Energy Value of a Mixed Glycosidic Linked Dextrin Determined in Rats

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A series of in vivo and in vitro experiments was conducted to determine the available energy of a bulking agent, Fibersol 2 (FS-2). Fibersol 2 is obtained by a combination of heat and enzyme treatments of cornstarch to produce a low-viscosity, low-digestible dextrin with an average molecular massweight of 2000 Da. Although the substance is of the type known in Japan as an indigestible dextrin, it also comes within the U.S. Food and Drug Administration's (FDA) definition of "maltodextrin" as found in the FDA Generally Recognized as Safe (GRAS) affirmation regulations. Chemical analysis shows FS-2 to contain glucopyranosyl units with 1,6-anhydro-β-D-glucose (levoglucosan) at some of the reducing terminals. Linkages in the molecule are randomly distributed among units consisting of α- and β-(1→4), (1→6), (1→2), and (1→3) glycosidic bonds. In vitro digestion of FS-2 with successive treatments of salivary α-amylase, a gastric juice preparation, pancreatic α-amylase, and intestinal mucosal enzymes gave 89.8% recovery of the starting material. Rats gavaged with FS-2 were found to have only a 5% increase in plasma glucose concentrations over 120 min compared to rats administered an equal amount of glucose. Approximately 38% of FS-2 administered to rats by gavage is recovered in the feces. Growth rates of rats fed FS-2 indicate less than <10% of the dextrin is contributing net metabolizable energy. FS-2 has an energy value of 2.2 kJ/g.

Keywords: *Dextrin; maltodextrin; bulking agent; energy and; rats*

INTRODUCTION

Indigestible dextrins have a wide application in the manufacture of foods and drugs (*Food Ingredient Reference Book*, 1995). One important use is as bulking agents. Use of the term "bulking agent" can include any mono, di-, oligo-, or polysaccharide, providing either no or a significant reduction in energy after ingestion compared to a totally digestible and metabolized carbohydrate. Bulking agents have lower molecular mass compared to carbohydrate fat mimetics (Glicksman, 1991). There is no rule or standard on the level of energy reduction that should be provided by a bulking agent to meet food product or regulatory specifications. A bulking agent with zero energy is ideal, and two examples have been reported (Livesey and Brown, 1995, 1996). There are many available bulking agents with different properties, and each is obtained from a different source or produced by a different method (Deis, 1994). The application and acceptance by food processors and consumers of these bulking agents in foods is dependent on knowing their net metabolizable energy content.

Dextrins result from the treatment of commercially available starches with heat. They have been found to have a well-developed branch structure more complex than that of starch (Ohkuma et al., 1990) and resistant to digestion (Evans and Wurzburg, 1967). The process of pyrolysis, which also produces hydrolysis, causes reducing end groups to undergo intramolecular dehydration, leading to the formation of new glycosidic bonds with other hydroxyl groups. These molecular rearrangements can lead to the formation of α and β-(1→2) and α and β-(1→3) glycosidic linkages in addition to some changes to β configuration of existing α-(1→4) and α-(1→6) bonds in starches (Bryce and Greenwood, 1963).

Various in vivo methods are available for measuring the net metabolizable energy of saccharides and other food ingredients intended to reduce the energy content of foods. Briefly stated, they include, but are not limited to, (1) recovery of the test substance or its products from the terminal intestine or expired air, (2) whole body calorimetry, (3) comparative analysis of carcass composition, (4) comparative analysis of growth rates, and (5) measurement of $^{14}CO_2$ resulting from the metabolism of radionuclide labeled starting material in expired air. Each method has its advantages and disadvantages (Hobbs, 1987; Oku, 1990).

Fibersol 2 (FS-2) is the commercially available dextrin evaluated in this study. It is accepted in Japan as an indigestible dextrin (Tsuji, 1995). It is a nonsweet saccharide polymer that consists of D-glucose units linked with α and β glycosidic bonds. It is produced under conditions to have it qualify as a Generally Recognized as Safe (GRAS) maltodextrin in the United States (*Maltdodextrins*, 1996). FS-2 is not a homogeneous polymer, and, for this reason, no one method is totally applicable for determining its energy content. FS-2 is being used as a bulking agent and source of dietary fiber for a new meal program. This total meal

