



Title	Ingestion of difructose anhydride III partially suppresses the deconjugation and 7 α -dehydroxylation of bile acids in rats fed with a cholic acid-supplemented diet
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1 **Ingestion of difructose anhydride III partially suppresses the deconjugation and 7 α -**
2 **dehydroxylation of bile acids in rats fed with a cholic acid-supplemented diet**

3

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18 **Abbreviation**

19 BA, bile acid; BSH, bile salt hydrolase; CA, cholic acid; DCA, deoxycholic acid; DFAIII;

20 difructose anhydride III; MCA, muricholic acid; MS, mass spectrometry; NCDs, non-

21 communicable diseases; LC; liquid chromatography; SCFA, short-chain fatty acid; TCA,

22 taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid;

23 TUDCA, tauroursodeoxycholic acid; T α MCA, tauro- α -muricholic acid; T β MCA, tauro- β -
24 muricholic acid; T ω MCA, tauro- ω -muricholic acid;

25

26 **Abstract**

27 Difuctose anhydride III (DFAIII) is a prebiotic involved in the reduction of secondary bile
28 acids (BAs). We investigated whether DFAIII modulates BA metabolism, including
29 enterohepatic circulation, in the rats fed with a diet supplemented with cholic acid (CA), one
30 of the 12 α -hydroxylated BAs. After acclimation, the rats were fed with a control diet or a diet
31 supplemented with DFAIII. After 2 weeks, each group was further divided into two groups
32 and was fed diet with or without CA supplementation at 0.5 g/kg diet. BA levels were
33 analyzed in aortic and portal plasma, liver, intestinal content, and feces. As a result, DFAIII
34 ingestion reduced the fecal deoxycholic acid level via the partial suppression of
35 deconjugation and 7 α -dehydroxylation of BAs following CA supplementation. These results
36 suggest that DFAIII suppresses production of deoxycholic acid in conditions of high
37 concentrations of 12 α -hydroxylated BAs in enterohepatic circulation, such as obesity or
38 excess energy intake.

39

40 *Keywords:*

41 Difuctose anhydride III

42 Bile acid

43 Deconjugation

44 7 α -Dehydroxylation

45

46 **Introduction**

47 According to the World Health Organization, non-communicable diseases (NCDs) are
48 responsible for almost 70% of deaths reported worldwide [1]. The number of deaths is
49 expected to increase worldwide from 38 million in 2012 to 52 million by 2030 [2]. Unhealthy
50 diets, including those with high portion of sugars or saturated fats (30%), drinking alcohol
51 (30%), physical inactivity (10%), and smoking (10%) are the primary factors that raise the
52 risk of NCDs. Dietary intervention is proposed as one of the suitable strategies to prevent
53 NCDs [3]. Epidemiologic studies have revealed that the consumption of an energy-dense diet
54 increases obesity rate as well as the risk of diseases, including insulin resistance and
55 metabolic syndrome [4, 5].

56 One of prominent changes induced by energy-dense diet is the alteration in bile acid (BA)
57 metabolism. BA is a steroid synthesized from cholesterol in the liver and contributes to lipid
58 absorption [6]. Primary BAs are conjugated with taurine or glycine in the liver and stored in
59 the gallbladder. The conjugated BAs are secreted in response to diet consumption into the
60 intestine through the bile duct to assist lipid absorption. The secreted BAs are reabsorbed
61 through the portal vein into the liver and reused in the process called as enterohepatic
62 circulation [7]. In the large intestine, some gut microbes produce bile salt hydrolase (BSH),
63 which deconjugates BA and liberates a taurine or glycine moiety [8]. The production of BSH
64 depends on the species of bacteria, intestinal environment, and BA concentration [9]. The
65 “free BA” becomes weakly acidic and undergoes 7 α -dehydroxylation to produce secondary
66 BAs [10].

