubated at 37°C for 3 days in an anaerobic steel-wool jar (6) filled with an atmosphere of oxygen-free CO₂. Three agar plates of TATAC, PEES, and NAC were incubated aerobically at 37°C for 48 hr; TS and DHL agar plates were incubated at 37°C for 24 hr.

After incubation, each plate was examined for bacterial colonies. The identification of 16 bacterial groups, yeasts, and molds was performed with colonial and cellular morphologies, gram-reaction, spore formation, and aerobic growth. For the bacterial species identified, the bacterial count per gram of wet intestinal contents was calculated and converted into a logarithmic equivalent. The total viable count was calculated from the sum of the counts of each bacterial species.

Cecal weight and water content. After each animal on FD14 and FD28 was killed, the cecum was removed and weighed. The cecal water content was calculated as the wet weight minus the dry weight of contents. The drying of cecal contents was performed by the vacuum drying oven (VO-3-5, Shimizu Rikagaku Co., Ltd., Tokyo).

Cecal pH. The cecal pH was measured *in situ* as the cecum was dissected, using a combination electrode (Horiba Co., Ltd., Tokyo).

Ammonia concentration in cecum. The ammonia concentration in the cecum of rats was determined by use of an ammonia test kit (Wako Pure Chemical Industries, Ltd., Osaka).

Serum cholesterol and lipids. Serum cholesterol (1) and triglycerides (5) were measured by enzyme techniques. HDL and LDL cholesterol were measured by use of the precipitation method with heparin, Ca²⁺, and an anion-exchange resin (9, 10). Phospholipids were determined by a combined enzymatic method using phospholipase D, choline oxidase and peroxidase (15).

RESULTS

Effects of Acarbose and BAY-m-1099 on the Small Intestine and Cecal Microflora of Rats

The compositions of the small intestinal and cecal microflora of 10 rats in four groups by FD 14 are shown in Table 1. In the small intestine, a significant reduction of Enterobacteriaceae ($P < 0.05$) was found in the Acarbose 40 mg group compared with the control group. The cecal microflora of rats fed Acarbose (20 mg and

Table 1. Effects of Acarbose and BAY-m-1099

Intestinal flora	Group A		Group B	
	Small intestine	Cecum	Small intestine	Cecum
Total bacteria ^a	8.8±0.3 ^b (100) ^c	10.2±0.1 (100)	8.9±0.2 (100)	10.0±0.3 (100)
Anaerobic bacteria				
Bacteroidaceae	3.5 (10)	10.0±0.3 (100)	4.3±1.8 ^d (70)	9.8±0.5 (100)
<i>Eubacterium</i> spp.	(0)	7.3±1.6 (40)	(0)	8.3±0.5 (80)
<i>Peptostreptococcus</i> spp.	(0)	8.1±0.6 (80)	6.7 (10)	9.1±0.7 (90)
<i>Bifidobacterium</i> spp.	(0)	6.8±2.9 (30)	(0)	(0)
Lecithinase-positive <i>Clostridium</i> spp.	2.4±0.1 (20)	2.7±0.4 (60)	3.0 (10)	2.9±0.7 (30)
Lecithinase-negative <i>Clostridium</i> spp.	3.2±0.6 (80)	6.3±1.8 (100)	2.9±0.7 (40)	7.2±1.5 (100)
Curved rods	(0)	9.3±0.5 (60)	(0)	(0)
Spiral-shaped rods	(0)	9.3 (10)	(0)	(0)
Fusiform bacteria	(0)	9.0±0.5 (100)	(0)	9.0±0.6 (100)
Aerobic bacteria				
<i>Lactobacillus</i> spp.	8.8±0.3 (100)	9.0±0.2 (100)	8.9±0.2 (100)	9.2±0.3 (100)
Enterobacteriaceae	4.7±1.0 (100)	5.7±0.5 (100)	4.3±1.0 (100)	5.1±0.5 ^e (100)
<i>Streptococcus</i> spp.	4.4±0.9 (100)	5.0±0.4 (100)	4.0±0.3 (100)	5.3±0.9 (100)
<i>Staphylococcus</i> spp.	6.0±0.7 (100)	6.2±0.8 (100)	5.5±0.6 (100)	5.9±0.7 (100)
<i>Corynebacterium</i> spp.	4.9 (10)	6.6 (10)	6.3 (10)	6.2 (10)
Yeasts	(0)	(0)	2.2 (10)	(0)
Molds	2.3±0.3 (30)	2.5±0.2 (30)	2.4±0.1 (20)	2.6±0.5 (40)

^a: No veillonella and megasphaera were detected.

