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Molecules of Interest

Stevioside

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Abstract

Stevioside is a natural sweetener extracted from leaves of *Stevia rebaudiana* (Bertoni) Bertoni. The literature about *Stevia*, the occurrence of its sweeteners, their biosynthetic pathway and toxicological aspects are discussed. Injection experiments or perfusion experiments of organs are considered as not relevant for the use of *Stevia* or stevioside as food, and therefore these studies are not included in this review. The metabolism of stevioside is discussed in relation with the possible formation of steviol. Different mutagenicity studies as well as studies on carcinogenicity are discussed. Acute and subacute toxicity studies revealed a very low toxicity of *Stevia* and stevioside. Fertility and teratogenicity studies are discussed as well as the effects on the bio-availability of other nutrients in the diet. The conclusion is that *Stevia* and stevioside are safe when used as a sweetener. It is suited for both diabetics, and PKU patients, as well as for obese persons intending to lose weight by avoiding sugar supplements in the diet. No allergic reactions to it seem to exist.

Keywords: *Stevia rebaudiana*; Stevioside; Sweetener; Biosynthesis; Toxicology

1. Introduction

Stevia rebaudiana (Bertoni) Bertoni is a perennial shrub of the Asteraceae (Compositae) family native to certain regions of South America (Paraguay and Brazil). It is often referred to as ''the sweet herb of Paraguay''.

Stevioside, the main sweet component in the leaves of *Stevia rebaudiana* (Bertoni) Bertoni tastes about 300 times sweeter than sucrose (0.4% solution). Structures of the sweet components of *Stevia* occurring mainly in the leaves are given in Fig. 1. Their content varies between 4 and 20% of the dry weight of the leaves depending on the cultivar and growing conditions. Stevioside **3** is the main sweet component. Other compounds present but in lower concentration are: steviolbioside **2**, rebaudioside A **4**, B **5**, C **6**, D **7**, E **8**, F **9** and dulcoside A **10** (Kennelly, 2002; Starrat et al., 2002). The presence of steviolbioside and rebaudioside B in extracts might be due to artifacts of the extraction procedure (Refs. in Kennelly, 2002).

Details on the genus *Stevia*, its botany, its sweet and non-sweet constituents, modifications of the naturally occurring sweeteners to improve the taste can be found in the recent excellent book by Kinghorn (2002). Both the *Stevia* plant, its extracts, and stevioside have been used for several years as a sweetener in South America, Asia, Japan, China, and in different countries of the EU. In Brazil, Korea and Japan *Stevia* leaves, stevioside and highly refined extracts are officially used as a low-calorie sweetener (Mizutani and Tanaka, 2002; Kim et al., 2002). In the USA, powdered *Stevia* leaves and refined extracts from the leaves have been used as a dietary supplement since 1995. In 2000, the European Commission refused to accept *Stevia* or stevioside as a novel food because of a lack of critical scientific reports on *Stevia* and the discrepancies between cited studies with respect to possible toxicological effects of stevioside

Abbreviations: ADI, Allowable daily intake; BW, Body weight; CHL, Chinese hamster lung fibroblast cell line; GA, gibberellin; Glc, Glucose; ICH, International Council of Harmonisation; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; EST, expressed sequence tags; IPP, isopentenyldiphosphate; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LD50, Lethal dose at which 50% of the animals die; MEP, 2-C-Methyl-D-erythritol-4-phosphate; MVA, mevalonic acid; NOEL, No-observable effect level; OECD, Organisation for economic co-operation and development; PKU, Phenylketonuria; Rha, Rhamnose; Xyl, Xylulose

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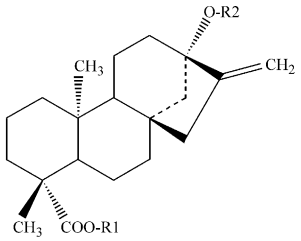