67 A significant increase in 12 α -hydroxylated BAs, especially taurocholic acid (TCA) in the
68 bile and deoxycholic acid (DCA) in the large intestine, is observed in the rats fed with a high-
69 fat diet in our previous study [11]. This observation suggests that the increased TCA flows
70 into the gastrointestinal tract and undergoes deconjugation and 7 α -dehydroxylation, resulting
71 in an increase in the excretion of DCA in feces. Such an alteration in BA metabolism was

72 observed in the rats fed with CA and was accompanied with an alteration in the gut
73 microbiota composition [12]. In the intestine, a variety of gut microbes are involved in BA
74 metabolism [13]. Ingestion of a prebiotic may modulate the gut microbiota and possibly alter
75 BA metabolism and host health because some of the secondary BAs are risk factors
76 associated with colon [14] and liver cancer [15].

77 Difructose anhydride III (di-D-fructofuranose-1,2':2,3'-dianhydride, DFAIII), a non-
78 digestible disaccharide, is mainly derived from inulin [16]. DFAIII is highly fermentable and
79 enhances short-chain fatty acid (SCFA) synthesis during microbial fermentation in the cecum
80 [17]. Intestinal acidification after DFAIII supplementation [18,19] increases DFAIII-
81 assimilating bacteria and decreases fecal secondary BA concentration [20]. However, no
82 information is available on BA metabolism, including enterohepatic circulation. In this study,
83 we investigated whether DFAIII ingestion affects BA metabolism in combination with CA
84 supplementation.

85

86 **Material & Methods**

87 *Animal and diets*

88 The animal experiment was approved by the Institutional Animal Care and Use Committee
89 of National University Corporation of Hokkaido University (14-0026). All animals were
90 maintained according to the Hokkaido University Manual for Implementing Animal
91 Experimentation. Male Wistar rats ($n = 32$; 3-week old; Japan SLC Inc, Hamamatsu, Japan)
92 were individually housed in stainless steel cages in a controlled environment of temperature
93 ($22 \pm 2^\circ\text{C}$), humidity ($55 \pm 5\%$), and light (from 8:00-20:00). Feeding schedule of the entire
94 experimental period is presented in Fig. 1. Rats had free access to water and a control diet (C)
95 based on AIN-93G [21] (Table 1) during the acclimation period for a week. The rats were
96 then divided into two dietary groups, namely, C ($n = 16$) and D (DFAIII-supplemented diet)

97 ($n = 16$). After feeding for 2 weeks, each group of rats was further divided into two groups
98 and were fed diet with or without CA supplementation at 0.5 g/kg diet. Body weight and food
99 intake were measured every 2 days. On day 33 of the test period, the rats were orally
100 administered with a chromium (III) chloride hexahydrate (Wako Pure Chemical Industries,
101 Ltd., Osaka, Japan) [22] solution with ethylenediamine tetra acetate 2Na (Dojindo
102 Laboratories, Kumamoto, Japan) for the determination of urinary chromium (Cr) excretion.
103 Urine samples for 24 h were obtained after 24 h from oral administration for gut permeability
104 test. Fecal samples were collected from day 34 to 35 and stored at -30°C for the evaluation of
105 BAs. On day 35, the rats were sacrificed under anesthesia by an intraperitoneal injection of
106 sodium pentobarbital (50 mg/kg body weight, Somnopentyl, Kyoritsuseiyaku Corporation,
107 Tokyo, Japan). Blood plasma samples were collected using syringe from both abdominal
108 aorta and portal vein with aprotinin (final concentration of 500 KIU/mL) and heparin (final
109 concentration of 50 IU/mL). The blood samples were centrifuged and the supernatant
110 collected. Cecum was collected and the weights of whole cecum and cecal contents were
111 measured. The cecal contents were diluted four times with deionized water and homogenized
112 with Teflon homogenizer. The pH of homogenates was measured using a semiconducting
113 electrode (ISFET pH sensor Argus; Sentron, Roden, Netherlands). Cecal contents were kept
114 in liquid nitrogen and stored at -80°C for BA analysis. Epididymal adipose tissue was
115 dissected and weighed. Liver tissue weight was measured and the tissues were kept in liquid
116 nitrogen and stored at -80°C for BA analysis.

117

118 *Gut permeability*

119 The concentration of Cr in urine samples was measured using an atomic absorption
120 spectrophotometer (Z-5310, Hitachi High-Tech., Tokyo, Japan). The proportion of urinary Cr

121 excretion following oral administration of Cr-ethylenediaminetetraacetic acid solution was
122 calculated as an indicator of gut permeability [23].

