The energy conversion factor of D-tagatose

Summary of the scientific evidence prepared for
Nutrilab NV, Bekkevoort, Belgium

Author: Albert Bär PhD
Bioresco Ltd.
Bundesstrasse 29
4054 Basel
Switzerland

Date: April 7, 2008
Contents

1. Introduction ......................................................... 4
2. Energy conversion factors of carbohydrates in nutrition labeling ........................................... 6
   2.1 Terminology .................................................... 6
   2.2 Methods for the estimation of energy conversion factors ........................................... 7
3. Estimation of the energy value of D-tagatose on basis of in-vivo studies .................................... 9
   3.1 Energy balance study in growing rats ...................... 9
   3.2 Growth study in adult rats ............................... 10
   3.3 Energy balance study in growing pigs .................. 10
   3.4 Interpretation and conclusions of in-vivo studies .... 11
4. Estimation of the caloric value of D-tagatose with the factorial calculation model .................. 13
   4.1 Absorption of D-tagatose in the small intestine... 14
   4.2 Metabolism of absorbed D-tagatose ................. 18
   4.3 Fermentation of unabsorbed D-tagatose ......... 18
   4.4 Increased excretion of fecal dry matter ............ 20
      4.4.1 Increased bacterial mass (biomass) ........... 21
      4.4.2 Increased turnover of intestinal mucosal cells and increased synthesis of mucin .... 23
      4.4.3 Increased non-bacterial mass ................. 24
   4.5 Calculation of the energy value using the factorial method ........................................ 26
5. Conclusions .......................................................... 27
6. References ............................................................ 29

Figures 1 - 2

Tables 1 - 3
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>bw</td>
<td>Body weight</td>
</tr>
<tr>
<td>DE</td>
<td>Digestible energy</td>
</tr>
<tr>
<td>FOS</td>
<td>Fructooligosaccharides</td>
</tr>
<tr>
<td>GE</td>
<td>Gross energy</td>
</tr>
<tr>
<td>ME</td>
<td>Metabolisable energy</td>
</tr>
<tr>
<td>NME</td>
<td>Net metabolisable energy</td>
</tr>
<tr>
<td>RE</td>
<td>Retained energy</td>
</tr>
<tr>
<td>RQ</td>
<td>Respiratory quotient</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short-chain fatty acid</td>
</tr>
</tbody>
</table>

1.
INTRODUCTION

D-Tagatose is a monosaccharide, an enantiomer of D-fructose (inversion at C-4), which has been authorized as a novel food in the EU in June 2005 based on an opinion of the UK Advisory Committee for Novel Foods and Processes (ACNFP) (FSA, 2005). D-tagatose has also been evaluated by JECFA which allocated an ADI "not specified" to this sugar in 2004. In Australia/NZ, D-tagatose has been authorized as a novel food in 2004. In the US, the use of D-tagatose in a variety of foods is considered "generally recognized as safe" (GRAS Notice No. 0078) (FDA, 2001).

The estimated daily intakes of D-tagatose which have been considered in the different safety assessments vary from about 4 - 5 g/d for the mean consumer to about 10 g/d for the 90th percentile consumer. However, also higher intakes (15 - 20 g/d) are conceivable (FSA, 2005). At even higher doses, intestinal side effects (flatulence, laxative effects) may limit the further intake of this poorly absorbed sugar.

The intake of D-tagatose may, therefore, reach similar levels as the intake of polyols, fructooligosaccharides, inulin or other carbohydrates for which energy conversion factors different from the general value of carbohydrates (4 kcal/g, 16 kJ/g) have been allocated.

In recognition of the need for the allocation of an appropriate energy conversion factor, the US FDA has accepted a value of 1.5 kcal/g (6 kJ/g) for D-tagatose in 2001 (FDA, 1999). In Australia/NZ, a factor of 11 kJ/g (2.5 kcal/g) was attributed in 2004 (FSANZ, 2004 a, b). The reason for these discrepant values is that FSANZ' model for the calculation of energy conversion
factors does not take into account all losses of energy that are associated with the fermentation of not absorbed material by the intestinal microflora, while the US FDA does.

In the summary of the UK ACNFP's assessment of D-tagatose as novel food, note was taken of the applicants proposed energy value of 1.5 kcal/g (6 kJ/g). It was acknowledged that this value would be significantly lower the value of 4 kcal/g (16 kJ/g) that applies currently for the nutrition labelling of all sugars. However, the ACNFP did not speak out on the scientific substantiation of the proposed energy value of D-tagatose, because this matter was not in the scope of its mandate of the safety assessment of D-tagatose.

For establishing a more accurate energy value for D-tagatose than the currently applicable value of 4 kcal/g (16 kJ/g) for sugars generally, a scientific evaluation by EFSA may, therefore, be needed before Directive 90/496/EEC can be amended accordingly. In the present dossier, the data are compiled which are required for assessing the energy conversion factor of D-tagatose.
2. ENERGY CONVERSION FACTORS OF CARBOHYDRATES IN NUTRITION LABELING

For the purpose of nutrition labeling, an energy value of 4 kcal/g (16 kJ/g) is attributed to fully digestible and metabolized sugars (e.g., glucose, sucrose) and other carbohydrates (Directive 90/496/EEC). This value is approximative and represents the metabolizable energy of these carbohydrates.

Sugars, oligosaccharides and polysaccharides which are poorly digested, absorbed, and/or metabolized, have a lower physiological energy value than the fully digestible and metabolized carbohydrates. For nutrition labeling, lower energy values apply, therefore, for all polyols (2.4 kcal/g, 10 kJ/g), except erythritol, for which 0 kcal/g (0 kJ/g) is proposed, polydextrose (4 kJ/g, 1 kcal/g) and inulin (8 kJ/g, 2 kcal/g). Being poorly absorbed, also D-tagatose would belong to this group of carbohydrates with an energy value of less than 16 kJ/g (4 kcal/g).