	Compound name	R1	R2
1	steviol	H	H
2	steviolbioside	H	β-Glc-β-Glc(2→1)
3	stevioside	β-Glc	β-Glc-β-Glc(2→1)
4	rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
5	rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	rebaudioside C (dulcoside B)	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	rebaudioside E	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	dulcoside A	β-Glc	β-Glc-α-Rha(2→1)

Fig. 1. Structures of stevioside and related compounds. In rebaudioside D and E R1 is composed of 2 β-Glc-β-Glc(2→1). In rebaudioside A, B, C, D, E and F in group R2 an additional sugar moiety is added on carbon 3 of the first β-Glc. In rebaudioside F one β-Glc is substituted for by-β-Xyl.

and especially its aglycone steviol **1** (Fig. 1) (Kinghorn, 2002; Geuns, unpublished). The advantages of stevioside as a dietary supplement for human subjects are manifold: it is stable, it is non-calorific, it maintains good dental health by reducing the intake of sugar and opens the possibility for use by diabetic and phenylketonuria patients and obese persons.

2. Biosynthesis of stevioside

The *ent*-kaurene skeleton of stevioside and hence also of gibberellins (GAs) is formed via the recently discovered 2-C-Methyl-D-erythritol-4-phosphate pathway (MEP; Totté et al., 2000). The genes of the enzymes catalysing the first two steps of this pathway, 1-deoxy-D-xylulose-5-phosphate synthase (DXS) and 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) were cloned using reverse transcriptase-PCR. DXS and DXR from *Stevia* both contain an N-terminal plastid targeting sequence and show high homology to other known plant DXS and DXR enzymes. Furthermore, it was demonstrated through heterologous expression in *Escherichia coli* that the cloned cDNAs encode functional proteins (Totté et al., 2003). Kim et al. (1996a) found a high activity of HMG-CoA reductase, a key enzyme of the mevalonic acid (MVA) route to IPP, in the chloroplast fraction from *Stevia*. They suggested that steviol was synthesised via MVA, but no direct proof was given to support this assessment. The results concerning the involvement of the MEP pathway in the biosynthesis of steviol cast doubt on their hypothesis as no contribution of the MVA pathway could be detected. These conclusions were confirmed by Brandle et al. (2002) who sequenced 5548 expressed sequence tags (ESTs) from a *Stevia* leaf cDNA library. The ESTs were classified according to their function in primary or secondary metabolism. In the last category many candidate genes specific to the MEP pathway and no members of the MVA pathway were identified, suggesting that the primary source of IPP for diterpene biosynthesis is via the MEP pathway. A kaurene oxidase, a cyt P450 type

of enzyme, was highly represented. Other P450s involved in GA biosynthesis, like *ent*-kaurenoic acid oxidase, were not found among the ESTs. These results demonstrate the divergence of steviol and GA biosynthesis after the production of kaurenoic acid. Kim et al. (1996b) claimed to have purified and partially characterized the *ent*-kaurenate 13-hydroxylase, the key enzyme in steviol biosynthesis. However, use of the published N-terminal sequence for constructing primers led to fructose-bisphosphate aldolase instead of an *ent*-kaurenate 13-hydroxylase (Totté, personal communication).

Looking at the accumulation percentage of the sweet glycosides, it is clear that a large fraction of total plant metabolism is committed to the synthesis of these structurally complex molecules. In contrast, gibberellins such as GA_{20} are present in *Stevia* leaves at concentrations of 1.2 μg/kg fresh weight, over 10,000 times lower than steviol glycosides (Alves and Ruddat, 1979). How can these differences in the occurrence of such structurally related products be explained? Richman et al. (1999) concluded that profound changes in the regulation of copalylphosphate synthase and kaurene synthase expression in *Stevia* leaves have enabled the synthesis and accumulation of high concentrations of sweeteners. The fact that expression levels are highest in mature tissues as compared to young rapidly growing tissues raises the possibility of temporal and spatial separation, preventing an overlap of steviol and GA biosynthesis.