123

124 *BA analysis*

125 BA extraction and liquid chromatography (LC) separation were performed with Dionex
126 UltiMate 3000 UPLC system (Thermo Fisher Scientific corporation, San Jose, CA, USA)
127 according to our previous study [24]. Mass spectrometry (MS) was performed using Orbitrap
128 mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray ionization probe
129 under negative-ion mode. Full-scan MS spectra (from m/z 200–700) were acquired with
130 Orbitrap analyzer after accumulation to a target value of $1e6$ in the linear ion trap. Resolution
131 in Orbitrap system was set to $r = 17,500$. Standard mass spectrometric conditions for all
132 experiments were as follows: spray voltage, 3.8 kV; sheath gas flow rate, 35 L/h; aux gas
133 flow rate, 10 L/h; heated capillary temperature, 250°C; aux gas heater temperature, 450°C; s-
134 lens RF level, 100%. Individual BA concentration was measured using nordeoxycholic acid
135 (23-nor-5 β -cholanic acid-3 α ,12 α -diol) as an internal standard. BAs were measured as
136 follows: 5 β -cholanic acid-3 α ,7 α ,12 α -triol (CA); 5 β -cholanic acid-3 α ,6 β ,7 α -triol; 5 β -cholanic
137 acid-3 α ,6 β ,7 β -triol (β -muricholic acid, β MCA); 5 β -cholanic acid-3 α ,6 α ,7 β -triol (ω -
138 muricholic acid, ω MCA); 5 β -cholanic acid-3 α ,6 α -diol; 5 β -cholanic acid-3 α ,7 β -diol; 5 β -
139 cholanic acid-3 α ,7 α -diol; 5 β -cholanic acid-3 α ,12 α -diol (DCA); 5 β -cholanic acid-3 α -ol; 5 β -
140 cholanic acid-3 α ,6 α ,7 α -triol; 5 β -cholanic acid-3 α ,7 β ,12 α -triol; 5 β -cholanic acid-3 α ,12 α -diol-
141 7-one; 5 β -cholanic acid-3 α -ol-7-one (7-oxo-LCA); 5 β -cholanic acid-3 α -ol-12-one; 5 β -
142 cholanic acid-12 α -ol-3-one (3o12 α); 5 β -cholanic-3 α ,7 α ,12 α -triol-*N*-(2-sulphoethyl)-amide;
143 5 β -cholanic-3 α ,6 β ,7 α -triol-*N*-(2-sulphoethyl)-amide (tauro- α -muricholic acid, T α MCA); 5 β -
144 cholanic-3 α ,6 β ,7 β -triol-*N*-(2-sulphoethyl)-amide (tauro- β -muricholic acid, T β MCA); 5 β -
145 cholanic-3 α ,6 α ,7 β -triol-*N*-(2-sulphoethyl)-amide (tauro- ω -muricholic acid, T ω MCA); 5 β -

146 cholanic-3 α ,6 α -diol-*N*-(2-sulphoethyl)-amide; 5 β -cholanic-3 α ,7 β -diol-*N*-(2-sulphoethyl)-
147 amide (taoursodeoxycholic acid, TUDCA); 5 β -cholanic-3 α ,7 α -diol-*N*-(2-sulphoethyl)-
148 amide (taurochenodeoxychlic acid, TCDCA); 5 β -cholanic-3 α ,12 α -diol-*N*-(2-sulphoethyl)-
149 amide (taurodeoxycholic acid, TDCA); 5 β -cholanic-3 α -ol-*N*-(2-sulphoethyl)-amide; 5 β -
150 cholanic-3 α ,7 α ,12 α -triol-*N*-(carboxymethyl)-amide; 5 β -cholanic-3 α ,6 α -diol-*N*-
151 (carboxymethyl)-amide; 5 β -cholanic-3 α ,7 β -diol-*N*-(carboxymethyl)-amide; 5 β -cholanic-
152 3 α ,7 α -diol-*N*-(carboxymethyl)-amide; 5 β -cholanic-3 α ,12 α -diol-*N*-(carboxymethyl)-amide;
153 and 5 β -cholanic-3 α -ol-*N*-(carboxymethyl)-amide.

