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Stevia (*Stevia rebaudiana* B.) as a medicinal plant and its antioxidant and lipid-modulating effects – with focus on ruminant production. A review

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Abstract

Penchev, P., Enchev, S., Nenova, R., Ilieva, Y., Ivanova, D. & Schreiner, M. (2025). Stevia (*Stevia rebaudiana* B.) as a medicinal plant and its antioxidant and lipid-modulating effects – with focus on ruminant production. A review. *Bulg. J. Agric. Sci., 31*(3), 595–604

A comprehensive literature search was conducted, and the expertise and achievements with stevia and some other medicinal plants of our plant and animal breedeing teams were taken in consideration. The objective to summarize the background of the medicinal applications of stevia was generally fuilfilled, the found available sources treating the effects on ruminant products is limited. Besides its direct benefits to consumers, indirect effect of its glycosides should be especially sought on production of ruminant origin, which is notorious for its saturated lipids. Except its immunomodulatory, anti-inflammatory, anti-oxidative and anti-tumor properties, this review implies stevia's capacity to modulate the anti-oxidant and nutritional qualities of foodstuffs. Not many proofs have been found for the influence of stevia on fatty acid composition of ruminant products, but its effects on rumen processes suggest application as a natural modulator of lipid quality via manipulation of rumen environment and functions. All this necessiates further in-depth research, but to date the available scientific evidence affords the presumption that it is worthwile to grow stevia for such purposes, justifying the peculiarities of its cultivation (vegetatively bred and maintained *in vitro*) and the efforts in the latitudes of Bulgaria, where it needs to be treated like an annual, not like a perennial plant as in its continent of origin.

Keywords: buffalo; medicinal plants; stevia, antioxidant; fatty acids

Introduction

The essential nutritional values of foodstuffs of ruminant origin are indisputable but still there is criticism against the mass consumption of milk and dairy products, because of the saturated nature of their lipids (Givens & Shingfield, 2006). In this context, according to FAO, saturated fatty acids should provide no more than 10% of total calories (FAO/WHO, 2003).

It is noteworthy that milk contains highly desirable oleic acid and conjugated linoleic acid (CLA), with anti-cardiovascular, anti-carcinogenic, anti-diabetic and immunomodulating effects (Belury, 2002; Parodi, 2004). Mainly Δ9-desaturase enzyme in the mammary gland (Bauman et al., 2006) and ruminal biohydrogenation (Shingfield et al., 2005; Kalač & Samková, 2010) are responsible for the expression of the effect of feeding on fatty acid profile of milk (Fernandes et al., 2007; Beyero et al., 2015) and meat (Daley et al., 2010; Nogoy et al., 2022).

Since 1970s, encapsulated/protected proteins and lipids have been used in cattle breeding achieving significant changes in the fatty acid profile in ruminant (Ashes et al., 1992; Scollan et al., 2003; Smith et al., 2010). Nevertheless, due to the hazards they cause to animals, plants and humans, the World Health Organization (WHO) encourages farmers to use medicinal plants and herbs to substitute or minimize their use, as a global trend that turns towards nature (Allam et al., 1999).

In the search for immunomodulatory agents to alleviate diseases, for many years the focus has been on finding medicinal plants that match the philosophy of 'Rasayana' and 'Ayurveda' and that possess such properties (Patwardhan et al., 1990; Diasio & LoBuglio, 1996).

In ruminants, herbal and medicinal plants have been also used to stimulate appetite and ruminal function resulting in health benefits for the animals (Cedillo et al., 2015; Salem et al., 2017). Moreover, phytogenic compounds have been used as alternatives of antibiotics, and to increase feed intake, blood chemistry and productivity (Matloup et al., 2017; Khattab et al., 2020).

In a study of Fahim et al. (2022) on buffalo cows, fennel was found to significantly increase the concentrations of trans-10, cis-12 C18:2, total CLA in milk without affecting other fatty acids. A dose of 75 g per capita per day of fennel or ginger improved nutrient digestibility and milk production of buffaloes.

Our experiment with a buffalo herd in Bulgaria supplemented a herbal preparation of the Piper genus (AyuFertin) showed a trend to affect the fatty acid profile of the milk of the treated buffaloes (Ilieva et al., 2022). The results of another our recent experiment with Curcumin C3 Complex on buffaloes (Penchev et al., 2022), showed increased concentration of the valuable vaccenic, rumenic acid and total trans-isomers in the raw milk after supplementation.