Various plant-growth regulator effects, mostly gibberellin-like, have been described of stevioside, steviol and isosteviol or of their metabolites (Hersenhorn et al., 1997; de Oliveira et al., 1999; Bearder et al., 1975; Gianfagna et al., 1983; Orihara et al., 1991; Ruddat et al., 1963; de Oliveira and Strapasson, 1996).

3. Acute and chronic toxicity

The toxicology and safety of stevioside used as a sweetener were recently reviewed (Geuns, 2002; Huxtable, 2002). An acceptable daily intake (ADI) of 7.9 mg stevioside/kg BW was calculated (Xili et al., 1992). However, this ADI should be considered as a minimum value as the authors did not test concentrations of stevioside higher than 793 mg/kg BW.

Neither those scientific studies where Stevia extract or solution of pure stevioside were injected in animals, nor those studies employing perfusion experiments of organs, are considered relevant for the use of Stevia or stevioside as a food additive and are not discussed in this review. Stevioside has a very low acute oral toxicity in the mouse, rat and hamster. An oral LD50 between 8.2 and 17 g/kg BW was found (Mitsuhashi, 1976; Medon et al., 1982; Toskulkao et al., 1997).

In hamsters the LD_{50} of steviol (90% purity), the aglycone of stevioside, was 5.2 and 6.1 g/kg BW for respectively male and female animals. In rats and mice the LD_{50} was above 15 g/kg BW demonstrating that of the tested animals hamsters are much more sensitive to steviol (Toskulkao et al., 1997).

In chronic subacute toxicity studies with rats during 3 months (Mitsuhashi, 1976; Akashi and Yokoyama, 1975; Aze et al., 1991) or with hamsters over several generations (Yodyingyuad and Bunyawong, 1991) a NOEL higher than 2.5 g/kg BW was found. From this an ADI of 25 mg/kg BW can be deduced (safety factor 100).

4. Steviol, the controversial metabolite of stevioside

Mutagenic effects of steviol, the aglycone of stevioside, and/or its metabolites were reported in *Salmonella typhimurium* TM677 (Pezzuto et al., 1985; Compadre et al., 1988; Matsui et al., 1996a; Temcharoen et al., 1998; Terai et al., 2002). After metabolic activation it was shown that so far unknown steviol metabolites caused mutations in *Salmonella typhimurium* TM677, i.e. transitions, transversions, duplications and deletions at the guanine phosphoribosyltransferase (*gpt*) gene (Matsui, 1996b). However, stevioside and steviol were inactive in various TA strains of *Salmonella typhimurium*, *Escherichia coli* WP2 *uvrA*/pKM101 and the *rec*-assay using *Bacillus subtilis* even when activated microsomal fraction was present (Matsui et al., 1996a; Klongpanichpak et al., 1997). The direct mutagenic activity of 15-oxosteviol was refuted by Procinska et al. (1991), but confirmed by Terai et al. (2002). The activity of steviol in *Salmonella typhimurium* TM677 was very low and was only about 1/3000 that of 3,4-benzopyrene and that of steviol methyl ester 8,13 lactone was 1/24,500 that of furylfuramide (Terai et al., 2002). Although a weak activity of steviol and some of its derivatives was found in the very sensitive *S. typhimurium* TM677 strain, the authors concluded that the daily use of stevioside as a sweetener is safe. Moreover, the presence in the blood of the chemically synthesised steviol derivatives after feeding stevioside is not proven at all. Very high doses of steviol (90% purity) intubated to hamsters (4 g/kg bw), rats and mice (8 g/kg BW) did not induce micronucleus in bone marrow erythrocytes of both male and female animals. However, these doses showed some cytotoxic effect to the female, but not to the male of all treated animal species (Temcharoen et al., 2000). It is not excluded that the toxicity is due to the 10% impurities present. After metabolic activation of steviol some gene mutation and chromosomal aberration was found in Chinese hamster lung fibroblasts (Matsui et al., 1996a). It has to be said that of all animals tested hamsters had the most sensitive response. Moreover, in hamster several metabolites of stevioside were found that are not formed in rats or humans. Therefore, the relevance of experiments with hamsters should be questioned.

