Original Article

Metabolic effects of D-psicose in rats: studies on faecal and urinary excretion and caecal fermentation

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D-Psicose (D -*ribo*-2-hexulose), a C-3 epimer of D-fructose, is one of the "rare sugars" present in small quantities in commercial carbohydrate complex or agricultural products. We investigated the absorption and excretion of D-psicose when orally administrated (5g/kg body weight) to Wistar rats, and the fermentation of D-psicose was measured as caecal short-chain fatty acids (SCFAs) when fed to rats in controlled diets (0, 10, 20 and 30%). Urinary and faecal excretions of D-psicose over the 24 h, following a single oral administration, were 11-15% of dosage for the former and 8-13% of dosage for the latter. Serum D-psicose concentration and D-psicose in the contents of stomach and small intestines decreased progressively after administration. D-psicose in caecum contents was detected after 3 h and 7 h administration, but not after 1 h. Rats fed on D-psicose diets showed short-chain fatty acid production with caecal hypertrophy. These results suggest that D-psicose is partly absorbable in the digestive tract and is excreted into urine and faeces. As with other poorly absorbed dietary carbohydrates, D-psicose is fermented in the caecum by intestinal microflora.

Key words: D-psicose, D-fructose, fermentable sugars, short chain fatty acids, faecal/urinary excretion, fermentation, Japan

Introduction

D-Psicose (D -*ribo*-2-hexulose), a C-3 epimer of D -fructose, is one of the "rare sugars" present in small quantities in commercial mixtures of D-glucose and D-fructose obtained from hydrolysis of sucrose or isomerization of D-glucose.[1] D-psicose is also present in processed cane and beet molasses,[2] and is found in wheat,[3] *Itea* plants,[4] and in the antibiotic psicofranine.[5] Because of the very small amounts of D-psicose in natural products, few studies have examined D -psicose metabolism in animals.

Recently, we developed a new method to produce D-psicose enzymatically on a large scale.[6] Moreover, we have suggested that certain D-psicose supplements suppress hepatic lipogenic enzyme activity and reduce intra-abdominal fat accumulation compared to D-glucose or D-fructose in rats.[7] Wistler *et al*.,[8] reported that when D-[U-^{14}C] psicose was orally administrated by stomach tube into fasting rats, 39% of the radioactivity was retained by the carcass for the following 72 h. This finding suggested that a large portion of D-psicose was metabolized by intestinal microorganisms into by-products, some of which would be absorbed into the animal's metabolic system. However, D-psicose metabolism in animal digestive tracts (absorption, excretion, or fermentation) remains largely unknown.

In this study, we investigated the absorption and excretion of D -psicose by high performance liquid chromatograpgy (HPLC) when given orally, and fermentation of D-psicose when fed to rats in controlled diets. We found that in rats D-psicose is a fermentable sugar and that it produces short-chain fatty acids (SCFAs) in the caecum.

Methods

All procedures involving animals were approved by the Experimental Animal Care Committee of the Kagawa University.

Three experiments were conducted using male Wistar rats obtained from Japan SLC (Shizuoka, Japan). Animals were individually housed in a room regulated at a temperature of 24 ± 2˚C and lit from 08:00 to 20:00 h. Rats were fed CE-2, a commercial rodent diet (CLEA, Tokyo, Japan) before experimental treatment.

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Experiment 1: Urinary and faecal excretion of D-psicose after oral administration to rats

Animals and experimental design

Eight rats (5 weeks of age) were fed CE-2 and water ad libitum until 6 weeks of age (body weight, 138±4 g), then fasted for 12 h from 22:00 h. Rats were orally administered D-psicose at a dose of 5 g/kg body weight. Thereafter, urine and faeces were collected at intervals of 24 h for 72 h, with food (CE-2) ad libitum.