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Table 1. Consecutive Hydrolytic Conditions Used To Evaluate the Digestibility of Fibersol 2 and Pinedex 3[a]

enzyme system	dosage[b]	buffer system	sample[c]/buffer ratio, %	temp, °C	time, min	carbohydrate analysis
salivary α-amylase (human)	160	45 mmol/L Bis-Tris, 0.9 mmol/L $CaCl_2$, pH 6.0	4.55	37	30	Somogyi (1945)
artifical gastric juice		16.7mmol/L HCl−KCl, pH 2.0	0.73	37	100	Somogyi (1945)
pancreatic α-amylase (porcine)	400	45 mmol/L Bis-Tris, 0.9 mM $CaCl_2$, pH 6.6	0.45	37	360	Somogyi (1945)
intestinal mucosa (rat)	86	45 mmol/L sodium malate, pH 6.6	0.45	37	180	Miwa et al. (1972)

[a] Digestibility sequence conducted as described by Okada et al. (1990). [b] Enzyme activity in glucose units, mmol, released from a 1% starch solution per minute per milligram enzyme. [c] FS-2 or PD-3.

program has been shown to have beneficial effects in helping people to manage high blood pressure, plasma cholesterol, and glucose (McCarron et al., 1997).

The difficulty in determining the net metabolizable energy available from any bulking agent resides in the fact that many compounds are fermented in the large intestine. There is debate as to the amounts of energy lost in intestinal fermentation and the actual amounts of energy available from the main products of fermentation, the short-chain fatty acids (SCFA) (Gibson and Macfarlane, 1989). The objective of the present study was to determine the net metabolizable energy value of FS-2. A combination of methods was used to achieve this objective. Methods employed in this study were (1) determining its digestibility with a series of in vitro enzyme digestions, (2) estimating the amount digested and absorbed effecting blood glucose levels, (3) a balance method to measure the amount excreted after a single dose, and (4) the growth rate in rats.

MATERIALS AND METHODS

Materials. FS-2 is a product of Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan). Cornstarch is treated with suitable acids, enzymes, and heat in a process resulting in a saccharide of ≈2000 Da. A pure concentrated solution or dry powder can be prepared, and the latter was used in this study. Methylation and subsequent gas−liquid chromatography analysis shows the polymer to have a random distribution of α and β-(1→4), (1→6), (1→2), and (1→3) glycosidic bonds (Hakomori, 1964). The molecule consists of all glucopyranosyl subunits with the one exception that some of the reducing ends are 1,6-anhydro-β-D-glucose (levoglucosan). FS-2 has a dextrose equivalent (DE) of 8.0 and a total dietary fiber (TDF) value of 42% as determined by the method for TDF (Prosky et al., 1992). FS-2 meets GRAS requirements as set forth in 21 CFR 184.1444 (*Maltodextrins*, 1996).

In Vitro Digestion. FS-2 was compared to a maltodextrin, Pinedex 3 (PD-3) produced by Matsutani Chemical Industry Co. Ltd. PD-3 has a DE of 25 and a TDF value of 3%. Four digestion systems were employed in sequential manner on each saccharide to mimic digestion in the mouth, stomach, and small intestine. These were as follows: (1) oral, salivary α-amylase, human, Type IX-A, Sigma, St. Louis, MO; (2) stomach, artificial gastric juice, 16.7 mmol/L HCl−KCl, pH 2.0; (3) proximal small intestine, pancreatic α-amylase, porcine, Boehringer Mannheim, Germany; and (4) small intestine, dried and reconstituted intestinal mucosa, Sigma. The conditions employed for these digestions are reported in Table 1 and have been described elsewhere (Okada et al., 1990).

This sequence of digestions was intended to simulate the in vivo digestion process. After each digestion, salts were removed from the digested and hydrolyzed solutions by mixed bed ion exchange chromatography (Amberlite 200C, H type, and Amberlite IRA-68, OH type, Organo, Japan). Solutions were concentrated by freeze-drying. The increase in reducing equivalents (Somogyi, 1945) after the first three consecutive digestions was measured to determine percent hydrolysis of the starting material. The final digestion product was measured for glucose concentration (Miwa et al., 1972) and reported as percent total digestion. Duplicate digestions were accomplished. The highest variation between the duplicate final digestions (e.g., intestinal mucosa digestion) for FS-2 varied by 4% for total glucose.