5. Uptake and metabolism of stevioside

It has been shown that oral stevioside is not taken up by the human body or the uptake is extremely low (Yamamoto et al., 1985; Bracht et al., 1985; Koyama et al., 2003b; Geuns et al., in press a) and none of the digestive enzymes from the gastro-intestinal tract of different animals and man are able to degrade stevioside into steviol, the aglycone of stevioside (Wingard et al., 1980; Hutapea et al., 1997; Koyama et al., 2001, 2003a).

Nevertheless, in feeding experiments with rats and hamsters stevioside was metabolised to steviol by the bacterial flora of the caecum. Steviol was found in the blood of the animals with the maximum concentration occurring after 8 h (Nakayama et al., 1986; Koyama et al., 2003a). In the cited studies, it was not indicated that coprophagy, occurring in rodents, was prevented, so it is not clear whether the steviol occurring in the blood was taken up directly from the colon or indirectly from the ingested faeces (after passing through the intestines again). Although bacteria isolated from the human colon are able to transform stevioside into steviol in vitro (Hutapea et al., 1997; Koyama et al., 2001, 2003a; Gardana et al., in press), it has never been proven that this is also the case in vivo nor that the steviol possibly formed in the colon is taken up directly from it. Moreover, studies with roosters (Pomaret and Lavieille, 1931) and chickens (laying hens and broylers, Geuns et al., 2003) indicated that stevioside was rapidly eliminated from the body, largely untransformed. Opposed to these results, in pigs oral stevioside was completely degraded into steviol that was the only metabolite in the faeces. However, no stevioside or steviol were found in the blood (Geuns et al., in press a). From the references cited above it is concluded that only the bacteria from the caecum or colon were able to degrade stevioside into steviol (caecum of mice, rats, hamsters and chickens; colon of pigs and man). In one experiment, the bacteria from the human colon also formed steviol epoxid in vitro, that was again metabolised to steviol (Hutapea et al., 1997). However, in vivo this epoxid formation most probably will not occur due to the anaerobic conditions of the human colon. It was correctly concluded that steviol is the only metabolite in faeces and is not further metabolised (Hutapea et al., 1997; Koyama et al., 2001, 2003a; Gardana et al., in press; Geuns et al., 2003, in press a). Anyway, steviol epoxid has been tested in mutagenicity studies and showed to be inactive (Pezzuto et al., 1985). In contrast to the above studies is the work by Hutapea et al. (1999) who found, besides stevioside and steviol, steviol-16,17α-epoxide, 15α-hydroxysteviol, isosteviol and steviolbioside in the faeces 24 h after force-feeding hamsters with 1 g stevioside/kg BW. In the urine steviol-16,17α-epoxide, stevioside, 15α-hydroxysteviol, steviolbioside and isosteviol were found. The plasma contained steviol-16,17α-epoxide, stevioside, 15α-hydroxysteviol and steviolbioside. It is not possible to deduce the amounts of metabolites from the chromatograms given. As all the extract fractions were treated with type H-5 β-glucuronidase/sulfatase at 55 °C for 3 h some of the metabolites might possibly originate from this treatment. For steviol was the only metabolite of stevioside when bacteria from the hamster caecum were incubated (Hutapea et al., 1997). Moreover, it is unlikely that compounds as steviol-16,17α-epoxide or 15α-hydroxy-steviol are formed in the anoxic intestines of hamsters. Isosteviol might be an artifact due to acid conditions and is normally not detected even not after incubating stevioside in human stomatal juice for 6 h (Geuns, unpublished). The peaks in the chromatograms of plasma and urine identified as stevioside were impurities cochromatographing at the same RT as shown later (Hutapea, personal communication).