^b: Mean of log₁₀ no. of bacteria/g of intestinal content ± SD.

^c: Frequencies of occurrence (%).

^d: Statistically significant at the $P < 0.05$ level when compared with the bacterial number in

^e: Statistically significant at the $P < 0.05$ level when compared with the bacterial number in

^f: Statistically significant at the $P < 0.001$ level when compared with the bacterial number in

^g: Statistically significant at the $P < 0.001$ level when compared with the bacterial number in

40 mg per 100 g of feed, respectively)-supplemented diets showed a significant reduction of Enterobacteriaceae ($P < 0.05$) and an increase of lecithinase negative (*Clostridium* spp. and *Peptostreptococcus* spp.

Decreased numbers of *Lactobacillus* spp. ($P < 0.05$) and increased numbers of *Staphylococcus* spp. ($P < 0.001$) were found in the small intestine of rats fed BAY-m-1099-supplemented diets. Significantly increased numbers of *Staphylococcus* spp. ($P < 0.001$) were found in the cecum of the BAY-m-1099 group compared with that of controls. Additionally, the intake of BAY-m-1099 also induced the significantly increased numbers of fusiform bacteria ($P < 0.05$) and *Staphylococcus* spp. ($P < 0.001$) compared with that of Acarbose.

The small intestinal and cecal microflora of 10 rats in each group by FD 28 is listed in Table 2. Significant reductions of Enterobacteriaceae ($P < 0.05$) in the small intestine of all animals fed Acarbose- and BAY-m-1099-supplemented diets were found in the present study. The numbers of *Staphylococcus* spp. from the cecum of animals in the Acarbose-40 mg and BAY-m-1099 groups were significantly higher than those in the controls. The counts of *Bifidobacterium* spp., *Peptostreptococcus* spp.,

on the intestinal flora of rats after two weeks

Group C		Group D	
Small intestine	Cecum	Small intestine	Cecum
8.6 $\pm$ 0.3 (100)	10.3 $\pm$ 0.3 (100)	8.4 $\pm$ 0.4 (100)	10.2 $\pm$ 0.1 (100)
4.4 $\pm$ 1.7 (60)	9.7 $\pm$ 0.4 (100)	2.9 $\pm$ 1.0 (40)	9.9 $\pm$ 0.3 (100)
(0)	8.7 $\pm$ 0.5 (50)	(0)	6.4 $\pm$ 2.5 (60)
7.0 (10)	9.5 $\pm$ 0.6 (100)	(0)	8.8 $\pm$ 0.8 (80)
(0)	(0)	(0)	5.9 $\pm$ 4.4 (20)
(0)	(0)	3.2 $\pm$ 1.6 (20)	2.8 $\pm$ 0.3 (80)
3.4 (70)	7.2 $\pm$ 0.8 (100)	2.6 $\pm$ 0.5 (50)	6.6 $\pm$ 1.4 (100)
(0)	9.3 $\pm$ 0.8 (100)	(0)	9.6 $\pm$ 0.4 (70)
(0)	(0)	(0)	(0)
(0)	8.9 $\pm$ 0.5 (100)	(0)	9.4 $\pm$ 0.5 (100)
8.6 $\pm$ 0.3 (100)	9.1 $\pm$ 0.3 (100)	8.4 $\pm$ 0.3 ^a (100)	9.0 $\pm$ 0.2 (100)
3.7 $\pm$ 0.9 ^a (100)	4.8 $\pm$ 1.0 ^e (100)	4.1 $\pm$ 0.5 (100)	5.2 $\pm$ 0.7 (100)
3.9 $\pm$ 0.5 (100)	5.8 $\pm$ 0.7 (100)	4.1 $\pm$ 0.3 (100)	4.8 $\pm$ 0.6 (100)
5.9 $\pm$ 0.8 (100)	5.9 $\pm$ 0.9 (100)	7.3 $\pm$ 0.3 ^f (100)	7.8 $\pm$ 0.6 ^g (100)
(0)	(0)	6.5 $\pm$ 0.1 (20)	6.8 $\pm$ 0.6 (20)
(0)	2.2 (10)	(0)	2.9 $\pm$ 1.3 (50)
2.5 (10)	2.3 $\pm$ 0.4 (30)	4.4 (10)	2.4 (10)