Animals. Male Sprague−Dawley rats (Jel:SD, Japan CLEA, Osaka, Japan) were used for in vivo experiments. The age and corresponding size of rats were different for each animal trial, and the reasons for these differences are presented in the description of each experiment. Upon receipt, all rats were given a standard diet, closed formula (CE-2, Japan CLEA, Osaka, Japan) for specified periods before being placed on each experimental protocol. The proximate composition of the standard diet was 25% protein, 4.5% fat, 7.2% ash, and 63.3% carbohydrate−10% TDF.

Groups of rats were of equal mean weight for each experiment. Rats were individually housed in suspended stainless steel wire cages. Room temperature was maintained at 21 ± 1 °C. A 12 h photoperiod (lights on between 8:00 a.m. and 8:00 p.m.) was employed. All rats were housed and cared for under approved animal care conditions in compliance with the *National Research Council Guide for the Care and Use of Laboratory Animals* (1985).

Oral Test Load. Eighteen rats, 7 weeks old and weighing ≈250 g, were acclimated with the standard diet for 1 week. Animals of this age and size were used to facilitate easier blood drawing and yet not too large to have excess fat accumulation. Food was withheld for 16 h, and the rats were orally administered a 300 g/L solution of glucose, FS-2, or PD-3 at the rate of 1.5 g/kg of body weight. Blood was drawn from the external jugular vein of conscious rats 10 min before oral loading and 30, 60, and 120 min after being gavaged. Plasma glucose concentrations were measured with a Mutarotase-GOD test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan), which measures oxidized *o*-dianisidine (Miwa et al., 1972).

Fecal Excretion. The excretion of two test saccharides were measured in 12-week-old rats weighing ≈400 g to facilitate a larger recovery of feces. Rats were acclimated to the standard diet for 1 week and then divided into three groups, eight rats per group. The first group of rats (control) was orally administered 5.0 mL of water. Rats in the second and third groups were given 5.0 mL of a 400 g/L aqueous solution of FS-2 and 400 g/L aqueous solution of PD-3, respectively. Feces were collected from under each cage on water-absorbing paper for 72 h after administration. Feces were dried and acid hydrolyzed, and total sugars were determined by the phenol−sulfuric acid method of Dubois et al. (1956).

Growth Rate. Three-week-old rats were fed a basal diet for 5 days. Animals of this age are most responsive to energy changes in their diet as employed is this study (Rice et al., 1957). Composition of the basal diet is reported in Table 2. Rats were divided into five groups with an average starting weight of 70.0 ± 3.0 g in each group. This was a restricted feeding protocol executed for 15 days as initially proposed by Rice et al. (1957). Rats in the control group were fed 5.4 g of the basal diet per day. Rats in the second and third groups and in the fourth and fifth groups were fed 5.4 g of basal diet per day plus 0.5 and 1.0 g of glucose or 0.5 and 1.0 g of FS-2, respectively. Diets were given daily at 10:00 a.m. Feed

Table 2. Composition of Basal Diet To Measure Growth in Rats Fed an Indigestible Dextrin (Fibersol 2) and Glucose

component	amount, g	component	amount, g
cornstarch[a]	657	vitamin mix[f]	8
casein[b]	200	DL-methionine[b]	3
corn oil[c]	50	choline bitartrate[b]	2
cellulose[d]	20		
mineral mix[e]	40	total	1000

[a] Nihon Shokuhin Kako Co., Ltd., Tokyo, Japan. [b] Wako Pure Chemical Industries, Ltd., Osaka, Japan. [c] Corn oil, Ajinomoto Co., Inc., Tokyo, Japan, with added 0.01% butylated hydroxytoluene and 0.01% butylated hydroxyanisole. [d] Asahi Chemical Industry Co., Ltd., Osaka, Japan. [e] AIN-76 formulation with starch carrier supplied in g/kg of mineral premix: $CaHPO_4$, 500; NaCl, 74; potassium citrate, 220; K_2SO_4, 52; MgO, 24; $MnCo_3$, 3.5; $ZnCO_3$, 0.16; KIO_3, 0.01; Na_2SeO_3, 0.01; $Cr(SO_4)\cdot 12H_2O$, 0.55; and cornstarch to 1 kg (AIN 1977). [f] Vitamin premix with sucrose carrier supplied (mg/kg of diet): thiamin-HCl, 6; riboflavin, 6; pyridoxine-HCl 7; nicotinic acid, 30; calcium pantothenate, 16; folic acid 2; biotin, 0.2; cyanocobalamin, 0.01; retinyl palmitate, 8; DL-α-tocopheryl acetate, 200; cholecalciferol, 0.025; and menoquinone, 0.05 (AIN 1977).

spillage was weighed, and an equal amount of test diet was given back to ensure each rat consumed its allocated diet per day. Feed consumption was completed within a 2 h period. Rats were given free access to water throughout the experiment. The weights of the rats were determined at the start of the experiment and on days 5, 10, and 15.