Analysis

Urinary and faecal excretion of D-psicose were assayed by HPLC with refractive index detection described previously.[9] Urine samples collected for 24 h were diluted with distilled water to 100 mL, and 0.5 mL of the internal standard solution (0.05% L-sorbose) and 1.5 mL of 99% ethanol were added to 0.5 mL of diluted sample, then mixed well and centrifuged (10,000g, 15 min) (A). Faecal samples collected for 24 h were freeze dried, and 6 mL of distilled water and 3 mL of 0.2% L-sorbose solution as internal standard were added to 0.1 g of dried sample, then homogenized and centrifuged (10,000g, 15 min). The supernatant (0.7 mL) was added to 1.3 mL of 99% ethanol, and then mixed well and centrifuged (10,000g, 15 min) (B). The supernatant of (A) or (B) was aspirated to discard ethanol and then filtrated to membrane (0.22 μm). D-psicose concentration was determined by HPLC (Shimazu model LC 10 A, Shimazu Ltd., Japan) under the following conditions: packed column (Shodex SUGAR SP0810, 30 cm X 8mm, Showa Denko Ltd., Japan); sample volume, 10 μL; mobile phase, distilled water; flow rate, 1.0 mL/min; column temperature, 80˚C.

Experiment 2: Absorption from digestive tracts of D-psicose after oral administration to rats

Animals and experimental design

Eighteen rats (5 weeks of age) were fed CE-2 and water ad libitum until 6 weeks of age (body weight, 140 ± 4 g), then fasted 12 h from 22:00 h. Rats received orally 5 g/kg body weight of D-psicose. Six rats were killed by decapitation each time at 1h, 3h and at 7 h after oral administration. Blood was collected to obtain serum. The stomach, small intestine and caecum were quickly removed, and residual food was collected. D-psicose in serum and in the digestive tracts (stomach, small intestine and caecum) was determined as described in experiment 1. For serum pre-treatment, serum (0.5mL) was added to 0.5mL of 0.05% L-sorbose solution and 1.5mL of 99% ethanol, then mixed well and centrifuged (10,000g, 15min). The pre-treatment of contents in the digestive tract was the same as that of faeces (see Experiment 1).

Experiment 3: Fermentation of D-psicose in the caecum of rats fed several levels of D-psicose in diets

Animals and diets

Twenty-six rats (3 weeks of age) were randomized into four groups and fed CE-2 and water ad libitum until 4 weeks old (body weight, 76 ± 2 g). Rats were fed a synthetic high carbo-hydrate diet including 5% corn oil, 0, 10, 20 or 30% D-psicose and 65, 55, 45 or 35% corn starch (Table 1). D-psicose was prepared from D -fructose by immobilized D-tagatose 3-epimerase. Vitamin and mineral mixtures based on AIN-76A were used.[10,11] Butylated hydroxytoluene (0.01g/ kg diets) was added to all diets as antioxidants.

Experimental design

Each group of rats was given free access to the 0, 10, 20 or 30% D-psicose diets and water for 34 days. After 34 days of the experimental diet, the rats were fasted overnight (12 h) and killed by decapitation. The caecum was quickly removed and residual food was collected. Caecal weight, surface area and caecal content weight were measured.

Table 1. Composition of experimental diets[1]

Groups	0% D-psicose	10% D-psicose	20% D-psicose	30% D-psicose
Ingredients		*(g/kg diets)*		
Casein	200.0	200.0	200.0	200.0
DL-Methionine	3.0	3.0	3.0	3.0
Corn starch	650.0	550.0	450.0	350.0
D-Psicose	0.0	100.0	200.0	300.0
Cellulose	50.0	50.0	50.0	50.0
Corn oil	50.0	50.0	50.0	50.0
Vitamin mixture[2]	10.0	10.0	10.0	10.0
Mineral mixture[2]	35.0	35.0	35.0	35.0
Choline chloride	2.0	2.0	2.0	2.0
Total	1000.0	1000.0	1000.0	1000.0

[1]Butylated hydroxytoluene (0.01 g/kg diets) was added to all the diets as antioxidants; [2]Based on AIN-76A.