2.1 Terminology

The heat of combustion ($\Delta H_c$), i.e., the energy liberated when a compound is burned in oxygen to produce carbon dioxide and water, is 3.72 and 4.18 kcal/g for glucose and starch, respectively. In nutritional sciences, the term "gross energy" (GE) is used as a synonym for $\Delta H_c$. A small fraction of the GE ingested with food is excreted with feces. The difference between GE and fecal energy is called "digestible energy" (DE). After subtracting from DE the energy losses with urine, there remains the so-called "metabolizable energy" (ME) (Fig. 1). It is the ME which forms the basis for the energy conversion factors used for nutrition labeling, i.e., the so-called Atwater
factors of 17, 17 and 29 kJ/g (4, 4 and 9 kcal/g) for carbohydrate, protein and fat, respectively (FASEB, 1983). However, it is by now well recognized that the calculation of the ME according to the Atwater method does not give appropriate estimates of the energy values of dietary fiber and other incompletely digested, fermentable carbohydrates, because the ingestion of such products leads to additional energy losses, for example with gases (CH₄, H₂) and heat of fermentation (British Nutrition Foundation, 1990; Livesey, 1992). Correcting the ME for such substrate-induced energy losses yields the so-called "net metabolizable energy". The net metabolizable energy represents for the body the food-derived energy with which it can perform metabolic and physical work.

2.2 Methods for the estimation of energy conversion factors

The energy value of food or food components may be estimated either directly from results of in-vivo studies (e.g., energy balance studies) or from data on their metabolic fate (factorial calculation model).

Direct estimates for DE and ME values of complete diets or individual food components may be obtained from energy balance studies and other in-vivo techniques (e.g., indirect calorimetry). However, for measuring the ME of an individual food component, it must be administered at a high dose if the experimental error is to be kept at an acceptable level (Hobbs, 1988). Also, it may be necessary to analyze the carcass for changes of its composition (protein, fat). For these and other reasons, energy balance studies usually are conducted in experimental animals rather than in human volunteers.

For estimating energy values by the factorial model, the energetic contribution of each metabolic step is evaluated
separately using the collective data from all pertinent in-vitro and in-vivo experiments. The energy value is then calculated as the sum of all individual steps (Bär, 1990).

In this report, estimates of the energy value of D-tagatose are presented which are derived from energy balance studies in rats and pigs, and from calculations using the factorial model. In this context, "energy value" means the energy conversion factor that may be applied for nutrition labeling. In describing the scientific data, the more specific terms (DE, ME, etc.) are used, as appropriate. It is considered that the net metabolizable energy describes the caloric value of incompletely digested carbohydrates most adequately.

In the metric system, energy values are expressed in kJ/g. In the following, the more traditional expression in kcal/g is used (1 kcal/g = 4.18 kJ/g).
3. ESTIMATION OF THE ENERGY VALUE OF D-TAGATOSE ON BASIS OF IN-VIVO STUDIES

The results of three in-vivo studies on the energy value of D-tagatose are summarized in Table 1. Two studies were performed in rats and one in young pigs.

3.1 Energy balance study in growing rats

In the first rat study, two groups of 30 rats each were adapted for a period of 21 days to a basal diet (containing 31% sucrose) with supplements of sucrose (5%) or D-tagatose (10%). Each group was then split at random to three subgroups. One of the subgroups continued the treatment for 40 days, one discontinued the treatment (i.e., basal diet without supplement for 40 days), and one was killed for analysis of body composition. The daily intake of the basal diet was fixed. Body weights were determined at the start of the experiment, at the end of the 21-day adaptation period, and at the end of the 40-day balance period.

Fat, lean dry matter, and ash content of carcasses were measured at the start and end of the balance period. The GE (heat of combustion) of the basal diet, D-tagatose, and sucrose also were determined. From these data, it was calculated that D-tagatose has a net metabolizable energy ("NEV_s") of -0.12 kcal/g (Livesey & Brown, 1996). The NEV_s corresponds to the net metabolizable energy, i.e., ME minus "substance-induced energy expenditure".
3.2 Growth study in adult rats

Another rat study with D-tagatose was performed in retired breeders with a body weight of about 710 g ("fatty rats"). 18 male rats were used. After a 7-day acclimatization period, they received standard rodent diet with a supplement of 15% D-tagatose for a period of 14 days (adaptation period). The group was then divided into 3 subgroups of 6 rats each. Two subgroups received diets with 15% D-tagatose and 15% sucrose, for a period of 90 days (test period). The sucrose diet was pair-fed so as to match the intake of the 15% D-tagatose group. The third subgroup was killed for analysis of body composition (fat, protein, moisture, and ash). Body weight gains during the 90-day test period were significantly lower for rats of the D-tagatose group as compared to the sucrose group (55 g vs. 126 g). Analysis of body composition indicated that the fat deposition was significantly reduced in the D-tagatose group. The retained energy (RE) of D-tagatose was about 31% that of sucrose. Calculations of ME were not performed (Saunders et al., 1994).