The growth rate experiment ended on day 15 with the rats being anesthetized, laparotomized, and killed by cervical dislocation. Rats were not fed on day 15. The cecum was removed and weighed, the contents were removed, and the pH of this material was determined. The pH of 0.1 g of cecum contents was measured after dilution with 1.0 mL of distilled water.

The potential total energy of each diet and FS-2 was determined by bomb calorimetry (1261 adiabatic calorimeter, Parr Instrument, Moline, IL). The available energy provided by FS-2 to rats based on their growth was calculated in the following manner. Weight gain after 15 days of restricted feed intake, with and without cecum weight, was divided by total energy consumption in this period. Cecum weight included the weight of its content. Energy consumption was the product of the measured energy content of the diets provided each day multiplied by 14 days. The quotient values (QV) obtained by dividing weight gain by energy consumption for rats fed 0.5 or 1.0 g of glucose (G) or FS-2 were used in the proportional equation

$$16.7 \text{ kJ/g G}:X \text{ kJ/g FS-2} = \text{QV-G}:\text{QV-FS-2}$$

Solving for X provided the kilojoules per gram of FS-2 in rats fed this indigestible dextrin. Hobbs (1987) originally proposed this calculation based on the original studies of Rice et al. (1957).

Statistics. The statistical differences among groups in each experiment were assessed using Duncan's multiple range test after preliminary ANOVA (Shibata, 1974). Difference between mean values was considered significant at the 5% level ($P \leq 0.05$).

RESULTS

Significantly more FS-2 remained undigested compared to the maltodextrin PD-3 after four consecutive in vitro digestions. Only 10% of the FS-2 was digested compared to 96% of the PD-3 with the four consecutive enzyme systems (Table 3). Salivary α-amylase, gastric juice, and pancreatic α-amylase had little effect on FS-2. These combined enzyme systems resulted in ≈34% digestion of PD-3. Approximately 62% of the PD-3 was digested by the intestinal mucosa suspension compared to ≈8% digestion of the FS-2.

Table 3. Percent Hydrolysis of Individual Digestions and Cumulative Digestion Values of Fibersol 2 and Pinedex 3[a] in Vitro

	Percent Hydrolysis			
	FS-2		PD-3	
enzyme system	single hydrolysis,[b] %	cum hydrolysis,[b] %	single hydrolysis,[b] %	cum hydrolysis,[b] %
saliv α-amylase	0.8 ± 0.0	0.8	11.3 ± 0.1	11.3
isoltd gastr juice	0.4 ± 0.0	1.2	0 ± 0.0	11.3
pancr α-amylase	1.5 ± 0.0	2.7	23.0 ± 0.3	34.3

	Total Digestibility[c]	
	FS-2	PD-3
intestinal mucosa	10.2 ± 0.4	95.9 ± 0.6
indigestible residue[d]	89.8	4.1

[a] Digestibility sequence conducted as described by Okada et al. (1990). [b] Hydrolysis ratio in percent based on increase in reducing equivalents of starting material. [c] Total digstibility ratio in percent of starting material based on release of glucose. [d] Percent starting material not hydrolyzed or digested.

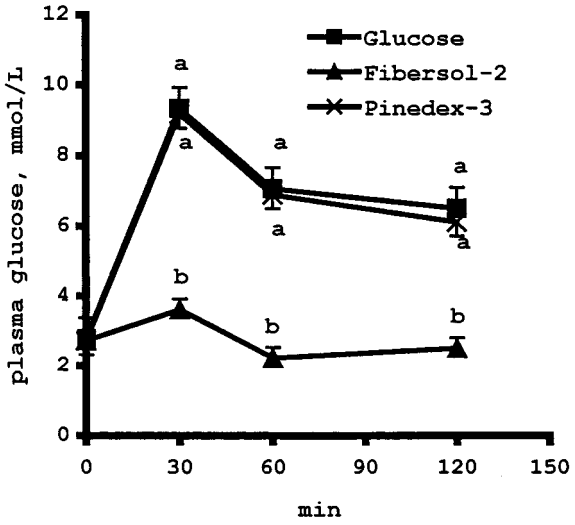

Figure 1. Plasma glucose response, mmol/L, in rats given an oral dose of FS-2, PD-3, or glucose at 0, 30, 60, and 120 min. Plasma glucose concentrations were significantly ($P \leq 0.05$) lower in rats administered FS-2 compared to rats given PD-3 or glucose ($a > b$).