When steviol was fed to rats is was easily taken up by the intestines (Wingard et al., 1980; Nakayama et al., 1986; Koyama et al., 2003b). The easy uptake of steviol by the gastro-intestinal tract was demonstrated in experiments with everted gastrointestinal sacs (Koyama et al., 2003b) and Caco-2 cell monolayers (Geuns et al., in press a). Although it was demonstrated that the absorptive transport of steviol was high in Caco-2 cell monolayers, and that stevioside fed to pigs (68 mg/kg BW) was completely converted in the colon into steviol, no steviol could be detected in the blood of the animals, suggesting that the possible uptake from the colon is very low (Geuns et al., in press a). The lack of steviol in the blood samples can probably not be attributed to metabolism during or after uptake as was the case with soy isoflavones that after uptake were metabolised to compounds that were hydrolyzable with a combined β-glucuronidase and sulfatase enzyme preparation (Setchell, 2002); indeed free steviol was detected in the plasma of rats up to at least 8 h after feeding stevioside or steviol (Compadre et al., 1988; Koyama et al., 2003b). Moreover, the in vitro conversion of steviol by liver microsomal fraction from Aroclor 1254-pretreated rats was rather low (about 0.3% after 2 h, Compadre et al., 1988). The intrinsic clearance of steviol by human microsomes was about 4 times lower than that of rat microsomes (Koyama et al., 2003b).

Taking into account the very low detection limits of steviol (50 pg) when analysed as the (7-methoxycoumarin-4-yl)methyl ester the amount of steviol possibly remaining undetected in blood samples of pigs fed stevioside can be estimated to be very low (below 1 μM, i.e. below 318 ng/ml; Geuns et al., in press a). This hypothetical maximum steviol concentration in the blood would probably not be toxic, as in hamsters fed steviol up to 250 mg/kg BW no toxic effects were found (Wasuntarawat et al., 1998). In this case steviol would have been easily taken up by the intestines. When steviol was fed to rats (45 mg/kg BW) a fast uptake was found

Table 1
Steviol concentration (μg/ml plasma) measured in the blood of rats (Koyama et al., 2003b) or estimated to be present in the blood of hamsters (Wasuntarawat et al., 1998) and pigs (Geuns et al., in press a) after the administration of steviol to the animals[a]

Amount administered (mg/kg BW)	After				
	15 min (μg/ml)	2 h (μg/ml)	4 h (μg/ml)	8 h (μg/ml)	16 h[a] (μg/ml)
45 (rats)	18.3	2.5	3.5	2.5	1
250 (hamsters)	102	13.6	19	13.6	5.4
9.7 (pigs)	3.94	0.5	0.7	0.5	0.2

[a] Amount estimated from the decay of the proceeding data points.

and the highest plasma concentration of 18.3 μg/ml was observed after 15 min (the first data point, Table 1; Koyama et al., 2003b). The plasma concentration declined to about 2–3 μg/ml at 8 h. Although we are aware that species differences might occur, we have extrapolated the data obtained in rats to hamsters and pigs (Table 1). Assuming a similar uptake and metabolism in hamsters the reported NOEL of 250 mg steviol/kg BW would correspond to a plasma concentration of 102 μg/ml 15 min after intubating steviol and about 13.6 μg/ml after 8 h. In pigs having at the most 9.7 mg steviol/kg BW available in the colon for uptake, this would then be 3.94 and 0.5 μg/ml respectively (Geuns et al., in press a). Although these concentrations are above the detection limit of the (7-methoxycoumarin-4-yl)methyl ester of steviol, no steviol was detected in the blood of these animals. Therefore, it was suggested that *in vivo* the uptake of the carboxy acid steviol from the colon is neglectible and that it is rather adsorbed to the compounds present in the colon (pH 7–7.5) of which the contents is being concentrated by withdrawal of water.

6. Stevioside and carcinogenicity

A weak mutagenic effect of steviol (only 90% purity) in one sensitive *Salmonella typhimurium* TM 677 strain (see above) does not mean that stevioside used as a sweetener should be carcinogenic in se, even if the stevioside might be transformed to steviol by bacteria in the colon. The safety of oral stevioside in relation to carcinogenic activity is evidenced by the work of Yamada et al. (1985), Xili et al. (1992), Toyoda et al. (1997) and Hagiwara et al. (1984) with rats. Very significant inhibitory effects of stevioside were reported on tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in carcinogenesis in mouse skin (Yasukawa et al., 2002). In 1999 the JECFA clearly stated that there was no indication of carcinogenic potential of stevioside (WHO, 1999).