3.3 Energy balance study in growing pigs

In a study with a cross-over design, six pigs received three diets containing either 20% sucrose, 10% sucrose and 10% D-tagatose, or 20% D-tagatose during three treatment periods. The first two treatment periods comprised a one-week adaptation period and a one-week balance period. The last treatment period comprised a one-week adaptation period and two one-week balance periods. During each period, body weights, energy intake from food, and energy losses with urine, feces, methane, hydrogen and heat were measured. On the 5th day of each balance period, the pigs were housed for 24 hours in a metabolic chamber for measurement of the respiratory quotient (RQ). RE was calculated
from the measured carbon and nitrogen balances. The heat production was calculated from the RQ and carbon balance. From the heat production, the energy for maintenance was calculated. The NE of D-tagatose, calculated as the sum of RE and energy for maintenance, was 1.05 kcal/g. Calculating the ME of D-tagatose as the difference between GE and energy lost with feces, urine and gases, a value of 1.9 kcal/g was obtained. Corrections were then made to account for the lower yield of ATP which is obtained from SCFAs, and for the loss of energy with the heat of fermentation (for explanation and rationale, see Section 4.3). Applying these corrections, a net metabolizable energy of 1.4 kcal/g was calculated for D-tagatose (Jørgensen & Laerke, 1998).

3.4 Interpretation and conclusions of in-vivo studies

A surprisingly low energy value (~0.12 kcal/g) was obtained from the energy balance study in growing rats (Livesey & Brown, 1996). This result was not matched by data from adult rats, in which D-tagatose had a retained energy value of about 31% that of sucrose. If the ratio of RE/ME was identical in the sucrose (control) and D-tagatose groups, the ME of D-tagatose would be 1.2 kcal/g. Two factors may explain the discrepancy between the two rat studies.

First, it is known that D-tagatose inhibits sucrase/isomaltase but not glucoamylase/maltase in vitro (Hertel, 1997a, b). Since the basal diet used by Livesey and coworkers contained 31% sucrose, it is conceivable that D-tagatose reduced the digestibility of sucrose and thus the energy value of the basal diet. Second, the basal diet used by Livesey and coworkers did not contain fermentable dietary fiber. Therefore, the D-tagatose mediated increase of fecal dry matter might have been
particularly prominent (see section 4.4 for further explanation).

The pig is considered to be a suitable model for evaluating the digestibility and caloric value of foods or food components. The anatomy and physiology of the porcine intestinal tract resembles that of humans (Graham and Aman, 1987; Giesecke, 1990). Therefore, particular relevance is attributed to the result of the pig energy balance study. With a net metabolizable energy value of 1.4 kcal/g, D-tagatose appears to have a lower caloric value than isomalt for which a value of 1.96 kcal/g was obtained in a similar pig study (Février & Pascal, 1991).
4. **ESTIMATION OF THE CALORIC VALUE OF D-TAGATOSE WITH THE FACTORIAL CALCULATION MODEL**

In order to estimate the caloric value of a carbohydrate using the factorial model, there must be knowledge about the different steps of the metabolic utilization of the product and about potential interference(s) of the product (or its metabolites) with other physiological processes that have implications on the energy balance. There must be quantitative data, for example, about:

- the fractional absorption of the ingested carbohydrate from the small intestine,
- the metabolism and excretion of the absorbed carbohydrate,
- the fraction of energy of unabsorbed carbohydrate which is made available indirectly, i.e., by microbial fermentation in the large intestine followed by absorption and utilization of the formed short-chain fatty acids (SCFAs),
- the excretion of unaltered carbohydrate in feces,
- any interference with the digestion or absorption of other food components, and
- the existence of energy consuming processes which are indirectly related to the intake of the carbohydrate (e.g., increased proliferation of intestinal bacteria, faster turnover of intestinal mucosa cells, increased mucin secretion).
4.1 Absorption of D-tagatose in the small intestine

Among the different dietary monosaccharides, glucose and galactose are absorbed by an active, energy-consuming transport mechanism. Fructose and xylose are absorbed by carrier-mediated, so-called "facilitated" diffusion. All other sugars are absorbed by passive diffusion.

The following observations demonstrate that D-tagatose belongs to the group of sugars which are absorbed by passive diffusion:

(a) D-Tagatose does not inhibit the absorption of fructose or glucose (at concentrations up to 100-times higher than those of fructose). A binding of D-tagatose to the glucose and fructose carriers followed by transport across the mucosa can, therefore, be excluded (Crouzoulon, 1978; Sigrist-Nelson & Hopfer, 1974; Tatibouët et al., 2000).

(b) Not more than about 20% of ingested $^{14}$C-D-tagatose is absorbed in rats (Saunders et al., 1999). Absorption rates of this magnitude are typical for monosaccharides and polyols of similar molecular size which are absorbed by passive diffusion (e.g., mannitol, L-fructose, L-gulose) (Bär, 1990).

(c) In rats fed diets with up to 20% D-tagatose, soft stool occurred during the first days of treatment (Kruger et al., 1999a,b). Soft stool is a typical side-effect of the ingestion of malabsorbed carbohydrates (sugars and polyols).
(d) In rats exposed to D-tagatose for the first time (i.e., unadapted rats), about 25% of ingested $^{14}$C-D-tagatose was excreted with the feces (Saunders et al., 1999).

(e) Less than 26% of ingested D-tagatose was absorbed in pigs according to measurements of (1) the disappearance of D-tagatose from the digesta (Laerke & Jensen, 1999) and (2) the appearance of D-tagatose in portal vein blood (Jensen & Laue, 1998).

(f) In 64% of unadapted humans tested, the acute intake of 29 g D-tagatose caused the same mild gastrointestinal side-effects (e.g., flatulence) which are seen typically after ingestion of malabsorbed carbohydrates (Buemann et al., 1999). An increased expiration of $H_2$, an indicator of colonic fermentation of malabsorbed carbohydrates, was observed as well (Buemann et al., 1998).