154

155 *Fecal DFAIII excretion*

156 Fecal DFAIII excretion in the DFAIII-fed rats was measured based on the method as
157 previously described [20]. Briefly, homogenized fecal samples were added to deionized water
158 and sonicated. After centrifugation, the supernatant was collected and degreased with
159 chloroform. The amount of DFAIII was analyzed using high-performance LC (Hitachi High-
160 Tech Science Co., Tokyo) with a TSKgel Amide-80 column (4.6 \times 250 mm).

161

162 *Statistical analysis*

163 All data are presented as the mean \pm standard error of means (SEM). Two-way analysis of
164 variance (ANOVA) test was used to represent significant interaction between two factors, BA
165 and DFAIII. Student's *t*-test was used for comparison of DFAIII excretion between two
166 groups of rats fed diet with or without CA supplementation. All statistical analysis was
167 performed using JMP software version 13.0 (SAS Institute Inc., Cary, NC, USA).

168

169 **Results**

170 *Reduction of fecal DFAIII concentration*

171 To investigate whether the ingested DFAIII was assimilated by the intestinal microbes, the
172 fecal DFAIII excretion was determined as shown in Fig. 2. Fecal excretion of DFAIII was the
173 highest on day 8 and gradually decreased thereafter. On day 21, the rats were divided into
174 two groups and fed with or without CA-supplemented diet to evaluate the effect of CA
175 supplementation on fecal DFAIII excretion. As a result, the fecal DFAIII was detected at
176 trace levels under CA supplementation condition. A significant difference was observed
177 between the groups on day 27, but only a trace level was detected.

178

179 *Alteration of BA metabolism in rats fed with the DFAIII diet*

180 Thirty molecular species of BAs were analyzed using LC-MS at the end of the experimental
181 period, as shown in Fig. 3. TCA was the most abundant BA in the liver, intestinal contents,
182 and portal plasma. CA supplementation increased the concentration of TCA at these sites.
183 Two-way ANOVA results revealed the synergetic effect of CA and DFAIII supplementation
184 on TCA concentration in the portal plasma. A similar tendency was observed in the ileal
185 contents. CA supplementation increased the concentration of TDCA in the liver; a significant
186 interaction was found between CA and DFAIII. Similar changes were observed in the cecal
187 and fecal DCA concentrations, as shown in the liver TDCA. A synergetic effect of CA and
188 DFAIII supplementation was observed on both CA and TCA concentrations in the cecal
189 contents and feces. The trend in fecal 3 α 12 α excretion resembled that observed for DCA. On
190 the other hand, the concentration of ω MCA decreased after CA and DFAIII supplementation.
191 Similar tendency was also observed in the feces.

192

193 *Biochemical parameters in the rats fed the DFAIII diet*

194 Growth and biochemical parameters are shown in Table 2. DFAIII-supplemented diets
195 decreased the total food intake, final body weight, and epididymal adipose tissue weight. The

196 weights of whole cecum and its contents were higher in the rats fed with DFAIII-
197 supplemented diets than in those from the other groups and a reduction in the pH of the cecal
198 content was observed. The pH reduction was significantly affected by CA supplementation.
199 CA supplementation enhanced gut permeability, as observed with urinary Cr excretion, but
200 no difference was reported in the rats fed with DFAIII-supplemented diet.

201

202 **Discussion**

203 Gas chromatography/MS mainly used for the determination of BA profiles necessitates the
204 derivatization step. This derivatization process breaks taurine or glycine conjugation of BAs
205 and poses difficulty in the discrimination between conjugated and unconjugated BAs [25]. In
206 the present study, we analyzed not only unconjugated BAs but also the conjugated forms of
207 BAs using LC/MS [24] that enabled us to elucidate the effect of DFAIII ingestion on BA
208 metabolism, especially in the organs related with enterohepatic circulation.