Analysis

Caecal SCFAs were analyzed by a method described by Demigne *et al*.,[12] and Younes *et al.*[13] Briefly, 1 mL of the internal standard solution (5.874 μmoL crotonic acid/mL of 0.01 M NaOH) was added to 0.2 g of sample, then homogenized and centrifuged (10,000g, 15 min). The supernatant (0.7 mL) was collected, extracted with chloroform and centrifuged. An aliquot of the aqueous layer (0.3 mL) was diluted with equal volume of 100g/L H_3PO_4 solution and SCFA concentration was determined by gas chromatography (Hitachi model 163 gas chromatograph, Hitachi Ltd., Japan)

under the following conditions: glass column (3 mm X 2,000 mm) packed with Unisole F-200, 30/60 mesh (GL Sciences, Japan); sample volume, 1 μL; carrier gas, nitrogen; flow rate, 32 mL/min; injection temperature, 170˚C; column temperature, 140˚C. Results were expressed in terms of SCFAs, which were acetic, propionic and butyric acids.

Statistical analysis

All data were analyzed by a factorial analysis of variance (ANOVA) and Scheffe's post hoc test was used to identify significant differences. Correlation of caecal weight or surface area versus caecal SCFAs was calculated using Pearson product-moment correlation coefficients.

Results

Experiment 1

Urinary and faecal excretion of D*-psicose*

Urinary and faecal excretion of D-psicose over the 24 h following a single dosage (5g/kg body weight) were 11-15% of dosage for the former and 8-13% of dosage for the latter (Fig. 1). D-psicose was not detected in urine and faeces collected 24-48 h and 48-72 h after administration (Fig. 1).

Experiment 2

Serum D*-psicose concentration and residual* D*-psicose in digestive tracts*

Serum D-psicose concentration decreased progressively after administration (Fig. 2). D-psicose concentration decreased quickly in blood 1 h after administration (Fig. 2). D-Psicose in stomach was higher at 1 h after administration than at 3 h (Fig. 2). At 7 h after administration, it was not detected in stomach (Fig. 2). D-psicose in stomach was 26-37% and 0.4-0.6% of dosage after 1 and 3 h, respectively. D-psicose in the small intestine was 6-10%, 2-3% and 1-3% of the dosage after 1, 3 and 7 h, respectively (Fig. 2). D-psicose in the caecum was detected after 3 and 7 h (Fig. 2). It was 11-18% and 10-19% of the dosage after 3 and 7 h, respectively.

Experiment 3

Body weight gain, food intake, food efficiency and caecal size

Body weight gain, food intake and food efficiency (body weight gain/food intake) decreased with increasing amounts of D-psicose in the diets (Table 2). Caecal weight and caecal surface area increased with increasing amounts of D-psicose in the diets, whereas caecal density did not differ among the groups (Table 2). The SCFAs acetic, propionic and butyric acids in the rat caecum increased with increasing amounts of D -psicose in the diets (Fig. 3).

Correlation between caecal weight or surface area and caecal SCFAs

Acetic acid, propionic acid and butyric acid in the caecum were positively correlated to caecal weight or surface area (Fig. 4), i.e., the higher the SCFAs in the caecal contents, the greater the caecal weight and surface area.