the small intestine of group A.

the cecum of group A.

the small intestine of group A.

the cecum of group A.

and lecithinase-negative *Clostridium* spp. in the cecum of rats in both Acarbose groups were higher than those in control and BAY-m-1099 groups.

The numbers of fusiform bacteria and Enterobacteriaceae from the cecum of rats fed BAY-m-1099-supplemented diets were significantly higher than those given the Acarbose diet, whereas the frequency of occurrence of *Bifidobacterium* spp. in the rats fed BAY-m-1099-supplemented diets was lower than that in the animals fed Acarbose.

Effects of Acarbose and BAY-m-1099 on the Body Weight of Rats

The gains of body weight during feeding of Acarbose and BAY-m-1099 were computed on a weekly basis as given in Fig. 1. After FD 14, no significant body-weight gains were found in each group. By FD 28, body weights of rats given food containing 20 mg and 40 mg of Acarbose were significantly lower than those of rats in the BAY-m-1099 and control groups.

Table 2. Effects of Acarbose and BAY-m-1099

Intestinal flora	Group A		Group B	
	Small intestine	Cecum	Small intestine	Cecum
Total bacteria ^a	8.6±0.5 ^b (100) ^c	10.5±0.2 (100)	8.6±0.4 (100)	10.4±0.3 (100)
Anaerobic bacteria				
Bacteroidaceae	4.1±2.1 (80)	10.1±0.4 (100)	3.9±1.0 (40)	10.2±0.3 (100)
<i>Eubacterium</i> spp.	(0)	7.8±2.6 (40)	(0)	8.3±1.1 (70)
<i>Peptostreptococcus</i> spp.	7.7±0.8 (30)	9.0±0.7 (100)	(0)	9.6±0.5 (100)
<i>Bifidobacterium</i> spp.	2.3 (10)	2.7±0.2 (30)	(0)	9.1±0.9 (30)
Lecithinase-positive <i>Clostridium</i> spp.	(0)	2.8±0.4 (40)	(0)	4.1±0.7 (30)
Lecithinase-negative <i>Clostridium</i> spp.	3.6±0.9 (40)	5.9±1.2 (90)	3.2±1.5 (80)	7.8±1.2 (100)
Curved rods	7.6 (10)	9.7±0.2 (90)	(0)	9.0±0.7 (50)
Spiral-shaped rods	(0)	(0)	(0)	8.8 (10)
Fusiform bacteria	(0)	9.7±0.4 (100)	(0)	9.4±0.5 (60)
Aerobic bacteria				
<i>Lactobacillus</i> spp.	8.6±0.4 (100)	9.3±0.5 (100)	8.6±0.4 (100)	9.2±0.6 (100)
Enterobacteriaceae	4.7±0.8 (100)	5.2±0.5 (100)	4.0±0.5 ^a (100)	5.0±0.5 (100)
<i>Streptococcus</i> spp.	5.4±0.9 (100)	5.3±0.6 (100)	4.2±0.6 (100)	4.4±0.9 (100)
<i>Staphylococcus</i> spp.	5.5±0.7 (100)	5.0±1.6 (100)	5.9±1.0 (100)	5.9±0.5 (100)
<i>Corynebacterium</i> spp.	(0)	(0)	2.3 (10)	5.4±1.1 (20)
Yeasts	(0)	(0)	(0)	2.5±0.7 (40)
Molds	2.1 (10)	2.4±0.1 (70)	2.1±0.1 (20)	2.2±0.2 (30)

^a: No veillonella and megasphaera were detected.