(g) A single dose of 20 g D-tagatose resulted in the same mild intestinal side-effects as a single dose of 20 g lactitol, a not absorbed disaccharide sugar alcohol (Lee & Storey 1999).

Having established qualitatively that ingested D-tagatose is absorbed incompletely, it is necessary to quantify the fractional absorption rate. In rats and pigs, about 20 - 25% of ingested D-tagatose is absorbed (Saunders et al., 1999; Laerke & Jensen, 1999; Jensen & Laue, 1998). This result is supported by an intestinal absorption rate of 25% for D-psicose in rats (Whistler et al., 1974). The absorption of D-psicose, a stereoisomer of D-tagatose, can be measured easily because absorbed D-psicose is not metabolized but is excreted completely in the urine.
In humans, the absorption of D-tagatose cannot be measured directly because absorbed D-tagatose is readily metabolized by the liver. However, human data on the absorption of L-rhamnose provide a good basis for an estimate. L-Rhamnose (6-deoxy-D-mannose) has a slightly lower molecular weight and is slightly more lipophilic than D-tagatose (Fig. 2). Since the rate of passive diffusion of a substance through the intestinal mucosa is determined by its molecular volume and lipophilicity (Hamilton et al., 1987), the intestinal absorption of L-rhamnose is expected to be somewhat higher than that of D-tagatose. The absorption of ingested L-rhamnose can be determined quantitatively from its urinary excretion because L-rhamnose is metabolized only slowly and thus incompletely. In humans and dogs, about 70% of an intravenous dose of L-rhamnose is excreted unchanged with the urine (Jenkins et al., 1994; Bjarnason et al., 1994; Hall & Batt, 1996). Studies in humans show that not more than about 12 - 17% of ingested L-rhamnose is excreted with the urine (Table 2). Consequently, not more than about 17 - 24% of ingested L-rhamnose is absorbed. Since the absorption of D-tagatose most probably is somewhat lower than that of L-rhamnose, the fractional absorption of D-tagatose in humans will hardly exceed 20%.

It is recognized that this value is not in line with the result of a study in which the absorption of D-tagatose was examined in ileostomie patients. In this study, only about 20% of ingested D-tagatose (15 g) was recovered from the 24-hour ileal effluent (Normén et al., 2001). This result was interpreted by the authors as indicative of an 80% intestinal absorption. However, similarly high absorption rates were suggested earlier also for sorbitol, maltitol and isomalt from studies in ileostomates, although there is strong evidence that these polyols are poorly digested and absorbed (Langkilde, 1994; Bär, 1990). Inconsistent results also were reported from a study on the absorption of L-
rhamnose in ileostomic patients. After intake of 1 g L-rhamnose, 17.6% of the ingested dose was recovered in the 24-hour urine, suggesting an absorption of about 25%. On the other hand, 51.1% of the ingested dose was detected in the total ileal effluent suggesting an absorption of nearly 50% (J Jenkins et al., 1994). Different factors have been invoked for explaining such excessive absorption rates in ileostomic patients, such as fermentation of the test compounds by the small-intestinal microflora (e.g., by filaments which are firmly attached to the mucosa) (Dowsett et al., 1990), incomplete analytical recovery of the test compound from the ileal effluent, and altered permeability of the intestinal mucosa. Although a definitive explanation is not yet available, it appears that the ileostomate model has a limited value for determining absorption rates of malabsorbed monosaccharides and polyols. For this reason, such data were disregarded in an evaluation of the caloric value of polyols (FASEB, 1994).

Finally, it needs to be examined whether the intestinal absorption rate of D-tagatose varies in relation to the administered dose. A priori, a dose-related decrease of the absorption rate only is expected for nutrients which are absorbed by a saturable transport mechanism but not for compounds which, like D-tagatose, cross the intestinal mucosa by passive diffusion. Indeed, sorbitol was absorbed from isolated intestinal loops at a steady rate for concentrations ranging from 1 to 200 µM (Lauwers et al., 1985). Only if very high doses of sugars are administered, the resulting hyperosmolarity of the intestinal contents may lead to an influx of water into the intestinal lumen, thereby reducing the absorption of solutes somewhat (Maxton et al., 1986; Menzies et al., 1990; Bjarnason et al., 1994).
The estimated 20% absorption rate of D-tagatose is derived from human studies in which single doses of about 0.5 - 5 g of L-rhamnose were ingested. Such doses correspond well to the estimated intake of D-tagatose per eating occasion. However, for the reason explained above, a similar absorption rate would also be expected at higher intakes.

4.2 Metabolism of absorbed D-tagatose

A metabolic study with $^{14}$C-D-tagatose in rats demonstrates that about 20% of absorbed D-tagatose, corresponding to about 4% of ingested D-tagatose, is excreted unchanged with the urine (Saunders et al., 1999). Similar data were obtained from a metabolic study in pigs (Jensen & Laue, 1998). In humans, 1 - 2% of ingested D-tagatose was recovered in the urine (Buemann et al., 1998, 1999b).

The rapid appearance of respiratory $^{14}$CO$_2$ in the metabolic study in rats (including germfree rats) demonstrates that absorbed D-tagatose is catabolized to CO$_2$ and H$_2$O much like glucose or other monosaccharides (Saunders et al., 1999, Rognstad, 1982).

For estimating the caloric value of D-tagatose by the factorial calculation model (Table 3), it is assumed that the absorbed portion of D-tagatose, less what is excreted in urine, is fully metabolized yielding a ME of 3.75 kcal/g.