Stevia rebaudiana, belonging to the sunflower family (*Asteraceae*), is a perennial herb native to the subtropical regions of South America that has been used for decades as a sweetener in many countries, predominantly in Latin America and East Asia (Lemus-Mondaca et al., 2012). The sweeteners produced from it have sensory and functional properties superior to the conventional sweeteners and have the perspective to prevail on the food market (Goyal et al., 2010). This is chiefly attributed to Stevioside (*13-[(2-O-beta-D-glucopyranosyl–alpha-D-glucopy-ranosyl oxy] kaur-16-en-18-oic acid-beta-D-glucopyranosyl ester*), which is one of the main glycosides in the green parts of *Stevia rebaudiana*, and which outstands with its safety, stability, low pH, high degree of sweetness, in the same time with low caloric value (Puri et al., 2011).

Also, the European Food Safety Authority (EFSA) approved the usage of steviol glycosides as a non-toxic alternative sweetener in food and beverages industry (Ilias et al., 2021). Moreover, stevia was established to contain other components that have therapeutic properties including vitamins, minerals, essential amino acids, fatty acids, flavonoids, alkaloids, chlorophyll, xanthophyll, aromatic acids, chlorogenic acid, oligosaccharides, free sugars, and phytosterol (Muanda et al., 2011; Ahmad et al., 2020).

The first extraction of steviosides from stevia was in 1931, done by French chemists (Carakostas et al., 2008). In 1964 and 1968, stevia was commercially cultivated in Paraguay and Japan, respectively, and used extensively in commercial products. Japan legalized usage of stevia as a sweetener in the 1970s, banning and replacing all other chemical sweeteners. Since then, cultivation of the plant has expanded to other countries including China, Malaysia, Singapore, South Korea, Taiwan, Thailand, Paraguay, Brazil, USA, Canada and Europe (Midmore & Rank, 2002; Chatsudthipong & Muanprasatq, 2009).

In 1980, stevia was introduced in Bulgaria from Japan and USA and became a subject of research at the Agricultural Institute – Shumen. As a result of the scientific studies, stevia was introduced into culture *in vitro* and appropriate nutrient media were developed for its micropropagation and rooting (Slavova et al., 2003; Kikindonov, 2013; Uchkunov et al., 2015). At the Institute of Organic Chemistry Sofia was done the extraction and purification of the sweet substances from the dry leaves, and at the Institute of Nutrition at the Medical Academy was analyzed the sweet substances for toxicity and safety (Nikolova-Damyanova et al., 1994).

For more than 15 years, plants of several stevia genotypes were maintained under *in vitro* conditions. As a result of the selection, the first Bulgarian variety „Stella" was created and certified legally in 2016, now being bred vegetatively.

The soil and climate conditions in Bulgaria allow for the successful cultivation of *S. rebaudiana* B. Due to its high sensitivity to low temperatures, stevia is grown only as an annual plant (Kikindonov, 2013). At a later stage, in greenhouse conditions, it can be vegetatively bred – by means of green rhizomes obtained from planted for the purpose roots that had passed one growing season in field conditions, but it can also be developed from adapted regenerates from *in vitro* maintained branches, and under favorable conditions it is possible to obtain seeds from it (Mehmed et al., 2021).

Our studies have shown that the yield of glycosides, especially stevioside, strongly depends on the total biomass yield, which in turn depends on good agricultural practices for growing the plant (Kikindonov & Enchev, 2012; Mehmed & Enchev, 2020).

The stevioside and rebaudiside are the main steviol glycosides, which make up more than 95% of the total amount of sweet substances. Pure stevioside is about 200 times sweeter than sucrose, and rebaudiside A is about 250 times sweeter (Brandie, 2004). Although these substances are extremely sweet, they do not contain calories (Enchev et al., 2018; Tanova & Kaschieva, 2018) and are also resistant to high temperatures (up to 198°C) (Benford et al., 2006). They are non-fermentable and do not induce carbohydrate cravings, making them suitable for diabetics (Curi et al., 1986; Gregersen et al., 2004).

Objectives

With this review we aimed to summarize the background of the medicinal applications of *Stevia rebaudiana B.* and its derivates for oxidative stress and immune activity – in mammals as a whole, and in livestock in particular and their effects on the products thereof, with focus on foodstuffs of ruminant origin. In this context, the focus is on the water buffalo (*Bubalus bubalis*) and the produced milk.