^b: Mean of log₁₀ no. of bacteria/g of intestinal content±SD.

^c: Frequencies of occurrence (%).

^a: Statistically significant at the $P<0.05$ level when compared with the bacterial number in

^e: Statistically significant at the $P<0.05$ level when compared with the bacterial number in

Effects of Acarbose and BAY-m-1099 on Cecal Contents, Cecal pH, and Ammonia Concentration in the Cecum

Effects of Acarbose and BAY-m-1099 on the cecal weight, cecal pH, cecal water content, and ammonia concentration in the cecum are shown in Table 3. The weight of the cecum in the Acarbose groups was significantly increased when compared with those in the BAY-m-1099 and control groups. The heaviest cecum was found in the Acarbose 40 mg group. The water content in the cecum of rats in the Acarbose-40 mg group for four weeks was higher than those of other groups. The cecal pH in the Acarbose-40 mg group was significantly lower than those in the BAY-m-1099 and control groups. The ammonia concentrations in the cecum of rats in the Acarbose groups by FD14 and FD28 were significantly lower than those in the BAY-m-1099 and control groups.

Effects of Acarbose and BAY-m-1099 on Serum Cholesterol and Serum Lipids of Rats

Effects of Acarbose and BAY-m-1099 on the levels of serum cholesterol and lipids of the rats are indicated in Table 4. The levels of total cholesterol, LDL cholesterol, and phospholipids of rats fed Acarbose-40 mg-supplemented diet were significantly

on the intestinal flora of rats after four weeks

Group C		Group D	
Small intestine	Cecum	Small intestine	Cecum
8.6 $\pm$ 0.3 (100)	10.5 $\pm$ 0.1 (100)	8.8 $\pm$ 0.5 (100)	10.6 $\pm$ 0.1 (100)
5.0 $\pm$ 2.5 (70)	10.3 $\pm$ 0.2 (100)	3.1 $\pm$ 0.6 (60)	10.3 $\pm$ 0.2 (100)
(0)	8.5 $\pm$ 1.1 (90)	(0)	9.3 (10)
(0)	9.7 $\pm$ 0.5 (100)	(0)	9.4 $\pm$ 0.3 (100)
6.9 $\pm$ 1.5 (60)	9.1 $\pm$ 0.3 (80)	6.2 (10)	(0)
(0)	3.2 $\pm$ 0.6 (60)	(0)	3.2 $\pm$ 0.4 (80)
5.0 $\pm$ 2.4 (40)	7.0 $\pm$ 0.9 (90)	3.2 $\pm$ 0.9 (80)	6.7 $\pm$ 0.9 (100)
(0)	9.5 $\pm$ 0.4 (60)	(0)	9.5 $\pm$ 0.5 (70)
(0)	(0)	(0)	10.2 (10)
(0)	9.5 $\pm$ 0.6 (100)	(0)	9.9 $\pm$ 0.2 (100)
8.6 $\pm$ 0.3 (100)	9.1 $\pm$ 0.5 (100)	8.8 $\pm$ 0.5 (100)	9.2 $\pm$ 0.5 (100)
3.7 $\pm$ 0.9 ^d (100)	4.9 $\pm$ 0.3 (100)	4.0 $\pm$ 0.4 ^d (100)	5.5 $\pm$ 0.3 (100)
4.3 $\pm$ 0.6 (100)	5.6 $\pm$ 1.0 (100)	4.6 $\pm$ 1.1 (100)	5.0 $\pm$ 0.4 (100)
6.1 $\pm$ 0.8 (100)	6.2 $\pm$ 0.6 ^e (100)	6.3 $\pm$ 1.3 (100)	6.3 $\pm$ 0.7 ^e (100)
(0)	(0)	6.6 (10)	(0)
2.2 $\pm$ 0.2 (20)	2.3 $\pm$ 0 (20)	(0)	(0)
2.2 $\pm$ 0.3 (40)	2.5 $\pm$ 0.4 (40)	2.2 $\pm$ 0.2 (40)	2.4 $\pm$ 0.3 (30)

the small intestine of group A.

the cecum of group A.

lower than those in the BAY-m-1099 and control groups. Significantly decreased levels of triglycerides were also found in the Acarbose groups.