7. Fertility and teratogenicity

The results of a decrease of live birth rate in rats (Planas and Kuæ, 1968) by *Stevia* decoctions were refuted by Shiotsu (1996) who did more reliable experiments with many more animals using methods as similar as possible to the methods used by Planas and Kuc. No effect on general condition, body weight, water consumption, live birth rate or litter size was found. No effects of stevioside were found on fertility or reproduction in mice (Akashi and Yokoyama, 1975), rats (Mori et al., 1981; Xili et al., 1992; Sinchomi and Marcorities, 1989) or hamsters (Yodyingyuad and Bunyawong, 1991).

No significant effect was found on spermatogenesis, nor on the interstitial cell proliferation and tumor formation in the testes of F344 rats fed a ration containing up to 1% stevioside (95.2% purity) for 22 months (Yamada et al., 1985).

Whereas Melis (1999) suggested a possible decrease of the fertility of male rats by a very high dose of *Stevia* extract, Oliveira-Filho et al. (1989) who administered extracts with similar stevioside content stated that there is certainly not an effect on male fertility. It is not sure that the observed effects were due to the stevioside present in the extract. It should also be mentioned that the used extract concentrations were extremely high, at the start of the experiments even 5.34% of the body weight (or around 5.3 g stevioside/kg bw). For an adult person of 65 kg this means 3.47 kg of dry *Stevia* leaves or about 34.7 kg fresh leaves/day, i.e. more than 50% of the body weight! The significance of such experiments where only one extremely high concentration was tested, should be questioned. Melis' results are also in contradiction with the above and below cited studies that could not reveal any effect on fertility of male or female animals.

Chicken embryos react very sensitively to administered toxicants. Fertile broiler eggs (Ross) were injected with stevioside or steviol (Geuns et al., in press b). At hatch (day 21) and 1 week later no influence of the different treatments could be found on embryonic mortality, body weight of the hatchlings, deformations (e.g. bone, beak and head malformations, abnormal feathering, open vent) or abnormal development of the gonads. The hatchlings developed normally. It was concluded that prenatal exposure to stevioside and steviol was not toxic to the chicken embryo.

Applied stevioside has no effect on fertility, mating performance, pregnancy, number of fetuses, nor on the growth and fertility of the offspring (Yodyingyuad and

Bunyawong, 1991; Mori et al., 1981; Oliveira-Filho et al., 1989; Sinchomi and Marcorities, 1989; Usami et al., 1995; Geuns et al., in press b). However, when steviol (the aglucone of stevioside) was given to hamsters on days 6–10 of pregnancy at doses of 500–1000 mg/kg body weight/day it induced toxicity (Wasuntarawat et al., 1998). The number of live foetuses per litter and mean foetal weight decreased. The maternal kidneys showed a dose-dependent increase in severity of convoluted tubules in the kidneys. This study with steviol has nothing to do with the use of stevioside as a sweetener. When stevioside is fed to hamsters, no toxic effects were found even not in 3 successive generations (Yodyingyuad and Bunyawong, 1991). When steviol is given in the feed, it can be resorbed directly by the intestines, whereas stevioside is not. Moreover, hamsters are known to be very sensitive to steviol and stevioside (Toskulkao et al., 1997), this is the reason that hamsters were chosen in this study. The NOEL of steviol was 250 mg/kg bw (Wasuntarawat et al., 1998), which corresponds to 625 mg stevioside/kg bw. Even under these very unfavourable conditions an ADI of 2.5 mg steviol/kg bw, which corresponds to 6.25 mg stevioside/kg bw, can be calculated, which is close to 7.9 mg/kg bw obtained for stevioside (Xili et al., 1992).