Material and Methods

A profound comprehensive search of scientific literature was conducted in order to study the problem in focus. In the present review was used information from publications obtained on the Internet – exclusively Google Scholar but mostly Research Gate, PubMed, Scopus and Web of Science – as well as journal editions worldwide and also editions in Bulgaria. More specifically, the expertise and achievements with stevia of the team of plant breeders at Agricultural Institute – Shumen and also the research experience and experimental results with medicinal plants in ruminants of our team were taken in consideration, as well.

Results of Literature Review

In connection with the changes in the food industry and the modern trend for a healthy lifestyle, there is an increased scientific interest in natural immunomodulators and antioxidants in nature. Many countries are conducting their own research in order to discover natural, low-calorie sweeteners of plant origin that are not harmful to human body.

Functional benefits of stevia (in vitro investigations, laboratory animals and human)

The uses and applications of stevia are increasing both on national and international scale. It has been found to possess physiological activity that can be used in human healthcare. As a healthy replacement of sucrose, stevia has the ability to regulate blood pressure and blood sugar (Ferri et al., 2006; Gupta et al., 2016; Rotimi et al., 2018). In addition, it was revealed that stevia had the potential to positively affect blood sugar and insulin levels, urine sodium excretion, lipid profile and body weight (Savita et al., 2004).

Except for its exceptional sweetness, stevia is valuable because of its nutritional components, especially phytochemicals and polyphenols (Chu et al., 2000; Singh & Rao, 2005; Edeoga et al., 2005) contributing for its outstanding antioxidant properties (Chatsudthipong & Muanprasat, 2009; Ahmad et al., 2010).

According to the review by Thomas and Glade (Thomas & Glade, 2010) the antioxidant capacity of stevia is even greater than DL-α-tocopherol and green tea extract, due to the activity against free radicals and superoxides (Xi et al., 1998 a, b). It contains high concentrations of total polyphenols and flavonoids and prevents also lipid peroxidation and DNA strand excision (Ghanta et al., 2007). Another research also demonstrated the potential of *Stevia rebaudiana* in functional foods production or as microbial or therapeutic agent, finding a favorable correlation between phenolics and flavonoids on one hand and antioxidant capacity on the other (Lemus-Mondaca et al., 2018).

An experiment in human medicine showed that supplementation of stevia led to a decrease in blood glucose, alanine and aspartate transaminase and an increase in blood insulin level (Shivanna et al., 2013). Stevia also reduced malondialdehyde concentration in the liver and improved its antioxidant status through antioxidant enzymes. The authors result in optimized glucose tolerance and insulin sensitivity. The antioxidant capacity of stevia has found confirmation in other studies as well (Shukla et al., 2012; Bender et al., 2015).

The immune system support of stevia leaf extracts is expressed in significant antiviral and antimicrobial and antifungal activity towards a wide range of pathogenic bacteria, including enterohemorrhagic *Escherichia coli*, without affecting normal intestinal flora (Tomita et al., 1997; Ghosh et al., 2008). Furthermore, stevia inhibits the replication of rotavirus *in vitro* by blocking viral attachment to cells (Takahashi et al., 2001).

A very recent medicinal *in vitro* study reveals the ability of steviol glycosides (stevioside and rebaudioside A) to affect the expression of genes involved in glucose and lipid metabolism (Kurek et al., 2023).

A number of studies indicated the decreasing effect of stevia on triglyceride (Aghajanyan et al., 2017) and fatty acid synthesis due to reduced levels of acetyl-COA carboxylase mRNA (Park & Cha, 2010). It reduces LDL cholesterol, triglyceride, and increases HDL cholesterol (Sharma et al., 2009), and hence can be used as an instrument to alleviate cardiovascular diseases (Ilias et al., 2021).

Besides anti-oxidant activity, stevia glycosides have been proven to posses wound healing, anti-viral, anti-inflammatory, anti-diabetic, and renal-protective properties, expressed in stimulation of the immune system functions like delayed type hypersensitivity response, macrophage phagocytosis, and B and T lymphocytes proliferation (Sehar et al., 2008; Cho et al., 2013). Isosteviol and the steviol glycosides, stevioside, rebaudioside A, rebaudioside C, and dulcoside A are responsible for inhibiting inflammation (Yasukawa et al., 2002; Mizushina et al., 2005).

Stevia's immunomodulatory activity, and anti-inflammatory properties were confirmed *in vitro* by the established decrease in TNF-α, IL-1β, IL-6 synthesis and inhibiting of nuclear transcription factor-kappa B (NF-κB), and *in vivo* by inhibiting NF-κB and the mitogen-activated protein kinase (MAPK) signaling, and the release of proinflammatory cytokines (Fengyang et al., 2012; Jiang et al., 2019; Kasti et al., 2022).