DISCUSSION

It is known that, after intake of Acarbose, human fecal microflora show increased numbers of carbohydrate-dependent bacteria and decreased numbers of protein-dependent bacteria (14). Results of the present study indicated that the cecal microflora of rats fed Acarbose-supplemented diets by feeding day (FD) 14 was characterized by significantly decreased numbers of Enterobacteriaceae and increased numbers of lecithinase-negative *Clostridium* spp. and *Peptostreptococcus* spp. By FD 28 of the Acarbose groups, the intestinal microflora showed a decrease of Enterobacteriaceae and increases of *Bifidobacterium* spp., lecithinase-negative *Clostridium* spp., *Peptostreptococcus* spp., and *Staphylococcus* spp. These findings indicated that Acarbose on FD 28 had resulted in increased gram-positive bacteria in the cecum of rats, the same as in the Acarbose feeding in humans (14). The main carbohydrate-dependent bacteria in the rat intestine were *Lactobacillus* spp. and *Bifido-*

Fig. 1. Effects of Acarbose and BAY-m-1099 on body-weight gains of rats.
a: Statistically significant at the $P < 0.001$ when compared with control.

bacterium spp. By FD 28, the higher numbers and frequency of occurrence of *Bifidobacterium* spp., but not *Lactobacillus* spp., in the cecum of rats fed Acarbose were observed. Yazawa et al (17) demonstrated that sugar sources played an important role in the intestinal proliferation of *Bifidobacterium* spp. in humans. The result in the present study indicates that the increased numbers of *Bifidobacterium* spp. are influenced by the concentrations of sugar in the intestine. It is not known why lecithinase-negative *Clostridium* spp. was increased by the Acarbose feeding.

The influence of BAY-m-1099 on the intestinal microflora of rats was indicated by the significantly increased number of *Staphylococcus* spp. Rats fed BAY-m-1099 showed more fusiform bacteria and Enterobacteriaceae than those fed Acarbose. The fecal microflora of rats fed BAY-m-1099-supplemented diets was similar to that of the controls. These changes in fecal microflora of rats fed BAY-m-1099 may be related to the absorption of BAY-m-1099 from the small intestines, especially the ileum.

The cecal weight of all rats fed Acarbose-supplemented diets in the present study was significantly higher than that of the controls and BAY-m-1099 group. The cecal weight of rats fed Acarbose diets increased dose-dependently. The sig-

Table 3. Effects of Acarbose and BAY-m-1099 on cecal properties of rats

Cecal properties	Group A		Group B		Group C		Group D	
	FD 14	FD 28	FD 14	FD 28	FD 14	FD 28	FD 14	FD 28
Cecum weight (g)	5.8±0.7	4.8±1.1	8.3±3.4 ^a	9.3±2.0 ^b	14.5±3.1 ^c	13.2±2.2 ^b	4.9±0.5 ^a	4.3±1.1
Cecal pH	6.5±0.1	6.4±0.3	6.3±0.2 ^a	6.3±0.1	5.9±0.2 ^c	5.8±0.2 ^b	6.6±0.2	6.8±0.2 ^d
Cecal moisture content (%)	77.9±1.3	78.7±1.9	80.3±1.9 ^e	78.9±1.3	78.2±1.6	81.1±1.9 ^f	79.0±1.9	79.2±2.1
Cecal ammonia (μg/g of wet feces)	361±85	335±51	216±54 ^e	147±44 ^b	164±34 ^c	116±23 ^b	370±80	368±52

Data were expressed as mean±SD.

^a: Statistically significant at the $P<0.05$ level when compared with group A on FD 14.

^b: Statistically significant at the $P<0.001$ level when compared with group A on FD 28.

^c: Statistically significant at the $P<0.001$ level when compared with group A on FD 14.

^d: Statistically significant at the $P<0.01$ level when compared with group A on FD 28.

^e: Statistically significant at the $P<0.01$ level when compared with group A on FD 14.

^f: Statistically significant at the $P<0.05$ level when compared with group A on FD 28.