Rats given a glucose bolus by gavage had plasma glucose concentrations reach their highest level, 9.33 ± 0.45 mmol/L, after 30 min (Figure 1). These values declined by ≈30% after an additional 90 min. A similar trend was observed in rats administered the PD-3 (Figure 1). Plasma glucose concentrations in rats given FS-2 remained relatively constant during the 120 min period, with only a small increase after 30 min. This increase in plasma glucose concentration after 30 min was not significantly different from values observed at 0, 90, or 120 min. The total area under the curve in Figure 1 for rats given FS-2 was ≈5% of areas observed in rats administered glucose or PD-3.

The average fecal weights among the three groups of rats during the 72 h period following the oral administration of FS-2 or PD-3 are reported in Table 4. Rats given FS-2 had fecal weights that were 9.3% ($P \leq 0.05$) and 4.9% (NS) higher compared to the two groups of rats administered PD-3 or water, respectively. The amount of fecal sugars excreted by rats given FS-2 was ≈2-fold and significantly ($P \leq 0.05$) higher compared to

Table 4. Fecal Weight and Total Fecal Sugar Recovery from Rats Given 2 g of Fibersol-2 or Pinedex-3

		fecal sugar recovered	
sample[a]	fecal wt,[b] g	g[b]	%[b,c]
control	18.6 ± 0.40[b]	0.45 ± 0.02[b]	
FS-2	20.5 ± 0.10[a]	1.21 ± 0.10[a]	38.3 ±1.0
PD-3	19.5 ± 0.81[ab]	0.42 ± 0.03	<0

[a] Animals received 5.0 mL of a 400 g/L solution of sample. [b] Mean ± SEM, $n = 6$. Means in a column not sharing a superscript are significantly different ($P \leq 0.05$). [c] Percent sugar recovered minus sugar recovered in feces of control animals.

Table 5. Body Weight and Cecum Weight among Groups of Weanling Rats Fed Restricted Amounts of Basal Diet with Added Glucose or Fibersol 2 for 15 Days

	basal diet +				
day	no addition	0.5 g of glucose	1.0 g of glucose	0.5 g of FS-2	1.0 g of FS-2
			Body Weight,[a] g		
1	69.3 ± 1.0	70.0 ± 0.5	69.8 ± 0.7	69.2 ± 0.9	69.3 ± 0.9
5	62.6 ± 1.0[c]	68.5 ± 0.6[b]	76.0 ± 0.6[a]	68.5 ± 1.8[b]	67.6 ± 1.9[b]
10	65.6 ± 0.6[d]	69.9 ± 1.4[c]	87.3 ± 1.0[a]	67.9 ± 1.8[cd]	75.4 ± 1.9[b]
15	68.4 ± 1.2[d]	75.1 ± 1.4[c]	95.1 ± 0.9[a]	72.6 ± 1.4[c]	81.6 ± 1.7[b]
			Cecum Weight,[a] g		
15	1.2 ± 0.1[c]	1.0 ± 0.1[c]	1.1 ± 0.1[c]	3.1 ± 0.2[b]	7.9 ± 0.2[a]
			Cecal pH[a]		
15	8.1 ± 0.0[a]	8.0 ± 0.1[a]	8.0 ± 0.1[a]	7.5 ± 0.1[b]	6.3 ± 0.1[c]

[a] Mean ± SEM, $n = 6$. Means in a row not sharing a common superscript are significantly different ($P \leq 0.05$).

the amounts determined in rats given PD-3 or no polysaccharide. The fecal sugars in rats given a water bolus served as the control level in feces to estimate digestibility as measured by sugar excretion in the FS-2 group of animals. A similar amount of fecal sugars determined in the control animals was expected in the group of rats that were administered PD-3. This suggestion is based on results observed in the in vitro digestion experiment. Subtracting the amount of fecal sugars in the control group from the amount of sugars in rats given FS-2 amounted to 0.76 g/24 h period. This indicates that ≈38% of the orally administered FS-2 was not available for energy and completely indigestible and nonfermentable. Rats in this experiment or the following experiment measuring growth rate showed no signs of diarrhea.