209 In the rats fed control diet, the proportion of 12 α -hydroxylated BAs was almost
210 comparable that of non-12 α -hydroxylated BAs in liver, ileal contents, and portal plasma (Fig.
211 3). On the other hand, an increase in the proportion of non-12 α -hydroxylated BAs was
212 observed in large intestine and in feces such as β MCA and ω MCA. Those results suggest that
213 12 α -hydroxylated BAs are selectively reabsorbed in ileal epithelial cells. This notion is
214 supported by observations in mice [26] that show enormous excretion of ω MCA in feces
215 rather than 12 α -hydroxylated BAs. As expected, the CA-supplemented diet raised the
216 concentration of 12 α -hydroxylated BAs, for example TCA in enterohepatic circulation and
217 DCA in cecal contents (Fig. 3). The DFAIII-supplemented diet reduced cecal and fecal
218 ω MCA concentrations. β MCA is converted into ω MCA in the large intestine by anaerobic
219 bacteria, such as *Eubacterium lentum* strain [27], suggesting that the ingestion of DFAIII
220 reduces conversion of β MCA into ω MCA by the gut microbes. Interestingly, in combination

221 of CA with DFAIII, massive increase of TCA concentration was observed in the organs
222 related with enterohepatic circulation accompanied by decreased fecal DCA excretion. These
223 results suggest that 12 α -hydroxylated BAs were selectively reused in enterohepatic
224 circulation.

225 Simple feeding with CA-supplemented diet resulted in an increase in BA concentration
226 mainly in the organs associated with enterohepatic circulation. CA supplementation increased
227 DCA concentration in the large intestine and feces, indicating that the ingested CA was
228 completely dehydroxylated into DCA by the gut microbes. On the other hand, the elevated
229 DCA concentration was normalized in the rats fed with DFAIII that resulted in the reduction
230 in the pH of the cecal contents. Minamida and colleagues [25] measured the conversion of
231 CA to DCA using thin-layer chromatography and found that DCA production was
232 significantly suppressed at pH 5.8 as compared to that observed at pH 7.5, suggestive of the
233 connection between low pH and reduction in 7 α -dehydroxylation under DFAIII-fed
234 condition. The decrease in pH of the large intestine is thought to inhibit 7 α -dehydroxylase
235 activities in the luminal contents, contributing to the reduction in DCA concentration. In
236 addition, DFAIII ingestion increased acetate and other organic acid levels in the cecal
237 contents that may serve as a contributing factor for the reduced pH of the cecal contents [20].
238 A significant increase in the population of *Ruminococcus productus* was observed in the
239 cecal contents of the rats fed with DFAIII that negatively correlated with the reduction in
240 DFAIII excretion in the feces [20]. On the other hand, *R. productus* was undetected in control
241 rats. These results suggest that DFAIII is consumed by, and contributes to the growth of, *R.*
242 *productus*. *R. productus* was shown to assimilate DFAIII under in vitro conditions. Almost all
243 BAs of the feces in control rats were secondary BAs but those in DFAIII-fed rats were
244 primary BAs [20], suggesting that the altered gut microbiota by DFAIII ingestion had less
245 ability to perform 7 α -dehydroxylation.

246 Deconjugation of BA is catalyzed by BSHs in the intestinal bacteria that hydrolyze the
247 amino bond and liberate taurine or glycine moiety from the steroid ring of BA [8]. In general,
248 an enormous decrease in conjugated BA levels was observed in the large intestine after
249 deconjugation; these deconjugated BAs were converted into secondary BAs in the subsequent
250 dehydroxylation step [24]. High concentration of TCA was observed in the large intestine of
251 the rats fed with CA and DFAIII, wherein BAs underwent 7 α -dehydroxylation by the gut
252 microbiota. The suppression of BA deconjugation reaction by DFAIII ingestion was
253 responsible for the subsequent reduction in secondary BA production.