Table 4. Effects of Acarbose and BAY-m-1099 on levels of serum cholesterol and lipids

Serum cholesterol and lipids	Group A		Group B		Group C		Group D	
	FD 14	FD 28	FD 14	FD 28	FD 14	FD 28	FD 14	FD 28
Total cholesterol (mg/dl)	49.7±2.0	53.5±2.0	52.3±3.9	49.9±2.1 ^a	44.8±3.2 ^b	45.6±1.3 ^b	50.8±2.5	51.4±2.4 ^c
LDL cholesterol (mg/dl)	60.3±16.9	106.8±20.9	42.7±33.5	51.7±24.1 ^a	18.8±11.0 ^b	36.9±14.5 ^a	84.4±10	113.0±30.7
VLDL cholesterol (mg/dl)	<10	<10	<10	<10	<10	<10	10.8±1.2	<10
HDL cholesterol (mg/dl)	35.6±5.3	37.0±2.2	48.3±11.5 ^a	37.0±2.8	30.6±7.2	30.7±1.8	34.7±4.4	36.1±2.8
Phospholipids (mg/dl)	136.8±6.4	132.0±5.6	135.3±14.0	127.5±7.2	101.9±7.5 ^b	112.3±5.6 ^a	137.2±7.3	138.3±10.2 ^c
Triglyceride (mg/dl)	156.2±27.1	173.4±21.0	129.5±28.3 ^e	132.7±26.9 ^f	69.8±24.6 ^b	81.5±23.4 ^a	145.1±16.9	187.4±36.7

Data were expressed as mean±SD.

^a: Statistically significant at the $P<0.001$ level when compared with group A on FD 28.

^b: Statistically significant at the $P<0.001$ level when compared with group A on FD 14.

^c: Statistically significant at the $P<0.05$ level when compared with group A on FD 28.

^d: Statistically significant at the $P<0.01$ level when compared with group A on FD 14.

^e: Statistically significant at the $P<0.05$ level when compared with group A on FD 14.

^f: Statistically significant at the $P<0.01$ level when compared with group A on FD 28.

nificant reductions of cecal pH of rats given Acarbose diets may be related to the increased number of *Bifidobacterium* spp. in the cecum of these animals. The significant decrease in cecal ammonia concentration of rats fed Acarbose-supplemented diets related to the decreased number of Enterobacteriaceae and the increased number of *Bifidobacterium* spp. and may be due to the dilutions by the large volume of cecal contents and an increase of the water content. Different functions between Acarbose- and BAY-m-1099-supplemented diets were exerted. Briefly, no animal fed BAY-m-1099-supplemented diets had low ammonia concentration and low pH values in the cecum.

It is widely known that the intake volume of feed with Acarbose is dose-dependently decreased as compared with that without Acarbose (3, 11, 13), and as a result, body-weight gains were reduced. The results of the present study were in close agreement with those of the previous study. Caspary and Kalisch reported that the highest dose of Acarbose induced diarrhea (2). However, no animal fed Acarbose (20 mg and 40 mg/100 g of feed) had diarrhea in this study.

On the other hand, the body weight of rats fed BAY-m-1099-supplemented diet was similar to that of the control group. Although the body-weight gains were reported to be dose-dependently reduced by BAY-m-1099-supplemented diet, body-weight gains the same as in the control group were observed in rats fed BAY-m-1099 in the present study.

It is known that the levels of serum cholesterol and triglycerides are lowered by the Acarbose supplementation. The results of the present study were in agreement with those in previous reports (4, 16). The levels of total cholesterol, LDL cholesterol, and triglycerides in the serum of rats fed Acarbose-supplemented diets were observed to be dose-dependently reduced. LDL cholesterol in the Acarbose group reflected the changes in total cholesterol. Our results, however, also showed that BAY-m-1099 supplementation did not decrease the levels of serum triglycerides and LDL cholesterol.

In conclusion, we have been able to show in this study that Acarbose supplementation is associated with significant changes in the composition of intestinal microflora, cecal weight, cecal pH values, cecal ammonia concentration, but the changes by BAY-m-1099 supplementation were not similar to those by Acarbose.

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