4.3 Fermentation of unabsorbed D-tagatose

Unabsorbed D-tagatose is fermented nearly completely by intestinal microorganisms to short-chain fatty acids (SCFAs). These SCFAs are absorbed almost completely and, therefore, are calorically fully available. No D-tagatose was found in the feces of pigs or humans after ingestion of this carbohydrate.
(Laerke & Jensen, 1999; Jørgensen & Laerke, 1998; Buemann et al., 1998). In adapted rats, fed a diet with 10% D-tagatose, about 2% of an ingested dose of $^{14}$C-tagatose was recovered from the feces (Saunders et al., 1999).

In-vitro fermentation of D-tagatose by intestinal microbiota of pigs and humans yields acetate, propionate and butyrate as the main end-products. Formate, caproate, valerate and lactate also were detected. After a 4-hour incubation with colon content of pigs, 48% of the energy of D-tagatose fermented was present in the form of these products (SCFAs) (Laerke et al., 2000). Measurement for 12 hours of SCFAs in the portal vein blood of pigs fed D-tagatose gave a similar conversion rate (Jensen & Laue, 1998). The in vitro fermentation of D-tagatose by microbiota of human feces also produced about 50% SCFAs (percentage calculated on basis of $\Delta H_c$ (Jensen & Buemann, 1998).

The fermentation of D-tagatose by intestinal and fecal microorganisms in-vitro yields a high amount of butyrate. When D-tagatose was fermented with colon content from adapted pigs, 46% of total SCFAs was butyrate (Laerke et al., 2000). With feces from humans, butyrate was 35% and 47% of total SCFAs after 4 and 48 hours of incubation, respectively (Jensen & Buemann, 1998). High amounts of butyrate also are formed by the intestinal fermentation of D-tagatose in vivo. Butyrate concentrations increased in the intestinal contents of the hindgut of pigs with increasing intake of D-tagatose (Jensen & Laue, 1998). In the portal blood of pigs, butyrate concentrations were more than twice as high after ingestion of D-tagatose than sucrose (administered at a dose of 20% of diet) (Jensen & Laue, 1998). The high formation of butyrate may have implications for the energy value of D-tagatose because butyrate has an important trophic effect on the intestinal mucosa and also has a stimulating effect on mucin production (Sakata et...
al., 1995; Finnie et al., 1995) (see Section 4.4 for further explanation).

For estimating the energy that becomes available to the host with absorbed SCFAs, it must be taken into account that less ATP is gained from the oxidation of absorbed SCFAs than from the oxidation of glucose. Glucose yields $53.5 \times 10^{-3}$ ATP/kcal, while acetic, propionic and butyric acid yield $48.0$, $46.5$ and $51.7 \times 10^{-3}$ ATP/kcal, respectively (Bär, 1990). The ratio between the ATP yield per kcal of glucose and the weighted average of SCFAs formed from D-tagatose by fermentation is about 0.9. A similar value (0.85) has been proposed as a general correction factor by others (Livesey, 1993).

### 4.4 Increased excretion of fecal dry matter

The ingestion of incompletely digested, yet fully fermented carbohydrates is associated with an increased excretion of fecal dry matter. This increase may be attributed to:

(a) the increased bacterial mass which is formed as a result of the increased availability of fermentable substrate,

(b) the potentially increased turnover of intestinal mucosa cells due to a growth stimulating effect of butyrate (Sakata et al., 1995),

(c) the potentially increased production of mucin (due to a stimulating effect of butyrate (Finnie et al., 1995), and

(d) the increased excretion of undigested food components due to a faster gastrointestinal transit or a less complete microbial degradation.
In relation to (b) and (c) above, it should be noted that shed mucosal cells and secreted mucin are subject, at least in part, to degradation by the intestinal micro-organisms. The mechanism mentioned under (d) plays a role only at high intakes of osmotically active carbohydrates (intake at or above the threshold dose of laxation).

4.4.1 Increased bacterial mass (biomass)

With the increased availability of fermentable substrate, bacterial growth is stimulated. The part of the energy of a fermentable substrate which is not present at the end of the fermentation in the form of SCFAs or gases (H\textsubscript{2}, CH\textsubscript{4}) has been used for the maintenance and proliferation of the bacterial cells.

The growth yield, i.e., the production of biomass resulting from the fermentative process, varies widely depending upon many factors, such as the substrate, type of fermentation, growth rate and presence of inhibitory substance(s). In-vitro experiments typically give growth yields of 20 - 30%, i.e., 20-30 g of biomass is formed per 100 g of substrate fermented (Bär, 1990).

In vivo, the growth yield appears to be at the low end of this range, or even lower. In humans, the ingestion of lactulose (56 ± 6 g/d) was associated with an increase of the fecal bacterial mass of 12.2 g (i.e., 21.8% of lactulose ingested) while the increase of total fecal dry matter was 29.8 g (i.e., 52.6% of lactulose ingested) (Weber et al., 1987). The ingestion of lactitol (47.5 g/d) led to an increase of total fecal dry matter of 6.77 g (i.e., 14.3%) of which probably only a part represents bacterial cells (i.e., growth yield: 

(van Velthuijsen & De Uyl, 1988). In rats, the intake of 10 g isomalt increased total fecal dry matter by 1 - 1.5 g (Livesey, 1990). Since only about 60 - 70% of isomalt reaches the colon, growth yield is less than 15 - 20%.

Interestingly the growth yields estimated from in-vivo or in-vitro studies are substantially higher than the incorporation in the biomass of $^{14}$C from $^{14}$C-labeled carbohydrates. In humans given $^{14}$C-labeled lactitol (20 g), only 6.5% of the label was excreted with the feces (Grimble et al., 1988). For $^{14}$C-isomalt (15 g), fecal excretion of the $^{14}$C was 10% of the ingested dose (Patzschke et al., 1975 cited in WHO, 1987). For $^{14}$C-maltitol (10 g), a value of 4.9% was found (Rennhard & Bianchine, 1976). Therefore, only about 5 - 10% of the C-atoms of fermented carbohydrates is expected to end up in the incremental biomass. This estimate is matched by data on the metabolism of orally administered $^{14}$C-D-tagatose in rats. It was found that not more than 6% of the $^{14}$C was incorporated in fecal biomass (calculated from the difference of fecal $^{14}$C between germfree and adapted conventional rats, or from analysis of the $^{14}$C-labeled compounds in feces) (Saunders et al., 1999).