Growth rate in rats fed restricted amounts of the basal diet, 5.4 g/day, decreased after the first 5 days (Table 5). The growth rate of these animals then increased during the next 10 days but only to their weight at the start of the experiment. Among the four groups of animals fed diets with glucose and FS-2, only the group of animals with 1.0 g of added glucose had a constant and positive growth rate for the 15 day experimental period. The final growth rate of these animals was the highest ($P \leq 0.05$) among the four test groups and 14% higher compared to the group of animals fed 1.0 g of added dietary FS-2 per day. Percent increases in weight gain of animals fed restricted diets with 0.5 and 1.0 g of added FS-2 after 15 days were 5% (NS) and 15% ($P \leq 0.05$), respectively. The relative ratios of weight gain in the rats fed diets with an additional 0.5 g of glucose, 1.0 g of glucose, 0.5 g of FS-2, or 1.0 g of FS-2 were 1:5:0.7:2.4, respectively.

The pH of cecum contents was 8.0 in rats fed the basal diet and the two basal diets with added glucose (Table 5). There was a significantly lower ($P \leq 0.05$) cecum pH in rats fed 0.5 and 1.0 g of FS-2 per day by 0.5 and 1.7 pH units, respectively, compared to values observed in the other three groups of rats.

The energy values of the basal diet, glucose, and FS-2 were determined to be 17.9, 16.6, and 16.7 kJ/g, respectively, by bomb calorimetry. A value of 16.7 kJ/g glucose was used in our calculations to calculate the energy value of FS-2 using the proportional equation. On the basis of the total weight gain in rats fed 0.5 and 1.0 g of FS-2 per day, the energy values of FS-2 were calculated to be 11.0 and 8.0 kJ/g, respectively (Table 6). These values are provided but are not correct because weight gain used in the calculations includes the increase in cecum weight and its contents. An increase in digestive organ weight is an example of a supplement-induced energy loss not contributing to net metabolizable energy (Brown and Livesey, 1994). The weight of the cecum and its contents in rats fed the basal diet and the basal diet with added glucose averaged 1.1 g. The cecum weights in rats fed the basal diet and FS-2 at levels of 0.5 and 1.0 g per day averaged 3- and 7-fold higher, respectively, compared to cecum weights in rats fed only basal diet and basal diet with or without glucose (Table 5). The weight of the cecum and its contents is subtracted from weight gain among all groups in calculating the net metabolazible energy of FS-2 using the proportional equation. Net metabolazible energy is the amount of energy in FS-2 available to the rat for growth. Conversely, the nonmetabolizable energy portion of FS-2 consists of those fractions lost in the urine and feces or as combustible gases and used in the increase of cecum weight and its contents. When the weight of the cecum and its contents was subtracted from weight gain during the 14 day period, the calculated energy values of FS-2 in rats given 0.5 and 1.0 g per day were 1.2 and 3.1 kJ/g, respectively. The average of these two values is 2.2 kJ/g.

DISCUSSION

Using a series of in vitro steps to mimic the digestive system in mammals, 90% of FS-2 was found to be indigestible. This low digestibility of FS-2 appears to be substantiated by the plasma glucose response observed in rats administered FS-2 by gavage. There was little change in plasma glucose levels of rats gavaged with a solution of FS-2. The area under the curve after 120 min in plasma glucose levels of rats given the FS-2 amounted to <5% of the plasma glucose levels observed in rats administered equal amounts of PD-3 or glucose. These two experiments, in vitro digestion and change in plasma glucose concentrations, would indicate that only ∼5−10% of the energy in FS-2 is potentially available through small intestine digestion. White et al. (1988) showed a high coefficient of correlation ($r = 0.98$) between in vitro enzyme digestibility of dextrose polymers and their in vivo digestibility and metabolizable energy.