The extracts used in a Korean-Indian experiment were established to provoke a non-specific immune response in rats, and furthermore improved humoral and cell-mediated immunity effectively (Shukla & Mehta, 2015). On that ground, the authors claim *S. rebaudiana* is a therapeuticly effective and safe immunomodulatory agent that can be used as a 'Rasayana' in traditional medicine. In adition, stevia is rich in valuable protein and amino acids, minerals and fibers (Abou-Arab et al., 2010; Wolwer-Rieck, 2012).

It has been demonstrated that stevia has inhibitory properties of its polyphenolic constituents (aglycones, steviol and isosteviol, and their metabolites) on tumor promotion and initiation by blocking Epstein-Barr virus early antigen (EBV-EA) induction and tumor formation (Konoshima & Takasaki, 2002; Akihisa et al., 2004; Takasaki et al., 2009). Stevioside, isosteviol, potently inhibit DNA replication and cancer cell growth *in vitro* (Mizushina et al., 2005). It is noteworthy that except non-carcinogenic, non-mutagenic effects, stevia glycosides are non-teratogenic and do not cause toxicity and genotoxicity (Momtazi-Borojeni et al., 2017; Sharif et al., 2017; EFSA FAF Panel, 2022). On regular basis, the European Food Safety Authority releases reports on stevia and its derivatives usage, based on scientific opinion and sets acceptable daily intake for steviol glycosides (E 960) of 4 mg per 1 kg of body weight per day expressed as steviol (EFSA FAF Panel, 2022).

The research team of Ramos-Tovar were the first to show that stevia can take control over Nrf2, thereby counteracting oxidative stress, and hence prevent necrosis and cholestasis, implying that it can be administered in the clinical treatment of acute and chronic liver diseases (Ramos-Tovar et al., 2018). Similarly, it was found that stevia has the potential to protect against oxidative stress and age-related conditions via the Akt/Nrf2/HO-1 pathway, as its residue extract significantly increased the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and total antioxidant capacity (T-AOC), and decreased acetylcholinesterase (AChE) activity and malondialdehyde (MDA) level in mice (Zhao et al., 2019). The established clear membrane-stabilizing effect of stevia is to add to its antioxidant activity (Simonyan et al., 2021).

These many results of the effects of stevia as a medicinal plant imply that improvements in productivity of livestock should be also expected. The finding of its benefits on cholesterol and hence on cardiovascular healthcare suggest association between stevia-based diet and fatty acid profile of milk of dairy animals.

Stevia in monogastric animals

Studies in pigs (Munro et al., 2000; Geuns et al., 2003a; Wang et al., 2014) and poultry (Geuns et al., 2003b; Atteh et al., 2008) have demonstrated that stevioside supplementation leads to higher feed intake and utilization, as well as to positive effect on animal health. This can be associated with the findings that stevia and its glycosides can improve appetite in the porcine species (Wang et al., 2011, 2014; Guo et al., 2016; Yu et al., 2020), poultry (Jiang et al., 2020), as well as in horses (Ma & Ma, 2009).

Except in better consumption and utilization, some of these experiments resulted in improved health status of the digestive tract in pigs (Wang et al., 2014). It was found that 5% of stevia residue supplementation regulated poultry digestive function (Shang, 2011). Daily gain of 42-day broiler chickens supplemented 80 ppm of stevia-based sweetener was significantly higher (Molina-Barrios et al., 2021). In addition, immune response to Newcastle disease virus was also higher but not to infectious bronchitis.

A recent study of Liu et al. (2022) indicated that a supplementation doze of 200 mg/kg of stevia residue extract significantly reduced the incidence of diarrhea in piglets. A higher doze of 400 mg/kg significantly reduced serum MDA content and significantly increased the total antioxidant capacity, total superoxide dismutase, and glutathione peroxidase activity in the serum and catalase and glutathione peroxidase activity in the liver. Moreover, this higher doze improved health status by regulating antioxidant reduction homeostasis of the piglets, due to an increase of potentially beneficial bacteria like *Prevotellaceae* and *Roseburia*.