The difference between the increased fecal excretion of biomass (about 15 - 20% of the fermented substrate) and the much lower incorporation of C-atoms from the substrate (about 5 - 10%) suggests that the growing microbial cells incorporate C-atoms from other sources while their growth is stimulated by the presence of additional, fermentable substrate. It is conceivable, for example, that the microbial cells incorporate preformed metabolites, such as deaminated amino acids, from the digesta. If microbial growth was not stimulated by additional substrate, these metabolites might have been absorbed by the host or could have been fermented to
absorbable SCFAs. In other words, the increased microbial growth may lead to an additional loss of 15 – 20% metabolizable energy caused by the incorporation in the biomass of material that otherwise might have been available to the host either directly (yielding about 4 kcal/g) or indirectly via fermentation to SCFA (yielding about 2 kcal/g).

For D-tagatose, the increase of fecal biomass is estimated at not more than 15 – 20% of D-tagatose fermented, and the incorporation of C-atoms at not more than 5 – 10% of D-tagatose fermented. The average difference of 10% represents other incorporated material, which results for the host in a energy loss of about 0.2 – 0.4 kcal/g of D-tagatose fermented, or 0.15 – 0.30 kcal/g of D-tagatose ingested (assuming that 25% is absorbed and 75% is fermented).

4.4.2 Increased turnover of intestinal mucosal cells and increased synthesis of mucin

The ingestion of fermentable dietary fiber or low-digestible carbohydrates leads to a decreased nitrogen excretion with the urine and an increased nitrogen excretion with feces. The higher fecal nitrogen excretion may be explained, at least in part, by a butyrate-induced faster turnover of mucosal cells and an increased mucin secretion (Brunsgaard & Eggum, 1995; Sakata et al., 1995; Finnie et al., 1995). The increased loss of endogenous protein into the gut lumen represents an increased loss of energy, even though the secreted protein and the shed mucosal cells may partially be degraded by the intestinal bacteria and reabsorbed as SCFAs.

The loss of endogenous nitrogen in the large intestine was estimated at 13.4 g/d for a growing pig (40 kg bw) fed an
ordinary diet (Nyachoti et al., 1997). This amount corresponds to about 84 g protein requiring about 90 kcal for its biosynthesis from amino acids (Webster, 1981, cited by Nyachoti et al., 1997). Even if all secreted protein (84 g) was fermented to absorbable SCFAs, the energy loss due to this protein secretion into the gut would still be about 260 kcal/d [(84 g x 2 kcal/g) + 90 kcal]. If the ingestion of D-tagatose (dose: 250 g/d/pig corresponding to 20% in the diet) increased the secretion of endogenous protein in the gut by 10%, the net metabolizable energy of D-tagatose would thereby be reduced by about 0.1 kcal/g. This hypothetical calculation demonstrates that dietary effects on intestinal protein secretion can have significant implications for the calculation of caloric values of foods and food components. However, in the absence of quantitative data on the effect of D-tagatose (or similar low-digestible carbohydrates) on protein secretion in the gut, it is difficult to quantify energy losses by this mechanism.

4.4.3 Increased non-bacterial mass

Total fecal dry matter increased by 0.27 g per g D-tagatose ingested in pigs fed a diet with 20% D-tagatose (Jørgensen & Laerke, 1998). If calculated per g of D-tagatose fermented (in pigs: 75% of the ingested amount), the increase of fecal dry matter was 0.36 g/g. Bacterial mass has not been determined separately. However, assuming that the bacterial growth yield is about 15 - 20% (i.e., 0.15 - 0.20 g/g D-tagatose), it follows that about half of the increased fecal dry matter represents bacteria (i.e., biomass), while the other half represents different material.

In pigs, the ingestion of 10% D-tagatose in the diet did not reduce the small-intestinal digestibility of starch, protein
and fat (Laerke & Jensen, 1999). Therefore, the increased excretion of fecal non-bacterial mass might not be the result of a reduced digestibility of nutrients.

Rather, it must be considered that the increased non-bacterial mass represents secreta from the large intestine, i.e., shed mucosa cells and mucin which were produced at a higher rate under the trophic effect of butyrate formed from D-tagatose by fermentation (Finnie et al., 1995; Sakata et al., 1995). For pigs receiving a diet with 20% D-tagatose, the increased fecal excretion of non-biomass material corresponds to about 0.15 to 0.2 g/g D-tagatose fermented. If this material was secreted protein (mucus, mucosal cells), the loss of energy would be 0.6 to 0.8 kcal/g of D-tagatose fermented, or 0.45 to 0.60 kcal/g of D-tagatose ingested (Table 3). (The energy lost with secreted protein is about 4 - 5.5 kcal/g (FASEB, 1983) plus about 1 kcal/g for its biosynthesis. About 1-2 kcal/g is recovered by (partial) fermentation followed by absorption of formed SCFAs).

If, on the other hand, the increased non-bacterial mass was not mucosa cells and mucin, but was food-derived digesta which, in the absence of D-tagatose, would have been made available to the host via bacterial fermentation, the loss of energy would be about 0.2 - 0.3 kcal/g D-tagatose ingested.