How much net metabolizable energy is available from compounds fermented in the cecum and large intestine? The SCFA produced in the intestine are considered to be the prime energy compounds produced through fermentation, available to the host, and providing net metabolizable energy. Although these acids increase with increased amounts of fermentable substrates, other energy expenditures associated with the removal of these SCFA from the colon must be considered. Davies et al. (1991) and Brown and Livesey (1994) examined the relationship between fermentation and net metabo-

Table 6. Energy Intake and Weight Gain among Groups of Rats Fed Glucose or Fibersol 2 with and without Cecum Weight and Contents To Determine the Energy Value of Fibersol 2

	basal diet +				
	no addition	0.5 g of glucose	1.0 g of glucose	0.5 g of FS-2	1.0 g of FS-2
	Daily Energy Intake, kJ per Rat				
	96.8	104.2	111.3	105.0	113.3
	Weight Gain,[a] g				
days 1−5	-6.7 ± 1.0^{c}	-1.5 ± 0.8^{b}	6.1 ± 0.5^{a}	-0.7 ± 1.4^{b}	-1.7 ± 1.6^{b}
days 1−10	-3.8 ± 1.4^{c}	-0.1 ± 1.6^{c}	17.4 ± 1.2^{a}	-1.3 ± 1.6^{c}	6.1 ± 1.4^{b}
days 1−15	-1.0 ± 1.8^{d}	5.1 ± 1.6^{c}	25.3 ± 1.0^{a}	3.4 ± 1.2^{c}	12.3 ± 1.4^{b}
	Weight Gain Minus Cecum,[a] g				
days 1−15	-2.1 ± 1.8^{d}	4.0 ± 1.5^{b}	24.2 ± 1.1^{a}	0.3 ± 1.3^{c}	4.6 ± 1.2^{b}
	QV Based on Total Body Weight				
weight gain/energy intake[b]		0.00350	0.01624	0.00231	0.00775
kJ/g[c]				11.0	8.0
	QV Based on Total Body Weight Gain Minus Cecum Weight				
weight gain/energy intake[b]		0.00274	0.01524	0.00020	0.00290
kJ/g[c]				1.2	3.1

[a] Mean ± SEM, $n = 6$. Means not sharing a superscript in a row are significantly different ($P \leq 0.05$). [b] Weight gain in 15 day period divided by total energy intake during the same period. [c] Calculated energy value of FS-2 using formula 16.7 kJ/g G:*X* kJ/g FS-2 = QV-G:QV-FS-2. The QV obtained by dividing weight gain by energy consumption for animals fed 0.5 or 1.0 g of glucose (G) or FS-2. Solving for *X* provides kJ/g of FS-2.

lizable energy. They found cellulose and guar gum to be partially fermented but provided no metabolize energy. Both guar gum and, to a lesser extent, cellulose yield energy upon fermentation, but this increase in available energy is expended in their degradation. In the case of guar gum, increased fermentation also results in an enlargement of the cecum. The contents of the cecum are increased and reflect more microflora mass. This increased microflora mass uses part of the energy made available through fermentation. These are examples of energy expenditures not contributing to net metabolizable energy. We observed increased fermentation, increased cecum weights, and increased cecum contents in rats fed FS-2. The determination of net metabolizable energy values for indigestible carbohydrates is not a perfect science (Juhr and Franke, 1992). These investigators used radiolabeled cellulose and found it to be fermentable and calculated an "available energy" value of 3.5 kJ/g compared to the zero energy value reported by Davies et al. (1991).

The balance technique used in this study indicated 38% of the ingested FS-2 was recovered in the feces. These data, along with data from the first two experiments, would suggest not more than 52% (e.g., 100% − 38% recovered in feces − 10% digested) of the ingested FS-2 is available for fermentation in the cecum and large intestine to provide energy. We do not know the distribution of this energy between expenditures for microflora growth, colon growth, lost as gases, and net metabolizable energy for the host. Wolin (1981) suggested 20% of fermentable saccharides are used for bacterial growth and maintenance in the human.

The size (values not reported) and weight of the cecum in rats fed FS-2 were significantly larger compared to those of the cecum in rats fed glucose. Organ enlargement is associated with consumption of fermentable carbohydrates (de Groot, 1987). Brown and Livesey (1994) comment on organ enlargement and suggest it reflects increased energy expenditure. The pH of the cecum contents in rats fed FS-2 compared to rats fed glucose was significantly lower. Both observations support the contention that FS-2 is not efficiently digested in the small intestine. Weight gain in rats fed 0.5 g of FS-2 per day was 33% lower compared to rats fed an equal amount of glucose, but this reduction was not significant. The 51% reduction in weight gain among rats fed 1.0 g of FS-2 per day compared to rats fed the same amount of glucose was significant ($P \leq 0.05$). This latter observation would suggest that, at a maximum, only half of the FS-2 reaching the large intestine is available as net metabolizable energy (e.g., 4.3 kJ).