254 A decrease in body weight was observed in the rats fed with DFAIII supplementation as
255 compared to those fed with DFAIII-free diets. As mentioned before, an increase in acetic acid
256 level was observed in the gut that served as a potent stimulator to secrete anorexic gut
257 hormones such as glucagon-like peptide-1 and peptide YY [28]. It was recently revealed that
258 acetic acid reduces appetite via direct influence on hypothalamus in the brain without
259 induction of these gut hormones [29]. In both cases, acetic acid is one of the candidates that
260 suppressed appetite in the rats fed with DFAIII, resulting in the reduction in body weight.
261 Furthermore, we observed a different influence of CA supplementation on BA concentrations
262 in portal and aorta blood. CA supplementation increased portal BAs but not aorta BAs. The
263 absorbed BAs in the ileal epithelia entered the enterohepatic circulation, as reflected in the
264 BA profiles of the portal blood. The supplemented CA also enters this route rather than the
265 systemic blood flow (aortic blood). This observation suggests that the effect of CA
266 supplementation on the host may be restricted in the organs related to enterohepatic
267 circulation. We observed that CA supplementation increased the gut permeability, but
268 DFAIII intervention had no significant influence on permeability (Table 2). DCA is reported
269 to disrupt the gut barrier in the large intestine [30] and cultured epithelial cells [31].
270 Regardless of the significant reduction in DCA concentration observed in the cecal contents

271 and feces following DFAIII supplementation in the present study, no influence was observed
272 on gut permeability following the CA supplementation (Table 2). In the rats fed with CA-
273 supplemented diet, an increase in DCA or TCA was found in cecal contents or ileal contents,
274 respectively (Fig. 3). TCA might induce leaky gut in small intestine, not in the large intestine.

275 In our previous study, we found that BA metabolism especially in the gut induced after CA
276 supplementation (0.5 g/kg) resembles that observed in the rats fed with a high-fat diet,
277 suggesting that the consumption of energy-dense diet may selectively increase DCA levels in
278 the large intestine [11]. Such increases in DCA levels in the stool were reported in the patient
279 with colorectal cancer, suggestive of the link between DCA production in the large intestine
280 and cancer development in the colon [32]. Only limited data are available on conjugated BAs
281 in feces. Under normal conditions, almost no conjugated BAs were observed in the stool of
282 humans and feces in experimental animals [33,34]. In our previous study, we measured the
283 levels of conjugated BAs in the feces of rats fed with soy pulp and *Bacillus coagulans* and
284 found that conjugated BAs were absent in the feces [35]. It may be rare to observe fecal-
285 conjugated BAs following dietary intervention, as observed in the present study. Therefore,
286 dietary DFAIII may be a prebiotic that reduces secondary BA concentration via suppression
287 of 7 α -dehydroxylation and deconjugation.

288 In conclusion, DFAIII altered BA metabolism, especially in the gut, through the
289 suppression of deconjugation and 7 α -dehydroxylation, which results in the reduction in DCA
290 level in the large intestine probably through the modulation of gut microbiota. Hence, DFAIII
291 may serve as a prebiotic source that reduces secondary BA production.

292

293 **Author contribution**

294 S.I., T.N., H.S., S.F, and A.Y. designed the experiments. DG.L., S.H., O.K., S.K., R.Y., Y.T.,
295 K.T., S.F. and S.I. performed experiments. All authors discussed the data. DG.L. and S.I.
296 wrote the paper.

297

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301

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- 389

390 Figure captions

391 ***Fig. 1. Feeding schedule***

392 After acclimation for 1 week, the rats were divided into two groups, including those fed with
393 control diet (C) and DFAIII-supplemented diet (D) for 2 weeks. Rats from each group were
394 further divided into two groups and fed the diet supplemented with CA (CB and DB) or
395 without CA (C and D) (n = 8 each) for another 2 weeks.

396

397 ***Fig. 2. Changes in fecal DFAIII excretion in the rats fed with DFAIII with or without CA***
398 ***supplementation***

399 The proportion of fecal DFAIII following DFAIII supplementation was calculated in D (open
400 circle, n = 8) and DB (closed circle, n = 8) groups. Asterisk represents significant differences
401 in the values between groups at the same time point ($P < 0.05$).

402

403 ***Fig. 3. Distribution of BAs in the rats fed with four different diets at the end of the***
404 ***experimental period***

405 BA concentrations were measured in the aorta plasma, portal plasma, liver, ileal contents,
406 cecal contents, and feces of the rats fed with four different diets. Values are the mean \pm SEM
407 (n = 7 or 8 rats per group). Two-way ANOVA was used to determine significant differences
408 ($P < 0.05$).