As yet, no data are available on the effect of D-tagatose on the fecal excretion of non-bacterial mass in humans. However following the ingestion of lactulose (56 g), the weight of the additional fecal non-biomass corresponded to 31% of the amount of lactulose ingested (Weber et al., 1987). This observation demonstrates that also in humans the fecal excretion of non-bacterial mass may be increased after ingestion of non-digestible, yet fully fermented carbohydrates.
4.5 Calculation of the energy value using the factorial method

Calculation of the energy value of D-tagatose according to the factorial method is presented in Table 3. Depending upon some of the underlying assumptions, only ranges of caloric values rather than specific values could be allocated for some of the relevant factors. The calculation demonstrates that losses of energy due to an increased fecal excretion of biomass and non-bacterial mass are significant and may not be neglected.

Overall, the energy value of D-tagatose was estimated at 1.1 - 1.4 kcal/g. This result provides an upper limit to the energy value of D-tagatose. If further studies would identify additional, pertinent mechanisms of energy loss, the calculations would have to be adjusted accordingly.
5. **CONCLUSIONS**

Ingested D-tagatose is incompletely absorbed from the small intestine of animals and man. The fractional absorption is about 20% in rats and 25% in pigs. In humans, the fractional absorption is estimated at not more than 20% based on data of a structurally related carbohydrate (L-rhamnose). The absorbed fraction of D-tagatose is readily metabolized through the glycolytic pathway yielding 3.75 kcal/g. The unabsorbed fraction of D-tagatose reaches the large intestine where it is completely fermented by the intestinal microflora. The formed SCFAs are absorbed almost completely, and are metabolized. The metabolic fate of D-tagatose resembles, therefore, that of other incompletely digested carbohydrates (e.g., polyols).

The energy value of D-tagatose was evaluated in two studies in rats and one study in pigs. A net metabolizable energy value of -0.12 kcal/g was obtained for D-tagatose in the first rat study. This unexpectedly low value was attributed to an interference of D-tagatose with the absorption of sucrose which was present in the basal diet and/or to the relatively low amount of fermentable fiber in the basal diet. The second rat study suggested an energy value of about 1.2 kcal/g. Most relevance was attached, however, to the pig study since the digestive tract of pigs and humans show many similarities. The pig study gave an energy value of 1.4 kcal/g for D-tagatose.

Estimation of the energy value of D-tagatose by the factorial method gave a range of 1.1 - 1.4 kcal/g. In this method, the energy contributed by each metabolic step is evaluated separately, taking into account data from all pertinent experiments (in-vitro, in-vivo, in humans, in experimental animals). The factorial approach also takes into account losses
of energy which are caused indirectly by the fermentation of D-tagatose (e.g., increased fecal excretion of biomass and non-bacterial mass).

While the energy balance study in rats may offer the most precise energy value (Livesey & Brown, 1996), its accuracy may suffer form the low fermentable fiber content and sucrose supplement of the basal diet. Moreover, while the rat is an accepted model for human metabolism, the pig may be better suited for studying the digestibility of nutrients. Therefore, the pig study in conjunction with the factorial method was chosen as the basis estimating the energy value of D-tagatose. On theoretical grounds, there is no reason to believe that the energy value found in the energy balance study in pigs would not be valid also for man. The results of the factorial calculations which are equally applicable to pigs and humans, are fully consistent with the observed in-vivo energy value of 1.4 kcal/g. On this basis, it appears justified to use a rounded energy value of 1.5 kcal/g (6 kJ/g) for the nutritional labeling of D-tagatose.

This conclusion is supported by two independent critical reviews of the published literature and unpublished reports which were commissioned by MD Food Ingredients amba (Livesey, 1999; Gordon, 1999).
REFERENCES


without affecting 24 hours energy expenditure, or respiratory exchange ratio. J. Nutr. 128: 1481-1486.


Jenkins, A.P., Menzies, I.S., Nukajam, W.S., and Creamer, B. (1994). The effect of ingested lactulose on absorption of L-


<table>
<thead>
<tr>
<th>Species</th>
<th>Type of study</th>
<th>Treatment/ Dose levels</th>
<th>Duration of treatment</th>
<th>Estimated caloric value of D-tagatose</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (n = 10)</td>
<td>Growth, carcass analysis</td>
<td>Basal diet, diet with 10% D-tagatose, diet with 10% sucrose</td>
<td>40 d</td>
<td>-0.12 kcal/g (for sucrose: 3.9 kcal/g)</td>
<td>Livesey &amp; Brown, 1996</td>
</tr>
<tr>
<td>Rat (n = 6)</td>
<td>Growth, carcass analysis</td>
<td>Basal diet, diet with 15% D-tagatose, diet with 15% sucrose (pair-fed)</td>
<td>90 d</td>
<td>RE about 31% that of sucrose</td>
<td>Saunders et al., 1994</td>
</tr>
<tr>
<td>Pig (n = 6)</td>
<td>Energy balance</td>
<td>Diets with 20% sucrose, 20% D-tagatose, or 10% sucrose plus 10% D-tagatose</td>
<td>14 d</td>
<td>1.4 kcal/g</td>
<td>Jørgensen and Laerke, 1998</td>
</tr>
</tbody>
</table>

1) Values are "net metabolizable energy"
## Table 2  Urinary recovery of ingested L-rhamnose