We did not measure the SCFA in the cecum of rats, although we did measure cecum pH. It is known that SCFA will increase in the portal blood in rats fed fermentable carbohydrates, but the conversion of fermentable saccharides in the colon to SCFA is not efficient (Oky, 1990; Livesey and Elia, 1995). The SCFA are the main products of fermentation in the large intestine and considered the compounds that could contribute to net metabolizable energy. There are no good in vivo quantitative data available to indicate the amounts of SCFA produced by a known amount of fermentable carbohydrate. Englyst et al. (1987) measured in vitro the amount of SCFA produced per unit carbohydrate using mixed human colonic bacteria. These investigators found that among four polysaccharides (e.g., starch, arabinogalactan, xylan, and pectin) the amount of SCFA produced per unit of carbohydrate averaged 50% (weight for weight) with a range of 35−59%. The value of 52% of the ingested FS-2 that reaches the large intestine, in the fecal excretion experiment, suggests that half of the residual FS-2 in the intestine is converted to SCFA on the basis of the calculations of Englyst et al. (1987) and Cummings and Macfarlane (1991). One gram of ingested FS-2 would yield a theoretical energy content of 4.3 kJ available as SCFA. Oku (1991) indicated the "apparent energy utilization" of SCFA produced by intestinal microflora to be 69%. This calculates to 3.0 kJ (4.3 kJ × 0.69) being available for net metabolizable energy to the host. This value, 3.0 kJ, plus the 1.7 kJ resulting from the 5−10% FS-2 estimated to be digested in the small intestine equals 4.7 kJ. This estimated energy value of FS-2 based only on our digestion and balance studies approaches the energy value we determined through our growth rate experiment.

The growth rate experiment provides the best method of determining the energy value of FS-2. All estimates or approximations are set aside, and the growth of the

animal is directly related to the net metabolizable energy in FS-2. The energy value of FS-2 in rats based on their growth rate, excluding cecum weight and contents, is 2.2 kJ/g.

The use of bulking agents and various ingredients to reduce the caloric content of foods is an important issue in the food industry and the nutrition community. There are growing numbers of fat substitutes (LaBarge, 1991), fat mimetics (Glicksman, 1991), and bulking agents (Deis, 1994) now being incorporated into foods. Not all are perceived in a positive manner (Blackburn, 1995). One fat substitute has been reported to impair the absorption of fat-soluble vitamins. The concern with the excessive amount of fermentable fat mimetics and bulking agents added to foods is that they can lead to flatulence. Although this would be an unacceptable side effect, consumption of these ingredients would be self-limiting. From a positive perspective, there are the prebiotic properties of indigestible saccharides (Gibson and Roberfroid, 1995) such as FS-2. The term prebiotic has been applied to indigestible and fermentable saccharides reaching the large intestine. The therapeutic action of these prebiotics is as sources of energy for intestinal bacteria through fermentation. Livesey and Elia (1995) comment on the significance of SCFA contributing to colonic health and function. Some genera of intestinal bacteria are now referred to as probiotic bacteria (O'Sullivan et al., 1992). The products of this fermentation process have also been identified as beneficial regulators of intestinal mucosal cells (Sakata and Setoyama, 1995). Butyric acid is considered one of the most important SCFA produced in the colon through fermentation of carbohydrates and may be directly involved in the prevention of colon cancer (Smith and German, 1995). Different colonic bacteria appear to utilize different types of fermentable saccharides. The complete prebiotic benefits of FS-2 and other bulking agents remain to be determined. The exact mechanisms or health potential for these indigestible saccharides may not be known. This topic has recently been reviewed for resistant starch (Gordon, 1997), a type of indigestible carbohydrate.

FS-2 is predominantly an indigestible carbohydrate. Approximately 40% was found to be nonfermentable in the rat. The fermentation of FS-2 will lower the pH in the colon. This lower pH is most likely due to the increased production of SCFA that may have beneficial effects on mucosal lining the intestine. Using a series of in vitro and in vivo experiments, we have shown that FS-2 has an energy value of 2.2 kJ/g. This value is 13% (2.2 vs 16.7 kJ/g) of the energy found in conventional food sugars and starches. FS-2 has a unique application in foods due to its functionality and low energy content.

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