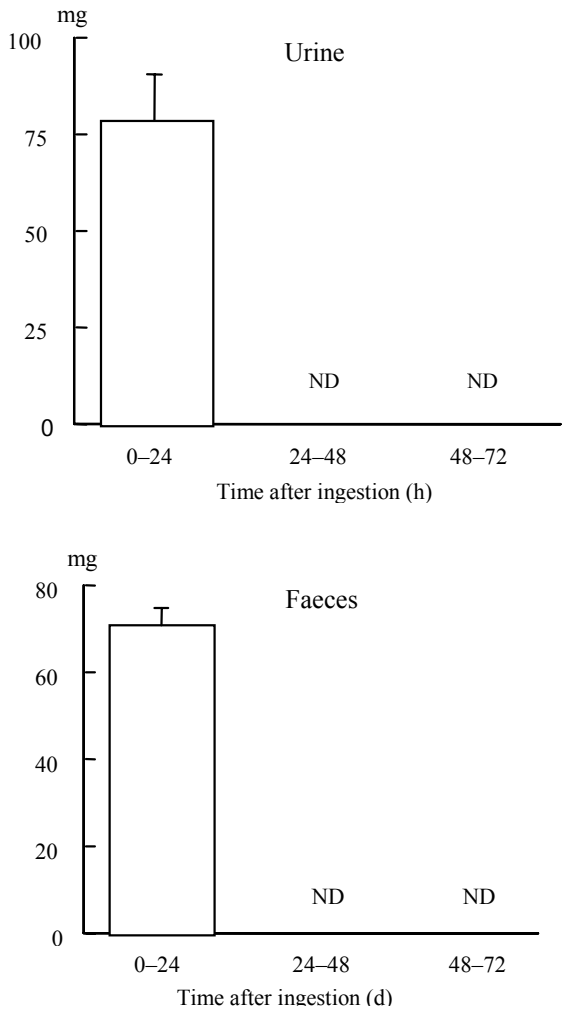

Figure 1. D-Psicose excretion in urine and faeces of rates after 5g/kg body weight of D-psicose administration. Values are means and SEM for rats. ND = not detected

Discussion

The present study demonstrates that D-psicose is partly absorbable in the digestive tract and is excreted into urine and faeces. These findings are in good agreement with the results obtained by Wistler *et al.*,[8] who administered D-[U-^{14}C] psicose to rats. In this study, we suggest that D-psicose is a fermentable saccharide because of the SCFA production in the caecum. Moreover, we showed that SCFAs in caecal contents correlated to caecal weight or surface area, which is similar to that of soluble dietary fibre.[14]

After oral administration of D-psicose, it disappeared from the stomach, small intestine and serum in that order in 1-7 h. D-Psicose in the caecum did not decrease even 7h after administration. Most of the D-psicose absorbed was rapidly excreted in the urine, where 11-15% of dosage was recovered within the first 24 h. Unfermented D-psicose was rapidly excreted in faeces, where 8-13% of dosage was recovered within the first 24 h. These results suggest that a large percentage of D-psicose administered remains in the rat body or is oxidized by the animal's metabolic system. Recently, we