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Dose</th>
<th>Urine sampling time</th>
<th>% recovery</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>8</td>
<td>100 mg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0-8 h</td>
<td>2.8 ± 1.5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Delahunty &amp; Hollander, 1987a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>150 mg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0-6 h</td>
<td>5.83 ± 0.16&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Delahunty &amp; Hollander, 1987b</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>250 mg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0-8 h</td>
<td>3.38 ± 0.60&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Meshkinpour et al., 1996</td>
</tr>
<tr>
<td>Dogs</td>
<td>9</td>
<td>1 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0-5 h</td>
<td>16.0 ± 6.9</td>
<td>Quigg et al., 1993</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1-2 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0-5 h</td>
<td>16.8 ± 5.9</td>
<td>Sorensen et al., 1993</td>
</tr>
<tr>
<td>Humans</td>
<td>6</td>
<td>5 g</td>
<td>0-6 h</td>
<td>16.3 ± 2.5</td>
<td>Delahunty &amp; Hollander, 1987a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.5 g&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0-5 h</td>
<td>11.7</td>
<td>Maxton et al., 1986</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>1 g&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0-6 h</td>
<td>10.1 ± 4.9</td>
<td>Howden et al., 1991</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.5 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0-5 h</td>
<td>17.4 ± 2.5</td>
<td>Bjarnason et al., 1991</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0-5 h</td>
<td>11.3 ± 3.2</td>
<td>Bjarnason et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 g&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0-10 h</td>
<td>13.7 ± 4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1 g&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0-5 h</td>
<td>9.3 ± 3.6</td>
<td>Bjarnason et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 g&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0-10 h</td>
<td>10.7 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>healthy</td>
<td>13</td>
<td>0-5 h</td>
<td></td>
<td>8.4</td>
<td>Mooradian et al., 1986</td>
</tr>
<tr>
<td>diabetic</td>
<td>48</td>
<td>0-5 h</td>
<td></td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1 g</td>
<td>0-5 h</td>
<td>14.7 ± 1.8&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Menzies et al., 1990</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1 g</td>
<td>0-5 h</td>
<td>16.7 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>ileostomates</td>
<td>10</td>
<td>1 g</td>
<td>0-5 h</td>
<td>11.8 ± 1.9</td>
<td>Jenkins et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 g</td>
<td>0-24 h</td>
<td>17.6 ± 2.7&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>1 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0-5 h</td>
<td>10.2 ± 3.0</td>
<td>Parrilli et al., 1987</td>
</tr>
</tbody>
</table>
Table 2 (continued)

Footnotes:

a) Given together with other incompletely absorbed sugars.
b) Iso-osmolar solution.
c) Given together with mannitol and lactulose.
d) Given together with mannitol, cellobiose, lactulose, and lactose.
e) Given together with lactulose.
f) Hyperosmolar solution.
g) Urine was not reported to be collected in dry-ice cooled beakers. Therefore, fermentation by microbes from fecal contamination may account for the low recoveries in rats.
h) Decreases if lactulose or mannitol is added.
### Table 3  Estimation of the energy value of D-tagatose using the factorial model

<table>
<thead>
<tr>
<th>Contributing factor(s)</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measured parameters and assumptions</strong></td>
<td></td>
</tr>
<tr>
<td>Fractional absorption</td>
<td>25%</td>
</tr>
<tr>
<td>Urinary excretion</td>
<td>5%</td>
</tr>
<tr>
<td>Metabolized by body</td>
<td>20%</td>
</tr>
<tr>
<td>Heat of combustion (ΔH&lt;sub&gt;c&lt;/sub&gt;)</td>
<td>3.75 kcal</td>
</tr>
<tr>
<td><strong>Energy gain from absorbed and metabolized D-tagatose</strong></td>
<td>0.75 kcal/g</td>
</tr>
<tr>
<td>Unabsorbed fraction</td>
<td>75%</td>
</tr>
<tr>
<td>Excretion of D-tagatose with feces</td>
<td>0%</td>
</tr>
<tr>
<td>Fraction of ingested D-tagatose which is fermented</td>
<td>75%</td>
</tr>
<tr>
<td>Yield of SCFAs (in % of GE of D-tagatose fermented)</td>
<td>50%</td>
</tr>
<tr>
<td>Correction factor for lower ATP yield from SCFAs</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Energy gain from absorbed SCFAs</strong></td>
<td>1.26 kcal/g</td>
</tr>
<tr>
<td>Growth yield of fermentation (in % of substrate fermented)</td>
<td>15 - 20%</td>
</tr>
<tr>
<td>Incorporation of metabolites derived from D-tagatose</td>
<td>5 - 10%</td>
</tr>
<tr>
<td>Incorporation of metabolites not derived from D-tagatose (% in relation to D-tagatose fermented)</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Energy loss due to increased fecal biomass (energy value: 2-4 kcal/g)</strong></td>
<td>(0.15 - 0.30 kcal/g)</td>
</tr>
<tr>
<td>Total increase of fecal dry matter (in % of D-tagatose fermented)</td>
<td>36%</td>
</tr>
<tr>
<td>Increase of non-biomass fecal dry matter (in % of D-tagatose fermented)</td>
<td>15 - 20%</td>
</tr>
<tr>
<td><strong>Energy loss due to increased fecal non-biomass (energy value: 2-4 kcal/g)</strong></td>
<td>(0.45 - 0.60 kcal/g)</td>
</tr>
<tr>
<td>Estimated energy value</td>
<td>1.1 - 1.4 kcal/g</td>
</tr>
</tbody>
</table>
Figure 1: Overview of food energy utilization and disposition

1) Modified from Wolfram, 1989
2) Ingested Energy (IE) = GE ingested
3) Corresponds to "energy for basal metabolism" or "maintenance energy"
4) Includes tissues, fat deposits, fetus, lactation, loss of hair, excretions of body
Figure 2  Molecular structures of D-tagatose and L-rhamnose

D-tagatose  
MW = 180.2

L-rhamnose  
MW = 164.2