Similarly, a series of Bulgarian-British experiments showed that stevia could be effective against oxidative stress also in broilers. As supplemented to the diet, it improved liver antioxidant status via increasing liver vitamin E and carotenoids (Pirgozliev et al., 2021a) and via increasing dietary antioxidant availability (Pirgozliev et al., 2021b). In the third study (Pirgozliev et al., 2022), the addition of stevia to the diet of laying hens lead to a richer color of the yolks and to an increased content of carotenoids in the eggs, but did not affect the palatability of the stored eggs. The authors conclude that increasing the dietary sources of lutein and zeaxanthin for humans can be achieved by including a stevia supplement in feed for laying hens to increase the concentration of these macular carotenoids in egg yolks.

Studying stevia plant as a potential fodder in livestock, Atteh et al. (2011) found that the fat percentage in the leaves was similar to other non-oil plants (2.6%), and it had high degree of unsaturated fatty acids – significantly higher then in the stem. The protein content in the leaves (16%) was higher than in the stems (6.7%) – in both cases moderately digestible, so retained in the broilers' tract. The high crude fiber content and low metabolisable energy of the stems renders stevia a good supplement in low nutritional diets for monogastric animals, as well as a sweet energy diluent. In the ruminants, that can digest high fiber diets stevia stems can be used as a source of energy. This study shows that minerals were higher in stevia leaves than stems, except for of Na and K that are lower. Noteworthy is the high Fe content of the leaves that can be used in anemic conditions. So, the authors concluded that stevia protein and gross energy content might be suitable for ruminants rather than for monogastric animals like pigs and chickens.

Stevia in ruminants

The studies with *Stevia rebaudiana* B. as a medicinal plant on large and small ruminants are scarce.

The first known studies with stevia in ruminants are published in 2014. One of them shows the effect of a feed containing 6.5% of stevioside (as an essential oil) and 25% of yeast culture on rumen and rectal temperature in steers (Cho et al., 2014).

According to an Italian study, stevia extract has negative effect on the rumen protozoa population only at low dietary doses (Sarnataro & Spanghero, 2020).

Stevia is a medicinal plant that can be used as a natural feed with environmental and bioactive functions. In a study on sheep, He et al. (2017) describe the *in vitro* rumen fermentation of stevia residue material as typical acetic acid type fermentation, which is capable of promoting carbohydrate fermentation in the rumen, improving energy utilization and the production of volatile fatty acids. As a result, it affords lower methane emissions due to rumen fermentation. The authors also observe that stevia stimulates the microbe metabolism of the rumen, hence the production of microbial protein.

A recent trial on dairy cows in China showed the replacement of only 6% of alfalfa hay with stevia hay did not affect nutrients intake but improved the fiber digestibility, and hence increased milk yield, milk fat and ruminal volatile fatty acids, while the 12% stevia supplementation did not have such beneficial effects (Jiang et al., 2022).

Another Chinese experiment (Han et al., 2019) established that with the supplementation of 400 to 800 mg/kg stevioside to the diet of dairy goats, the appetite and feed intake increased as well as the digestibility of fiber and portion of VFAs in ruminal content. In terms of the effect on the production of ruminant origin, it was observed that in Korean native beef the inclusion of 1% (on weight basis) stevioside and selenium improved polyunsaturated fatty acid, lipid oxidation during the storage and microbial count; and also growth performance, carcass traits, and meat quality (Shin et al., 2021).

According to a very recent trial (Zhang et al., 2023), 1.0% stevia stalk supplementation improves feed conversion and promotes rumen fermentation, and another study (Xie & Wang, 2010) established that a supplementation of 0.3% to 0.6% whole stevia plants for one month could improve the appetite of dairy cows, especially important during the period of negative energy balance.

Conclusions

In this review we summarized the history of the medicinal applications of *Stevia rebaudiana B.* and its derivates in animals, and in livestock in particular and their effects on the products thereof. The objective of this review found few result in the available scientific literature with focus on stevia as a supplementation in ruminants, and the species studied are few as well. Still, these findings imply that stevia would presumably play similar role in other ruminants as well. Yet, this calls for new research in the field of antioxidant and fatty-acid properties of milk and meat as affected by stevia supplementation in some other species, not studied to date, like the buffalo. Here is the place to note that growing stevia in Bulgaria has its peculiarities. Its cultivation is vegetative and it is maintained under *in vitro* conditions and moreover it needs more efforts in the latitudes of Bulgaria, where it should be treated like an annual, not like perennial plant – involving autumn hibernation and spring implantation of roots, as well as further development within one season. Though, based on the knowledge at this point, it seems worth growing stevia for purposes like ruminant foodstuff production because of the many benefits thereof.

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