Table 1. Diet compositions

	C	CB	D	DB
	g/kg diet			
Corn starch ^a	397.5	397.5	367.5	367.5
Casein ^b	200	200	200	200
Dextrin ^c	132	132	132	132
Sucrose ^d	100	99.5	100	99.5
Soybean oil ^e	70	70	70	70
Crystalline cellulose ^f	50	50	50	50
Mineral mixture ^g	35	35	35	35
Vitamin mixture ^h	10	10	10	10
L-Cystine	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5
Cholic acid	-	0.5	-	0.5
DFAIII	-	-	30	30

C, control diet; D, DFAIII-supplemented diet; CB, control diet with CA; DB, DFAIII-supplemented diet with CA.

^a Amylalpha, Chuoshokuryo, Japan

^b NZMP Acid Casein (Fonterra Co-Operative Group Limited, Auckland, New Zealand),

^c TK-16 (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan)

^d Nippon Beet Sugar Manufacturing Co., Ltd., Tokyo, Japan

^e J-Oil Mills, Inc., Tokyo, Japan

^f Crystalline cellulose (Ceolus PH-102, Asahi Kasei Chemicals Corp., Tokyo, Japan)

^g AIN93G mineral mixture [21]

^h AIN93 vitamin mixture (MP Biomedicals, USA) [21]

Table 2. Growth and other parameters in the rats

	C	CB	D	DB	Two-way ANOVA <i>P</i> -value		
					BA (B)	DFAIII (D)	B × D
Growth parameters (g)							
Total food intake	423 ± 8	407 ± 9	390 ± 4	386 ± 9	NS	0.0050	NS
Final body weight	221 ± 5	217 ± 6	200 ± 7	201 ± 3	NS	0.0003	NS
Tissue weight (g/100g body weight)							
Liver	4.28 ± 0.07	4.36 ± 0.09	4.39 ± 0.14	4.72 ± 0.14	0.0490	NS	NS
Epididymal adipose	2.01 ± 0.09	2.10 ± 0.11	1.80 ± 0.11	1.59 ± 0.11	NS	<0.0001	NS
Whole cecum	1.34 ± 0.04	1.50 ± 0.08	2.68 ± 0.19	2.55 ± 0.13	NS	<0.0001	NS
Cecal contents	1.12 ± 0.04	1.25 ± 0.08	2.26 ± 0.17	2.12 ± 0.12	NS	<0.0001	NS
Other parameters							
pH in cecal contents	6.65 ± 0.22	7.06 ± 0.16	6.23 ± 0.20	5.78 ± 0.09	NS	<0.0001	0.0224
Urinary Cr excretion (%)	2.54 ± 0.23	4.51 ± 0.53	2.71 ± 0.39	3.65 ± 0.28	0.0006	NS	NS

Values are shown as mean with SEM (n=8).

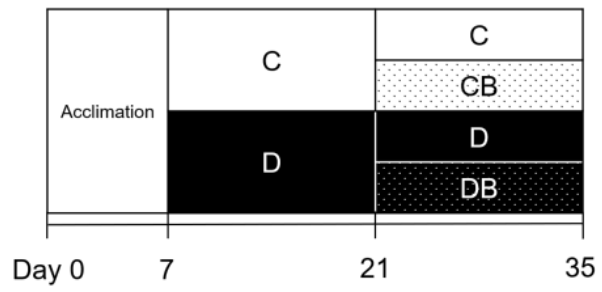


Fig. 1.

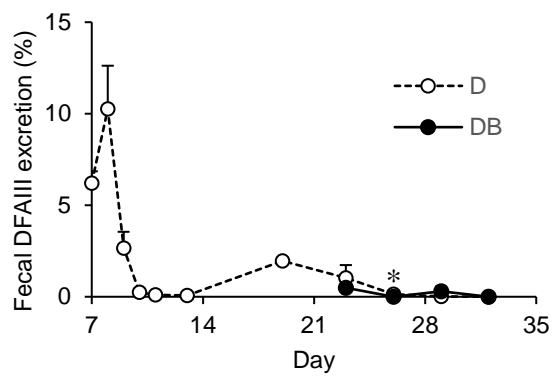


Fig. 2.