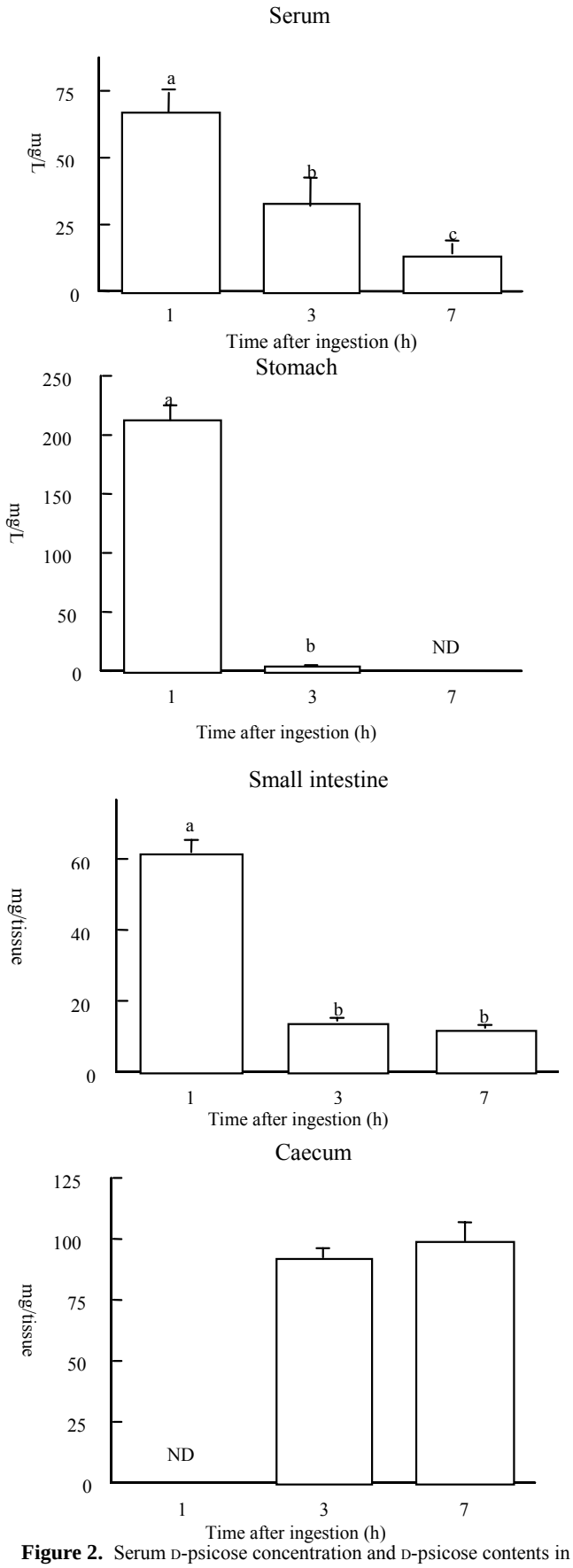

Figure 2. Serum D-psicose concentration and D-psicose contents in stomach, small intestine and caecum of rats after 5g/kg body weight of D-psicose administration. Values are means and SEM for 6 rates. ND = not detected. Values with different superscripts are significantly different (P<0.05, ANOVA and Scheffe's Tests)

Table 2. Body weight gain, food intake, food efficiency and caecal size of rats fed the D-psicose diets for 34 days[1]

Groups		0% D-psicose	10% D-psicose	20% D-psicose	30% D-psicose
Number of rats	(n)	6	7	7	6
Body weight gain	(g)	123 ± 4^{a}	101 ± 2^{b}	81 ± 4^{c}	42 ± 5^{d}
Food intake	(g)	435 ± 6^{a}	422 ± 3^{b}	367 ± 7^{c}	261 ± 15^{d}
Food efficiency	(mg/g)	283 ± 8^{a}	239 ± 4^{b}	221 ± 8^{c}	161 ± 12^{d}
Caecal weight	(g)	0.40 ± 0.02^{c}	0.64 ± 0.04^{c}	1.11 ± 0.16^{b}	$1.92 \pm .23^{a}$
	(%)[2]	0.20 ± 0.01^{c}	0.34 ± 0.03^{c}	0.70 ± 0.08^{b}	1.61 ± 0.20^{a}
Caecal surface area	(cm^2)	13.0 ± 0.6^{d}	22.4 ± 1.1^{c}	36.4 ± 1.5^{b}	53.0 ± 3.5^{a}
Caecal density	(g/cm^2)	17.7 ± 1.2	17.4 ± 1.5	15.7 ± 1.9	17.3 ± 1.5
Caecal contents	(g)	2.28 ± 0.16^{c}	3.85 ± 0.25^{c}	5.70 ± 0.68^{b}	9.08 ± 0.82^{a}
	(%)[2]	1.15 ± 0.09^{c}	2.16 ± 0.11^{c}	3.58 ± 0.40^{b}	7.67 ± 0.84^{a}

[1]Values are means ± SEM. Values in a row followed by distinct letters are significantly different (P<0.05).
[2]Calculated by dividing caecal weight or caecal contents weight by final body weight.

demonstrated that D-psicose provides no energy to growing rats.[15] Therefore, most of the D-psicose remaining in the rat body is probably fermented by bacteria in the caecum or large intestine (not shown in this study). Another possibility is that D-psicose is exchanged for glycogen in liver or muscles. A previous study demonstrated that a small percentage of D-psicose was exchanged for liver glycogen 6h after intraveneous injection.[8]

In experiment 3, caecal weight and surface area were greater in order of percentage of D-psicose in the diet. This finding is consistent with the results of Konishi *et al.*,[16] who reported that galactomannan, a soluble dietary fibre, increased caecal weight, whilst cellulose did not. We found that D-psicose increased caecal weight and surface area in a manner similar to a soluble dietary fibre. Moreover, we found that SCFAs in caecal contents were positively correlated to caecal weight or surface area, which showed that D-psicose is fermented in the caecum by intestinal microflora, producing SCFAs, similar to other soluble dietary fibres or polydextrose.[17,18] On the other hand, body weight gain, food intake and food efficiency decreased with D-psicose in diets. Calculated D-psicose intake was 0, 42.2, 73.4, or 78.3 g/34 days in the 0, 10, 20 or 30% D-psicose diet groups, respectively. These results suggest that a large consumption