8. Bio-availability of nutrients from the diet

Modern broiler chickens are intensively selected for growth rate and BW increases with a factor of more than 50 in a time span of 6 weeks, making these animals especially suited to study the influence of feed additives on growth. However, they have become very susceptible to even slight deviations from optimal environmental and nutritional conditions. If such aberrations occur, this is readily reflected in feed intake and growth rate. No effects of stevioside on the growth, feed uptake or feed conversion of broiler chickens were found (Wood et al., 1996; Geuns et al., 2003). It can be inferred that stevioside did not influence the uptake of other essential nutrients such as amino acids, vitamins, minerals etc. When stevioside was supplemented to the feed of laying hens (667 mg/kg), no significant differences were found for the total feed consumption, body weight gain, the total egg production nor for the feed conversion calculated as the ratio between total feed uptake and total gram of egg mass produced during the 10 days of the experiment (Geuns et al., 2003). The percentage of yolk and egg white was not significantly different between the control and the stevioside-treated group. These results showing the lack of effects on growth and hence on bioavailability of essential nutrients are in good accordance with studies performed with rats (Yamada et al., 1985; Melis, 1996; Xili et al., 1992; Oliveira-Filho et al., 1989; Das et al., 1992) and hamsters (Yodyingyuad and Bunyawong, 1991).

In all of the above cited experiments, no indications of any influence on the bio-availability of nutrients, nor on physiological effects were found. The animal feed used in the experiments did not contain sugar supplements. Therefore, no reduction of weight gain was observed in the experiments as *Stevia* or stevioside did not substitute for added sugar.

9. Stevia, stevioside and special groups of the population: nutritional significance

Boeckh-Haebisch (1992) concluded that concentrated *Stevia* extracts in normal doses to sweeten can be used without restriction by normal persons as well as by diabetics. The omission of excessively added sugar in the food is beneficial to diabetics by lowering the blood sugar content (Boeckh-Haebisch, 1992). *Stevia* and stevioside are also safe for phenylketonuria (PKU) patients as no aromatic amino acids are involved. Obese persons might lose weight by the fact that excessive sugar in the food is replaced by *Stevia* or stevioside. Omitting added sucrose in foods increases the relative proportion of polymeric carbohydrates. This has a beneficial effect for a balanced food intake and for human health (Anonymous, 1996).

Curi et al. (1986) reported that *Stevia* extracts from 5 g of dried leaves thrice a day administered for 3 days to healthy volunteers lowered the plasma glucose levels. However, care should be taken interpreting these results as the plasma glucose level of the *Stevia* treated group was already significantly lower before the administration of the extract. Intravenous administration of stevioside (95% pure, 50, 100 or 200 mg/kg BW) resulted in a significant hypotensive effect in spontaneously hypertensive rats without adverse effects on heart rate or serum catecholamine levels (Chan et al., 1998). In a study with humans stevioside (250 mg thrice a day) was administered for 1 year to 60 hypertensive volunteers (Chan et al., 2000). After 3 months the systolic and diastolic blood pressure significantly decreased and the effect persisted during the whole year. Blood biochemistry parameters including lipid and glucose showed no significant changes. No significant adverse effect was observed and quality of life assessment showed no detoriation. The authors concluded that stevioside is a well tolerated and effective compound that may be considered as an alternative or supplementary therapy for patients with hypertension. Liu et al. (2003) reported that the underlying mechanism of the hypotensive effect of administered stevioside in dogs (200 mg/kg BW) was due to inhibition of Ca^{2+} influx from extracellular fluid.

10. Stevioside and caries

From experiments with albino Sprague–Dawley rats Das et al. (1992) concluded that neither stevioside nor rebaudioside A is cariogenic.

Although rather high concentrations of stevioside and *Stevia* extracts were shown to reduce the growth of some bacteria, the concentrations used for sweetening purposes are rather low. Therefore, the benificial effect of the use of stevioside would rather be due to the substitution of sucrose in the food by a non-cariogenic substance.

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