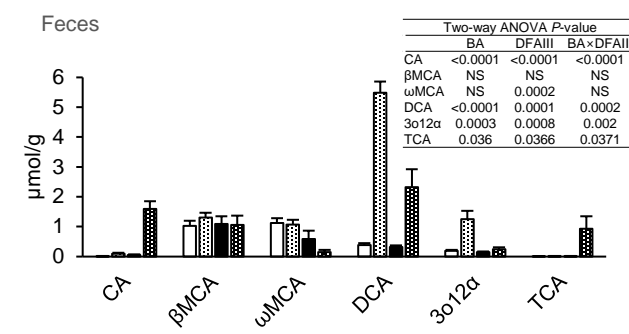
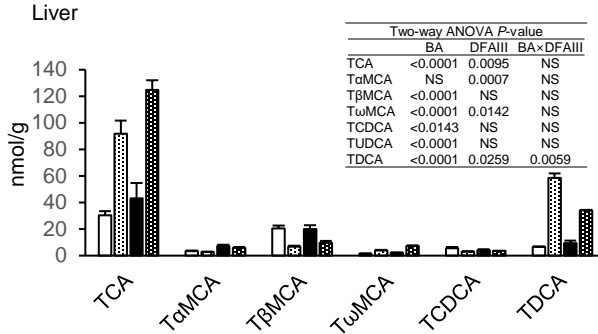
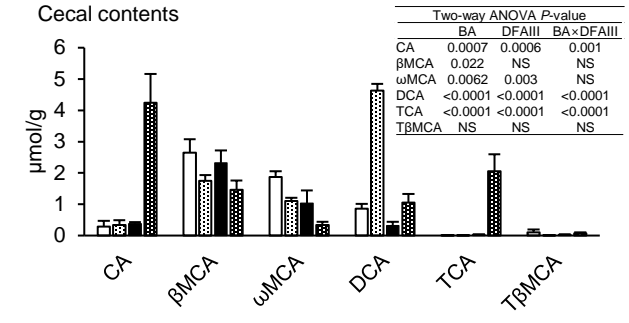
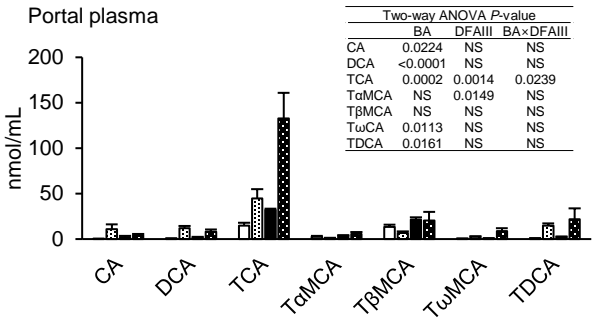
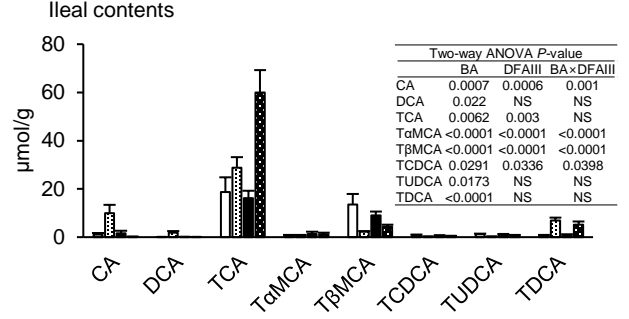
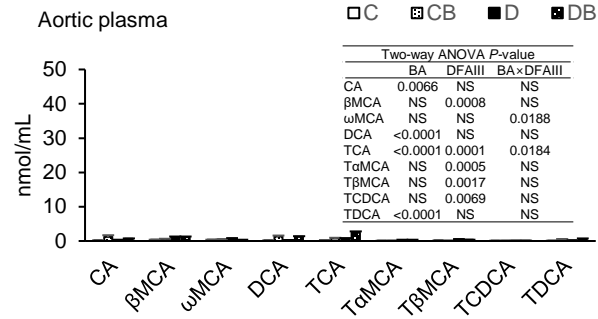


Fig. 3.