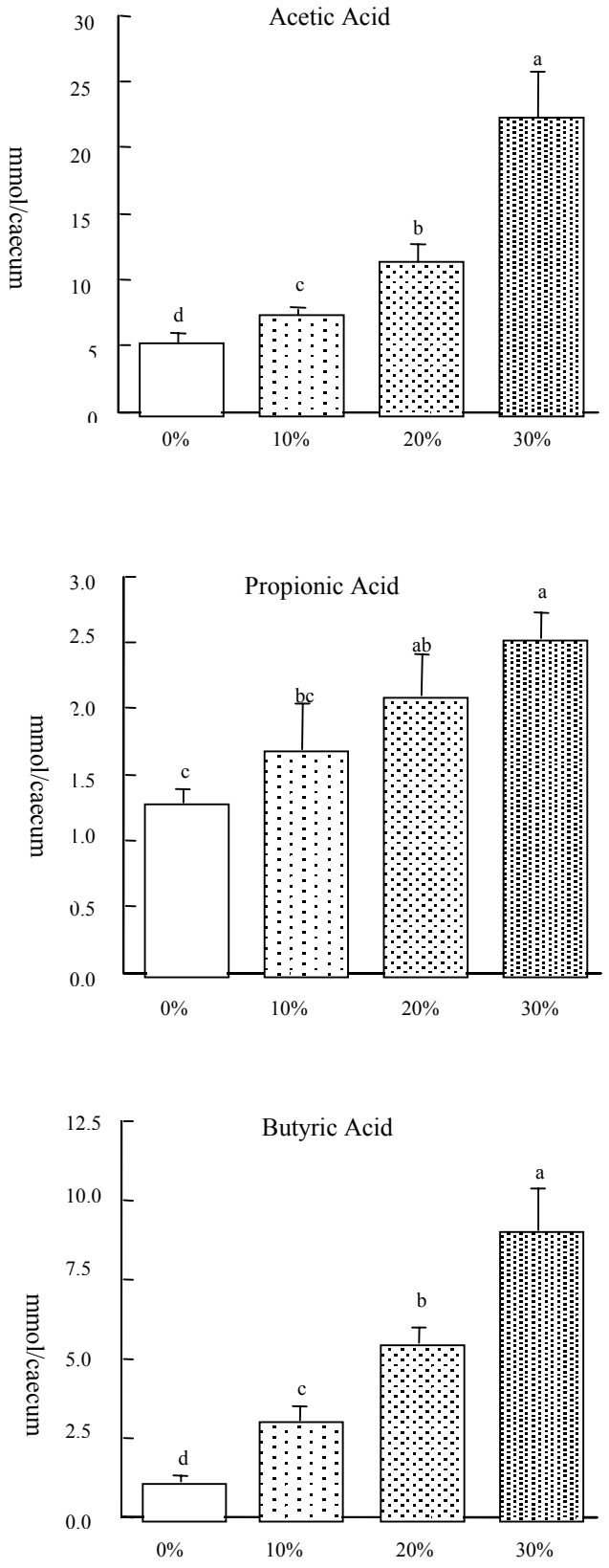

Figure 3. Caecal contents of acetic, propionic and butyric acids of rats fed several levels of D-psicose. Values are means and SEM for 6-7 rats. Values with different superscripts are significantly different (P<0.05, ANOVA and Scheffe's tests).

of D-psicose could inhibit rat growth and cause caecal hypertrophy, which could be harmful to the intestinal tract, as generally observed when dietary fibres are fed in large quantities.[14,19,20] However, rats seem able to adapt to some extent to the D-psicose diet, since rats fed on 30% D-psicose showed positive food consumption, body weight gain and food efficiency during the experimental period. In this study, diarrhoea was observed in only the 30% D-psicose group and only for the first 7 days (data not shown).

In conclusion, D-psicose is partly absorbable in the digestive tract and is excreted into urine and faeces. D-psicose is fermented in the caecum by intestinal microflora, producing SCFAs similar to soluble dietary fibres. In addition, the feeding of diets extremely high in D-psicose seems to be harmful to the intestinal tract, indicating that there must be reservations about its use as a dietary fibre-like substance or sugar substitute in food manufacturing.

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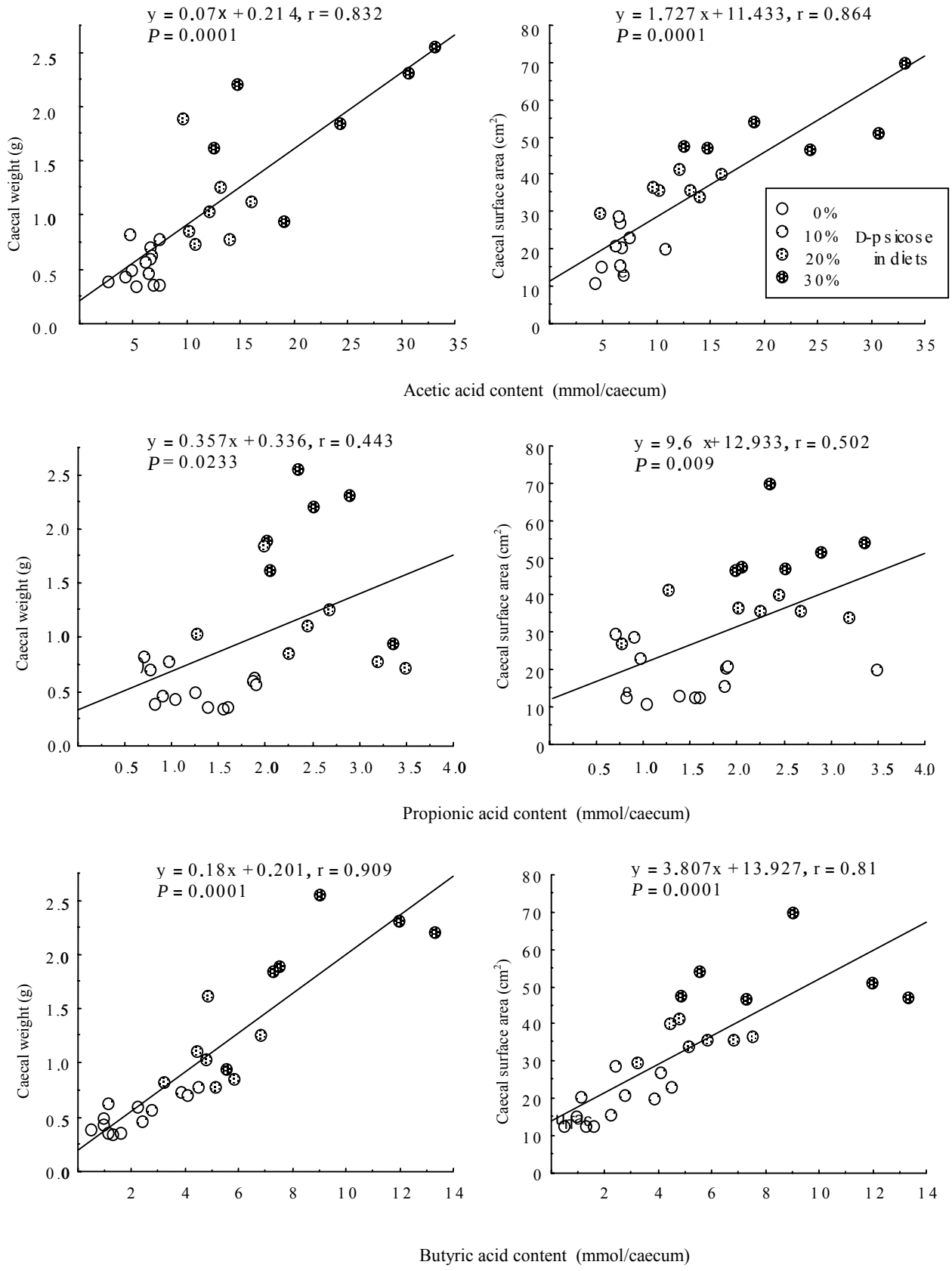

Figure 4. Correlation of caecal weight or surface area versus caecal contents of acetic, propionic and butyric acids of rates fed several levels of D-psicose.

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